PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1974 TO THE
USAEC DIVISION OF BIOMEDICAL AND
ENVIRONMENTAL RESEARCH
PART 2 ECOLOGICAL SCIENCES

Battelle

Pacific Northwest Laboratories
Richland, Washington 99352

DECEMBER 1974

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ANNUAL REPORT FOR 1974
TO THE
USAEC DIVISION OF BIOMEDICAL AND ENVIRONMENTAL RESEARCH
PART 2 ECOLOGICAL SCIENCES

By

Burton E. Vaughan, Manager
and
Staff Members of Ecosystems Department
Environmental and Life Sciences Division

December 1974

On January 19, 1975, research and development programs of the U.S. Atomic Energy Commission (AEC) became part of the newly formed Energy Research and Development Administration (ERDA). In this report, since it refers to work done in 1974, most references are to AEC programs.

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RICHLAND, WASHINGTON 99352

PREFACE

The Annual Report for 1974 to the U.S. Atomic Energy Commission's Division of Biomedical and Environmental Research is organized by major program categories according to our schedule-189 submissions. Each part is directed toward a particular DBER Research and Development Program: Part 1 to Biomedical Programs, Parts 2 and 3 to Environmental Programs, and Part 4 to Physical and Technological Programs. Each part of the Annual Report comprises project reports authored by scientists from several research departments, reflecting the interdisciplinary nature of the research effort. The Annual Report consists of four parts:

Part 1	Biomedical Sciences	Program Manager: R. C. Thompson Editor: D. L. Felton
Part 2	Ecological Sciences	Program Manager: B. E. Vaughan Editor: J. L. Engstrom
Part 3	Atmospheric Sciences	Program Manager: C. E. Elderkin Editor: E. L. Nichols
Part 4	Physical and Analytical Sciences	Program Manager: J. M. Nielsen Editor: G. M. Garnant

Reports previously issued are as follows:

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1953	HW-30437, HW-30464
1954	HW-30306, HW-33128, HW-35905, HW-35917
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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
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1969	BNWL-1306, Vol. 1, Pt. 1-2, BNWL-1307, Vol. 2, Pt. 1-3
1970	BNWL-1550, Vol. 1, Pt. 1-2, BNWL-1551, Vol. 2, Pt. 1-2
1971	BNWL-1650, Vol. 1, Pt. 1-2, BNWL-1651, Vol. 2, Pt. 1-2
1972	BNWL-1750, Vol. 1, Pt. 1-2, BNWL-1751, Vol. 2, Pt. 1-2
1973	BNWL-1850, Pt. 1-4
1974	BNWL-1950, Pt. 1-4

W. J. Bair
Program Director, Life Sciences

FOREWORD

This volume is the seventh annual report to DBER on work supported under its Land and Freshwater Sciences (RX-02-01) and Marine Sciences (RX-02-02) programs. A schedule of the earlier reports will be found in the preface. As in preceding annual reports, we have brought together in one volume all work funded under these two budget categories. As in past years, about three-fourths of the funded effort continues to be provided by Ecosystems Department, with one-fourth provided by cooperating staff located in other departments of the Pacific Northwest Laboratory (PNL). For convenience, organizational charts for several departments will be found at the back of this report.

New transuranium element studies were implemented during 1974, resulting in a comprehensive program drawing on several major discipline areas. Work is now in progress on: 1) the statistical difficulties of inventorying transuranium element releases from environmental data, chiefly at NTS; 2) measuring foliar interception and translocation of ^{238}Pu aerosols in green plants; 3) determining availability as a function of chemical and biochemical alteration; 4) measuring distribution and movement in a freshwater pond ecosystem; and 5) investigating geochemical factors affecting transuranium element behavior in marine environments at Thule and Eniwetak; 6) also during 1974, experiments were commenced to determine long-term effects on plant availability to natural climatic and ecological factors. Similar ^{239}Pu studies were commenced in 1973, and further arrays will be commenced in 1975. We anticipate that 10 years will be required for the development of full information from the "weathering/aging" experiments.

In the transuranium element program, perhaps the most important developments of the past year are: a) several lines of new evidence indicative of enhanced bioavailability of ^{238}Pu compared to ^{239}Pu ; b) demonstration of a plutonium complex distinct from plutonium chelate, and c) the wider recognition of soil to plant transfer ratios approaching or exceeding unity for americium chelates. Explicit consideration of these high soil/plant transfer ratios was also incorporated into the LMFBR impact assessment. In the several projects, the specific isotopes studied were those important in the recycling of fuel for the civilian power reactor program, principally ^{238}Pu and ^{241}Am .

The Marine Sciences program was expanded considerably on July 1, 1974, with the intent of better focussing PNL's oceanographic and marine biological capabilities on the problem of determining bioavailability of contaminants. To this end, Mr. Keith Abel, representing the oceanographic group in Radiological Sciences Department, was transferred to the Marine Research Laboratory (MRL) at Sequim, Washington. Another staff member of that Department will be transferred during the next year. The remaining Richland-based staff in marine biology was relocated to MRL, and the Marine Biology Program was consolidated at Sequim, under direction of the resident manager, Dr. Charles I. Gibson. MRL was also dedicated and accepted as part of the consolidated Pacific Northwest Laboratory, under terms of AEC Contract AT(45-1)1830.

In the Marine Sciences program, a rationale has been delineated for investigating combined and interaction effects, where as is usually the case, multiple stressors are present in the polluting effluents. To this end, Dr. Thomas O. Thatcher, formerly associated with the Aquatic Physiology Program in Richland, was transferred to the Sequim staff, with responsibility for bioassay development. Also, several staff members attended a joint EPA/Marine Technology Society workshop at Montauk, NY, in February, 1974, with this writer serving on Panel No. 4, concerned with synergism and modifying factors.

New work was developed in 1974 to better define certain problem areas relating to other energy technologies apart from the nuclear program. We have drawn heavily on experience gained in our extramural industrial programs in oil toxicology to develop suitable analytical and bioassay methodology for investigating for DBER the effects of oil effluents on communities of marine

organisms. Some rather advanced approaches have been implemented also, at Sequim and in Richland, for identifying the toxic fractions of oil in water, for monitoring these fractions during the course of experimental observations, and for controlling the complex variables in the multiphase bioassay system.

An important development of concern to hydroelectric energy development in the Northwest was the Workshop on Gas Bubble Disease hosted by Battelle and the AEC at Richland, October 8-9, 1974. The workshop provided valuable inter-agency exchange on current problems and state-of-the-art relating to fisheries effects of gas supersaturation. Fifty representatives, from state and federal agencies, utilities companies, and consulting firms participated in the workshop. Proceedings will be published shortly and will include both original manuscripts and summaries of the roundtable discussions. Following the workshop, members of the Aquatic Physiology program staff were invited to join the Nitrogen Task Force, an interagency group meeting several times yearly.* The Task Force coordinates related research and the operation of Northwest hydroelectric facilities to minimize adverse effects of dissolved gas supersaturation. PNL's contribution to these activities was an outgrowth of our participation 6 years ago in the tripartite research program of AEC, EPA, and the National Marine Fisheries Service (NMFS). It now includes cooperative effort with the U.S. Army Corps of Engineers and major state agencies.

Work on metal elements and their availability to biota has shown considerable development, with the potential for solubilization and movement by complexation reactions now established. While the primary DBER effort was directed to the power reactor program, as it concerns the transuranium elements, other DBER activities reported herein were directed at organic forms of mercury and iodine. Under other sponsorship the approaches have been further applied and extended to certain trace metals of environmental concern in connection with large-scale coal gasification and coal combustion plants. The Battelle study on inorganic pollutants from coal utilization has involved development of atmospheric and hydraulic dispersal models, models for soil/plant availability over the long-term of 21 trace elements, and estimates of resulting biohazards. This work was completed in December, 1974 and will be published shortly.**

Other important developments in 1974 were the revision and updating for ERDA of PNL's Biomedical and Environmental Research Plan, 1975-1981. While many aspects of this plan were touched on in comments above, the Terrestrial Ecology program was not. Synthesis of data is still in progress on environmental perturbations of the arid lands and grasslands within the ALE Reserve. These data and the experience gained over the past 6 years is highly relevant to the problems encountered in revegetation and economical restoration of lands likely to be strip-mined for coal. Based on this timely program, recommendations have been made to DBER for demonstration projects concerned with land rehabilitation.

In closing, mention should be made of Battelle's new Life Sciences Laboratory II (LSL II) which also will be part of the consolidated Pacific Northwest

* Federal Power Commission, Bonneville Power Administration, Bureau of Reclamation, U.S. Army Corps of Engineers, EPA, NMFS, State Dept. of Ecology, Washington State Dept. Fisheries, Oregon Dept. of Ecology, Fish Commission of Oregon.

** Environmental Impact of Inorganic Materials Entering the Environment as a Result of Coal Utilization. Burton E. Vaughan (ed.). Battelle Energy Program (manuscript in review, January, 1974).

Laboratory. Initial occupancy is scheduled for March 1975, with full occupancy scheduled later in the year. The laboratory will be shared with our sister department, Biology. Occupancy will particularly relieve crowding of our present laboratory for aquatic studies in AEC's Life Sciences Laboratory (Building 331) and presently restricted space available for the trace metals program. LSL II will provide essentially comparable facilities to Building 331.

Burton E. Vaughan
Manager, Ecosystems Department

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ANALYSIS OF NATURAL SYSTEMS

- **ANALYSIS OF NATURAL SYSTEMS**
- **QUANTITATIVE ECOLOGY OF IMPACT EVALUATION**

• ANALYSIS OF NATURAL SYSTEMS

Our long-term efforts to understand the quantitative aspects of natural systems are continuing, with emphasis on certain practical aspects, such as the environmental impact of electrical power generating systems. We have maintained our activities in developing a rationale and an improved methodology for sampling for contaminants in ecosystems and have stepped up the intensity of our studies directed to finding an optimal role for modeling in ecological research. We are increasingly convinced that statistical methods must play a much greater role in ecology, in general, and "systems ecology" in particular, if much of the present day field and laboratory research is not to be wastefully repeated in the future.

SAMPLING FOR CONTAMINANTS

Some Methodology for Appraising Contaminants in Aquatic Systems L. L. Eberhardt

The problem of contamination of aquatic systems by various substances is well-known and has been the subject of much discussion and research. In recent years considerable emphasis has been placed on the development of baseline data and on schemes for monitoring changes in aquatic pollution. Efficient design of such studies requires understanding of the dynamics of contaminants in natural and laboratory systems and thus should be aided by kinetic models. The purpose of the present study is to suggest the utility of some simple models and to discuss the use of such models in laboratory work and field studies.

The major value of kinetic models for laboratory studies would seem to be in intercomparing the behavior of a wide range of substances in the diverse life forms that must be investigated if we are to understand contamination well enough to protect our

living environment. The literature now abounds with reports on the uptake and retention of such substances as DDT, dieldrin, and mercury, but little has yet been done towards synthesis of the data in a form useful for the design of further studies and the application of the data to field investigations. One useful step along the way may be to fit models to the data in order to summarize the results in terms of a few parameters.

There have been a number of field studies of pesticides that indicate marked differences in concentration in the various trophic levels. Both design and analysis of such studies might be aided by estimates of the "turnover" parameters for particular species. Although the general principle of "biological magnification" of pesticides and other trace substances is now widely accepted, detailed understanding is as yet lacking and will no doubt require the synthesis of laboratory results as suggested above.

Global models of chlorinated hydrocarbons have been widely proposed,

but the virtual absence of accurate parameter estimates prevents such models from constituting more than useful speculations at present. No doubt it will be a long time before enough good rate estimates are available to implement a large-scale model, but the choice of organisms and of sampling intervals for a baseline program should be considerably facilitated by knowledge of loss rates for a range of species.

Kinetic models should also be useful in appraising dynamics in microcosms, inasmuch as "model ecosystems" (essentially aquarium systems) are apparently becoming popular for pesticide studies. The main problems to be dealt with in evaluating results in such model systems seem likely to be those associated with scaling. For example, the masses of the components of a model system are not at all likely to be directly proportional to those of any natural system. In particular, the real-world principle that the biota constitutes a very small fraction of the physical system will quite certainly no longer hold in a model system, so that exchanges of the test substance between components of the biota may be very different from those occurring in nature. The user of such systems thus needs to understand the observed patterns well enough to correct for the scaling phenomenon if possible.

The basic models used here have been employed for many years in studies of drug kinetics and of radionuclides, both as "tracers" in biomedical applications and in ecological research. The results given below were obtained through use of the "linearization" (or Taylor series or Gauss-Newton) method as described by Draper and Smith (1966); written in BASIC language for a Univac 1108 computer.

An important assumption in fitting models is that of the underlying "error structure." The method of least-squares is based entirely on minimizing the sum of squared deviations between data points and the model being fitted and thus requires no assumption as to the frequency distribution of these deviations. Any kind of statistical evaluation of the results does, however, depend on assumptions about error distributions. The available evidence and our previous

studies along these lines have led us to assume multiplicative, lognormally distributed errors, leading to use of a logarithmic transformation on the usual retention equation, which then becomes:

$$(1) \log y(t) = \log y(0) - \mu t + \log \epsilon$$

The retention model is thus assumed to be linear with normally distributed errors, so that ordinary linear regression may be applied to log-transformed data. For uptake data, we have ordinarily employed the simple uptake function:

$$(2) y = \frac{\lambda}{\mu} [1 - e^{-\mu t}]$$

for which non-linear least-squares fitting methods are required.

Table 1 provides a summary of our analysis of some published data, using equations (1) and (2). We recommend that the parameter estimates given in the table be treated as provisional values, subject to revision as better understanding evolves and more data is studied. Experience with various radionuclides shows that most animals should be considered as having a minimum of two body compartments (and normally several additional compartments may be considered). Nearly all of the fish used in these experiments were of very small size and, as we have previously demonstrated, there is a definite relationship between size and retention rate. On this basis, Eberhardt and Nakatani (1968) have proposed a steady decrease in magnitude of the loss-rate parameter (μ) as fish grow. We thus suppose that at least two major factors may tend to affect these curves, the existence of more than one body compartment (calling for a more complex model) and possible changes in parameters over the course of the experiment.

If one inspects the loss coefficient estimates in Table 1 with due attention to the magnitude of the associated standard errors, it appears that the most striking deviations in the overall pattern are associated with the coefficients for DDT in the two sets of trout data (Macek and Korn, 1970; Macek et al., 1970). There is also a suggestion that the dieldrin loss coefficients

TABLE 1. Uptake and Retention Coefficients for DDT and Dieldrin in Fish.

Reference	Fish	Size	Type of Study	Duration	Loss coefficients and standard deviations	
					DDT	Dieldrin
Gakstatter and Weiss (1967)	Goldfish	5 g	Loss	32 days	.016 + .002	.105 + .010
	Bluegills	7 g	Loss	32 days	.007 + .004	.083 + .019
Chadwick and Brocksen (1969)	Sculpin	2 g	Uptake	26 days		.038 + .022
	Sculpin	2 g	Uptake	26 days		.024 + .010
	Sculpin	2 g	Loss	89 days		.020 + .002
Macek and Korn (1970) and original data	Brook trout	6 in	Uptake	120 days	.008 + .001	
	Brook trout	6 in	Uptake	120 days	.008 + .002	
Macek et al. (1970)	Rainbow trout	15 g	Uptake	168 days	.008 + .002	.017 + .006
	Rainbow trout	15 g	Uptake	168 days	.007 + .001	
	Rainbow trout	15 g	Loss	90 days	.004 + .0004	.021 + .001
	Rainbow trout	15 g	Loss	90 days	.002 + .001	.021 + .003
Grzenda et al. (1970)	Goldfish	21 g	Uptake	192 days	.026 + .006	
	Goldfish	21 g	Loss	100 days	.023 + .001	
Grzenda et al. (1971)	Goldfish	20 g	Uptake	192 days		.008 + .007
Grzenda et al. (1972)	Goldfish	20 g	Loss	63 days		.035 + .003
Eberhardt et al. (1970)	Carp	Small	Loss	40 days	.032 + .008	
	Bullhead	Small	Loss	100 days	.011 + .004	
Reinert (1972)	Guppies	1/2 g	Uptake			.068 + .020

for the same two sets of data are smaller than for most of the remaining data. We believe that the most likely candidate for explanation of these differences is the effect of rapid growth on retention time, as mentioned above.

All of the laboratory data that we have been able to locate depend on fish on the order of 20 g body weight, or less. From the extensive evidence now available on the relationship between body size and retention time, we conclude that these data cannot safely be extrapolated to field conditions where much larger fish must be dealt with.

The remainder of our study consists of an analysis of several sets of field data based on fish of known age. We have investigated interrelations between body weight, age, and length in connection with concentrations of three pollutants (DDT, PCB's, and mercury). The findings generally support our thesis of the necessity for taking body size into account in

any analysis. A detailed report on the study has been prepared for publication and suggests various needs for further study along with methods for statistical analysis of field and laboratory data.

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Sampling and Analysis of Radionuclides in Ecological Systems
L. L. Eberhardt and J. M. Thomas

Most of our efforts on this project in the current year have been devoted to aquatic studies. One segment, reported elsewhere in this annual report, concerns our continued work with Dr. C. E. Cushing on radionuclide cycling by periphyton in a laboratory "microcosm." As an extension to that work, we have this year begun an analysis of some data on ^{65}Zn in simulated laboratory streams, originally collected by C. E. Cushing and D. G. Watson (1973). Where possible, transfer rates were estimated from the data, using non-linear least-squares methods. A water-periphyton-

carp food chain was then simulated by a simple computer program. The fact that replicate troughs were used for each of two levels of ^{65}Zn in water makes it possible to examine the variation between troughs relative to the simulation. So far as we know, this is seldom, if ever, considered in simulation modeling, and we can only conclude from this very limited start that more attention has to be paid to this feature in the future.

Structuring the zinc-in-troughs simulation model led us to examine the literature on several key features of that model, i.e., water versus food as a source of zinc uptake, effects of temperature, body weight and growth as influencing uptake and retention, and the effects of fish grazing on periphyton abundance. This brief review suggests that the available literature is either inadequate, inconclusive, or contradictory on several of these points, leading to the conclusion that further experimentation can be appreciably facilitated by use of a modification of the present model as a planning and design tool.

A third area of study in this program dealt with some data on the uptake and retention of ^{203}Hg by small fish, obtained in experiments previously conducted by D. G. Watson and A. J. Scott. Since many of these fish were individually marked, we were able to use the nonlinear least-squares computer routines to fit both "uptake" and "loss" curves for individual fish. Among the several interesting findings was the suggestion that temperature effects (7°C and 14°C were used in the experiments) seemed mainly evident as a "short half-time" component (of a two-compartment model), whereas the usual interpretation has been an influence of temperature on the "long-component." We thus suppose there is a need to look carefully at the temperature-retention-time relationship, especially in view of recent evidence showing a substantial effect of body-size on retention-time.

Reference Cited

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Some Quantitative Aspects of
Environmental Plutonium Studies
R. O. Gilbert and L. L. Eberhardt

Our continued involvement in environmental plutonium studies at the Nevada Test Site, along with frequent contacts with other such studies, has demonstrated the need for research on a series of as yet unresolved questions. Many of these questions came up at a 2-day workshop on ecological research for the transuranic nuclides held at Estes Park, Colorado on July 11 and 12, 1974. During the course of the workshop it became very clear that those participants actually conducting field studies in plutonium-contaminated areas have run into a number of difficulties in assessing field data on soils, vegetation, and animals. Our experience suggests that many of the problems have to do with the extreme variability encountered in plutonium content of environmental samples. In the presence of such variation, one needs to take special precautions to avoid insufficient or unrepresentative sampling.

When a survey has a single fairly simple objective, such as estimating the total quantity of plutonium in a well defined soil area, there are a number of well-established methods for designing an accurate and efficient sampling scheme. Unfortunately, most field studies have a number of rather different objectives, some of which seem not to have been studied from a statistical point of view. We have been attempting to define some of the problems of this sort, with the ultimate goal of designing suitable methods for field use.

In the discussion of our NTS work elsewhere in this report we indicate that a stratified random sampling plan designed to estimate plutonium inventory in soil at "safety-shot" sites does not necessarily yield a pattern of sampling locations that will also be optimally located for drawing plutonium contours over the region. The main problem seems to be in the low concentration areas immediately surrounding the higher concentrations near ground zero. For the low concentration region it is necessary that soil samples be collected in sufficient numbers and at appropriate locations if reasonably

accurate estimates of the contour surface are to be obtained.

While we have not yet had an opportunity to study the statistical computation problems in detail, it seems possible to design a single sampling program to accomplish the two objectives of inventory and distribution, at least when preliminary field surveys have indicated the general shape of the concentration surface. Probably more samples would have to be taken than necessary for the single objective of estimating inventory, but a better estimate of distribution would result from advance planning for contour estimation.

We have also been studying the problem of how systematic bias in plutonium concentrations can arise due to soil preparation and aliquoting procedures and how this bias can be estimated and adjustments made by taking additional samples. The bias we are concerned with arises due to a tendency for the larger soil particles to be under-represented in the aliquots of soil taken for analysis from the entire soil sample after ball-milling. Since the larger particles tend to have lower plutonium concentrations than the "fines," the concentrations reported by the analytical laboratories tend to be too high compared to the actual field condition. While it is possible to make adjustments for this bias, the precision of any quantity of interest such as an inventory estimate will necessarily be increased due to statistical variability in the adjustment process. Probably the best approach would be to develop ball-milling and aliquoting procedures that are less bias-prone.

Another area of study concerns the substantial variability observed between aliquots of the same soil sample, due presumably to the particulate nature of plutonium present. We were asked by one of the analytical laboratories to look at the variability present in their aliquot analyses and give guidance as to whether more than a single aliquot per soil sample need be analyzed for the purpose of estimating plutonium inventory. This led us to review the allocation of sampling effort for two-stage stratified random sampling plans and to prepare a draft of a document entitled "Allocation of

Sampling Effort in Soil Plutonium Inventory Studies."

There are a number of other problem areas in sampling for plutonium that have been identified during the past year but for which little time has been available for study. These include: 1) design and analysis of profile sample studies, and 2) the design of sampling plans for estimating long-term changes in geographical distribution of plutonium concentrations in soil or vegetation, possibly using the computer-estimated concentration contours discussed above.

Plutonium Studies on the Nevada Test Site*

R. O. Gilbert and L. L. Eberhardt

We have continued this past year to give statistical design and analysis support to the Nevada Applied Ecology Group's (NAEG) environmental plutonium studies on the Nevada Test Site (NTS). The NAEG is studying the dynamics of the plutonium and other transuranic contamination in desert soil, vegetation, animals, and air at 10 "safety-shot" sites on the NTS.** These studies are directed at determining whether the present contamination might be a potential health hazard to man.

Our efforts have been focused primarily on two objectives: 1) to estimate the total amount (inventory) of $^{239,240}\text{Pu}$ in the surface 5 cm of soil, and 2) to estimate the geographical distribution of plutonium concentrations in soil and vegetation. We have provided the statistical design support needed to set up the stratified random sampling plan used at each site to estimate plutonium inventory. This support included the design of FIDLER† grid surveys for demarcating strata and the specification of the number of field locations where soil and vegetation samples were collected. About 1100 surface soil samples and

an equal number of vegetation samples have been collected at the 10 sites. In addition, at each of 68 desert pavement locations 10 vertical profile samples (taken at 2.5 cm increments down to 25 cm) were taken to investigate the vertical distribution of $^{239,240}\text{Pu}$ and ^{241}Am . Radiochemical plutonium analyses are completed for Areas 13 and 5 (GMX) and have been used to estimate the Pu surface soil inventory for these sites. Plutonium determinations at the other eight sites are about 60% complete, but preliminary estimates of soil inventory have been made from the available data.

We are continuing our evaluation of "double sampling" as an alternative method to stratified random sampling for estimating plutonium inventory. The method would use either laboratory Ge(Li) gamma scans for ^{241}Am on soil samples or FIDLER readings taken at random locations in the field to predict $^{239,240}\text{Pu}$ soil concentrations. Results obtained to date suggest laboratory Ge(Li) counts on soils from all 10 sites could probably be used to estimate $^{239,240}\text{Pu}$ inventory with precision equal to or better than that obtained using the more expensive and time consuming radiochemical analyses for plutonium. The FIDLER counts are not as highly correlated with plutonium concentrations as the Ge(Li) scans, particularly for the lower contamination levels. For the higher contamination areas near ground zero, the FIDLER could possibly be used to estimate inventory, but the resulting estimate would probably be considerably less precise than that possible using Ge(Li) scans. The data now available will enable us to determine the extent of loss in precision likely to occur if the FIDLER were used.

A considerable effort has also been devoted to estimation of the geographical distribution of $^{239,240}\text{Pu}$ in surface soils as well as in vegetation using a computer contouring program. Figure 1 shows the estimated plutonium contours (isopleths) for soil in Area 13, while Figure 2 is a three-dimensional plot of the same estimated concentration surface. These contours are known to be biased in that the lowest isopleth lines tend to extend out too

* Funded in part by NTS

** Area 13, Area 5 (GMX site), Tonopah Test Range (sites Clean Slate 1, Clean Slate 2, Clean Slate 3, Double Track), and Area 11 (Sites A, B, C, D).

† Field Instrument for the Determination of Low Energy Radiation

FIGURE 1. Estimated Isopleth Lines of $^{239,240}\text{Pu}$ Concentrations (nCi/g) in Soil of Area 13.

FIGURE 2. Estimated $^{239,240}\text{Pu}$ Concentrations (nCi/g) in Soil of Area 13. Based on Wet Chemistry Analysis of Samples Chosen at Random Within Strata.

far from ground zero. Our experience thus far raises a number of questions regarding the design of efficient sampling plans for esti-

imating contour lines. We have observed for example, that a stratified random sampling plan designed to estimate plutonium inventory does not necessarily yield data points appropriately spaced over the land area to permit estimation of concentration contours.

Other statistical design and analyses work includes: 1) comparing $^{239,240}\text{Pu}$ concentrations in soil with those in vegetation, small mammals, and lizards; 2) the design of appropriate methods for sampling "blow-sand" mounds for purposes of inventory and distribution (vertical and horizontal); 3) comparing plutonium concentrations in desert pavement soils with those in mounds; 4) computation of $^{239,240}\text{Pu}$ to ^{241}Am ratios for soil and vegetation at all sites; 5) interlaboratory comparisons; 6) comparing plutonium concentrations in soils of <100 and >100 mesh size, and between sieved and unsieved soil and 7) making recommendations concerning the computation and reporting of counting errors. Certain of these topics are discussed in another section of this report.

For further information on transuranium element behavior refer to:

- 1) "The Potential for Plutonium Complexation in Soil and Uptake by Plants," Wildung et al., pp. 20-35.
- 2) "Suspended Particle Interactions," Klepper, Craig, Cataldo and Vaughan, pp. 49-55.
- 3) "Weathering and Aging of Transuranics in Soil," Rickard et al., pp. 191-203.
- 4) "Ecological Behavior of Plutonium and Americium in a Freshwater Ecosystem: Phase I Limnological Analysis; Phase II Isotopic Ratios," Emery et al., pp. 95-99.
- 5) "Biogeochemistry of Plutonium and Americium in Marine Systems," Robertson et al., pp. 148-150.

MODELING ECOLOGICAL SYSTEMS

Dairy Farm Study

J. M. Thomas and L. L. Eberhardt
Technical Assistance: M. I. Cochran

Efforts this past year have resulted in a draft manuscript which summarizes our modeling efforts in this study. Currently, we are preparing a draft of the companion document which will provide most of the data used to compute the summary tables and figures in the draft manuscript. Moreover, it will contain a more detailed exposition of the methods used (statistical, mathematical, and modeling).

Results from our simulation of fallout of ^{137}Cs on alfalfa plants (described in last year's annual report) are shown in Figure 3, while Figure 4 shows the simulated and observed milk values obtained when simulated alfalfa is fed to a dry lot milking herd in conjunction with grain and/or silage. With the exceptions of the first harvest in 1966 (Figure 3), the last harvest of 1963, and the first harvest of 1964 (Figure 4), it appears that observed and simulated data are generally in satisfactory agreement.

We do not believe such agreement constitutes full validation of our models or procedures. One method often suggested to cross-check models

FIGURE 3. Observed and Simulated Levels of ^{137}Cs for Each Alfalfa Harvest (pCi/m^2).

is to partition available data into two sets, one used to construct a plausible relationship and the other used for validation. Even with this precaution, biases in one set of data are very likely to persist through the second set. For this and other

FIGURE 4. Comparison of Observed and Simulated Levels for ^{137}Cs in Milk.

reasons we have resorted to comparisons of our results to those of other workers who either conducted similar studies or experimental work relevant to portions of our proposed model. Initial findings indicate that values used in the model are in reasonable agreement with those used by other investigators. Thus, we suppose that the overall framework of fallout radionuclide transfer through a dairy system is fairly well established, at least for the levels observed through 1966. There may be some question as to the adequacy of any model as a predictive tool for the fallout-milk food chain under conditions of "low level" fallout as a consequence of as yet uninvestigated mechanisms. We feel the statistical and mathematical methodology employed thus far in this study is appropriate to other fallout materials even though the environmental framework (model) may change depending on the actual field situation.

Alaskan Study L. L. Eberhardt

Our efforts to model the transfer of fallout ^{137}Cs through the Alaskan lichen-caribou-Eskimo food chain were largely devoted to re-examining some of the structural elements of the existing simulation model. We have changed the fallout input regime to reflect recent levels and experi-

mented fairly extensively with changes in transfer coefficients between lichen and caribou. Such changes will bring the observed and simulated data into much better accord than will use of a constant transfer rate throughout. We are, however, reluctant to institute arbitrary changes in transfer rates without having a clear idea as to the nature of the phenomenon responsible for this fluctuation.

Work on the model is in cooperation with Dr. W. C. Hanson (Los Alamos Scientific Laboratory), whose detailed personal knowledge of the system makes it possible to achieve the necessary detail in modeling. In a recent visit, Dr. Hanson provided a revised set of schedules for various segments of the model (e.g., harvest dates and caribou utilization rates), and the next step is to restructure the computer model accordingly. As soon as a sequence of new runs has been accomplished and evaluated, we plan to begin writing a detailed report on the model. We expect that this revision of our earlier report (Eberhardt and Hanson, 1969) will be helpful in planning future research on this and other food chains.

Reference Cited

Eberhardt, L. L. and W. C. Hanson. 1969. A simulation model for an arctic food chain. Health Physics 17: 793-806.

Overview and Synthesis Project L. L. Eberhardt

We have recently been asked to contribute to a conference titled, "Systems Ecology--Where Do We Go From Here?" to be held at Utah State University in February 1975. Inasmuch as the question posed is an important one for the future development of ecology and refers directly to a central theme of our quantitative ecology program, we have begun an intensive effort to review and synthesize some of the findings in this program for presentation at the conference.

In the last decade, there has been an increasing emphasis on modeling in ecology. Earlier the catchword was "Systems Analysis," but what actually was done under that heading seemed mostly to be computer simulation. The emphasis on "Systems Analysis" now

seems to be dying out, and perhaps "Systems Ecology" is the new key phrase. We have been emphasizing work on applied problems with real data, and believe that modeling and simulation ought to be tempered and supported by detailed statistical analysis wherever possible. As data accumulates and our understanding of ecological systems improves, through modeling, we should be able to efficiently design field studies (i.e., provide suitable sampling plans before field work begins). A key unanswered question, however, is one of how to optimally allocate field effort to estimation of relevant parameters in even the simplest of models. Although we do not expect to answer that question, we do hope to suggest some useful starting places, as well as to describe some particulars of our recent experience in sampling, modeling, and analysis.

STATISTICAL AND MATHEMATICAL ASPECTS OF ECOLOGY

Computation and Reporting of Radioactivity Counting Statistics*

R. O. Gilbert and L. L. Eberhardt

In large scale environmental sampling programs for radionuclides it is often the case that several analytical laboratories are required to handle the large number of samples collected. This is true, for example, with the Nevada Applied Ecology Group's (NAEG) environmental plutonium (and other transuranic) studies on the Nevada Test Site, for which three laboratories are currently analyzing samples.

It became apparent this past year that each laboratory in the NAEG program was using a different method of computing the "counting error" associated with each analysis, and that different definitions of the "limit of detection" were also in use. Consequently, we were asked to review each laboratory's method of computing "counting errors," "limits of detection," and other relevant statistics and to suggest any changes that might be appropriate so that reported results would be comparable between laboratories.

* Funded in part by NTS.

At the present time we have reviewed the counting systems (alpha spectrometry, gamma ray spectrometry, and beta counting) of one of the laboratories and have suggested certain changes in their methods for computing counting errors and limits of detection. We have also recommended that, whether or not the decision, "detected," has been reached, the net disintegrations per minute and its counting error be reported. In these situations it is often the case that a negative or very small positive number will be reported, but these results do contain information about the total variability associated with a given counting system. The reporting of "less than" numbers or the letters, "ND," is not recommended since a loss of information results. The simplest and most direct approach would be to report all results even though their counting errors may be large relative to the net count.

A rough draft of our recommendations formulated to date has been prepared and is being reviewed (Gilbert, R. O., 1974). We expect to continue this study during the coming year.

Reference Cited

Gilbert, R. O. 1974. Recommendations Concerning the Computation and Reporting of Counting Statistics for the Nevada Applied Ecology Group. Battelle-Northwest, Richland, WA. 69 pp.

Statistical Design, Sampling and Analyses for ALE-IBP Projects R. O. Gilbert

Statistical design and analysis support was provided throughout the past year to a variety of ecological studies on the Arid Lands Ecology (ALE) project, including the IBP Grasslands study site.

Our advice was sought early in the year as to how the sampling design on the IBP plot might be altered to study more thoroughly the recovery process of vegetation after being grazed by cattle. Several meetings were held with terrestrial ecologists to explore possible sampling designs. Considerable effort was also expended to improve on the field sampling design used in 1973 to measure

the effects of prior grazing on height of vegetation and seed leaves, length of seed heads and several other variables at the IBP site. The 1974 sampling procedure for this continuing study was designed to reduce sampling bias and to allow the estimation of differences in leaf height between the inner and outer sections of vegetation clumps. This later information will be useful in the design of future studies. Our attention was also directed to an analysis of root core data collected over the years 1971-73 at Lower and Upper Snively Fields on ALE. The data were collected to investigate whether double sampling might be used to estimate the important parameters with less cost than previously used methods.

The analysis of data from several environmental research projects was accomplished during the year including: 1) the analysis of shrub volume data and the percent of the ground's surface covered by vegetation at the three manipulation plots (irrigation, herbicide, and control) on ALE and at three different sites

elsewhere on the Hanford Reservation. These studies provide basic information relative to the potential for soils in these areas to be resuspended by wind action in both disturbed and undisturbed habitats; 2) the analysis of biomass data collected under different fertilizer treatments at two times during the 1974 growing season on ALE; 3) the analysis of root core data (mentioned above) to compare root growth at Lower and Upper Snively at different times over the years 1971-73; and 4) the analysis of data collected during 1974 on the length of seed and vegetative leaves and the number of seeds produced at the control and recovery plots at the IBP study site.

In addition to the above, we continue to supervise the routine analysis of biomass data collected during the growing season at the IBP site and at Lower Snively Ranch on ALE. The preparation of manuscripts for open literature publication has also been actively pursued during the past year in cooperation with the Terrestrial Ecology program staff.

For further information refer to Publications List in the Terrestrial Ecology section, pp. 204-206.

Line Transects for Population Studies L. L. Eberhardt

Our long-term study of line transects as tools for measuring animal population densities has been continued and extended this year. Work on a detailed account of our theoretical development of the method continues in cooperation with Professor D. S. Robson of Cornell University. As mentioned in last year's report, we find that a general statement of underlying principles has a much wider range of application than to line transects alone, thus leading to various complications in developing the basic equation.

We have been investigating several practical problems in conjunction with the overall study. One of these is in cooperation with the National Marine Fisheries Service research center at La Jolla, California. That study is concerned with the very important problem of losses of porpoises in nets set for tuna off the Pacific coast. Last winter NMFS and Department of Interior personnel conducted an aerial census of porpoise in the affected area, using the line transect method. We were consulted in the planning stages of the study and have been involved in analysis of the data and planning for further

research on porpoise census. In effect, porpoise schools actually need to be regarded in essentially the same light as inanimate objects insofar as the line transect method is concerned. This makes initial detection depend very much on the observer in contradistinction to the "flushing" response of animals that occupied a central role in our previous work with the method. In cooperation with Professor Robson, we have thus begun to look into this facet of the method.

A second area of cooperative work is through an extensive study of marine mammal census methods being conducted at the University of Washington, under the auspices of the Marine Mammal Commission. The initial aspects of the study are mainly concerned with a review of methods now being used for various marine mammals, and that investigation is being carried out by Dr. J. R. Gilbert of the Center for Quantitative Science at the University of Washington. Overall responsibility for the program is under the direction of Dr. D. G. Chapman, dean of the College of Fisheries and the author (in his capacity as affiliate professor at the University). Our cooperative effort, in terms of the present research program, is largely concerned with the line transect method as applied to marine mammals and probably will mainly center around the tuna-porpoise study in the immediate future, inasmuch as it constitutes an extremely important problem both ecologically and economically speaking. We have also begun to formulate plans for employing radiotelemetry in marine mammal studies (through the University of Minnesota) inasmuch as our initial appraisals clearly indicate that this kind of technology will eventually be required to test certain assumptions basic to census methods.

Bibliography of Mathematics and Statistics in Ecology

J. M. Thomas, L. L. Eberhardt, and M. I. Cochran

We have completed checking nearly all of the 2300 references now on hand for accuracy and completeness. In addition, we have individually

reviewed and coded each reference for entry into the following broad categories:

1) Age	13) Operations Research
2) Capture-Recapture	14) Ordination
3) Compartment Models	15) Patterns
4) Competition, Regulation	16) Plotless Methods
5) Computers and Data Processing	17) Population Dynamics
6) Cycles	18) Population Estimation
7) Diversity	19) Population Growth
8) Energetics	20) Sampling
9) Exploitation	21) Simulation
10) Frequency Distributions	22) Survival and Mortality
11) Growth	23) Taxonomy
12) Mathematical Models	

This task involved considerable time and effort and allows indexing not possible when the conventional methods of searching titles, reference fields, and the usually employed keywords are used. Professor Vincent Schultz (Washington State University) has continued his participation in this effort by checking citations, providing current references through 1973, and in supplying keywords (in addition to the categories above). We plan to enter references applicable to 1974 at mid-year 1975 to avoid many small and comparatively expensive "addition runs" to what is now a fairly large reference file. It is expected that between 100 and 200 references considered inappropriate for the bibliography or that would not be available from usual sources may be purged from the file later this fiscal year after all investigators concerned have examined each such reference.

The computer programs mentioned in last year's annual report have been modified to supply printed computer output suitable for direct reproduction and binding. Thus, we will have a preliminary draft document available by March 1975.

• QUANTITATIVE ECOLOGY OF IMPACT EVALUATION

In this program, started at mid-year of fiscal year 1974, we have begun to work in essentially two areas. One has to do with the essential issue of determining whether or not the present level of effort in evaluating environmental impacts at individual power plant sites can, in fact, actually reliably measure moderate impacts. The second area has to do with practical work on quantitative methodology for actual use in field studies. We are making progress in both areas, but it seems clear that long-term work is required to meet the goals of both NEPA and ERDA.

Biostatistical Aspects of Impact Evaluation

L. L. Eberhardt and J. M. Thomas

Our efforts in this segment of the program have primarily been devoted to an attempt to come to grips with the central issue of whether it is reasonable to suppose that a moderate impact of a single nuclear plant on an ecological system can actually be detected at the levels of effort now expended in the field. We thus devote the discussion in this report to the "design problem," and only briefly mention our work on actual field methods.

The issues in designing a study to evaluate the environmental impact of a single installation of some sort really revolve around the kinds of inferences one wants to attempt to make. In some cases, the National Environmental Policy Act is evidently interpreted as requiring an appraisal of possible or "probable" effects and little or no actual field work may be involved. In others, most notably those associated with nuclear power plants, detailed field studies extending over a number of years are part of the licensing requirements.

In such cases, the objectives of a study might be broadly classed as seeking to make inferences about either a particular population of some important species or about actual effects on some such population. In the first instance, the study plan might be patterned on the extensive experience available in the fields of fisheries and wildlife management or forestry. It needs to be pointed out, however, that management objectives usually have to do with sustained maximum exploitation of some particular population. The main inferences sought in management operations thus have to do with a particular population and much of the conceptual framework for a study can be borrowed from sample survey methodology. Since all sorts of changes from year to year are inevitably associated with natural populations, any design based solely on population studies may succeed in demonstrating "statistically significant" changes while failing to answer the crucial question: Can these changes be attributed to "impact?"

If the real goal in designing a study is taken to be one of detecting and measuring actual effects of the

construction and operation of some facility on a specific population, then the road to substantiating particular kinds of inferences has some well-known but often neglected pitfalls. The most traveled route is that of the classical experimental design, tracing back to R. A. Fisher and the Rothamstead experimental station. The main issue in the context is that the essential inferences in present-day experimental design rest firmly on the foundation of random assignment of experimental "treatments" and "controls" to a considerable number of test plots, animals, and batches of product. In the circumstances considered here, there is just one treated area and there may or may not be one or more "controls."

We find that most of the tests of significance reported in a large number of reports (virtually all unpublished, mimeographed, and of restricted distribution), now extant, on impact studies are not relevant to the question of whether or not there is an impact.

These tests are instead mostly attempts to detect changes in populations in time or differences in density from location to location. We already know that all populations change seasonally and vary in size from place to place. The relevant tests are thus only those that make comparisons with baseline (pre-operational) data or with control sites.

Inasmuch as no two areas can safely be said to be identical with reference to habitat and environmental factors, and because most natural populations do change in density from year to year, direct comparisons with baseline data and control areas are also subject to question. On the other hand, two areas that are not widely separated in space are ordinarily subject to much the same climatic factors, have populations with about the same genetic makeup, and generally the two populations can be expected to follow much the same trend over time, apart from human intervention. These premises lead to the conclusion that about the best we can do currently with the "single site" situation is to require that there be one or more control sites and that a "baseline" (pre-construction, pre-operational, etc.) period be used to establish the ratio of

population density in the prospective "impacted" site to that on the control site(s). The appropriate test for impact is then a comparison of pre-operational ratios (site/control) to post-operational ratios.

Most temperate zone vertebrates reproduce during a relatively short part of a year, and the subsequent course of the density of such a population is always downwards with the rate depending mainly on survival factors. Hence there would seem to be two critical measurements, one to measure reproductive success and the other to ascertain the seasonal low point in population size. In the longer-lived species the "recruitment" of juveniles to the breeding (and/or "harvestable") stock may require special consideration. Thus, for vertebrates, repeated sampling during a year amounts mostly to an effort to obtain insights as to the nature of an effect. Was it mainly felt in reproductive success or in survival, or both? One year is one data point, insofar as an arbitrary test of overall impact is concerned. For that kind of purpose one might elect to one of several choices: 1) census when field conditions are best for the particular method, 2) choose the low point in the annual cycle to include the combined effects on reproduction and survival, or 3) use several periods to spread the burden of field work.

For those species that reproduce continuously through much of a year there may be appreciable advantages in repeated sampling. Quite possibly the treatment effects may operate differentially during the course of the year. In aquatic environments thermal effects may be beneficial in one period but detrimental in another. Some evidence of such contradictory effects hopefully might show up in the ratios. Inasmuch as species groups like plankton show such dramatic cycles of abundance during a year, and because these cycles follow nearly identical patterns in comparable sites, the ratio scheme has the potential advantage of producing measurement variables that may be roughly independent in the statistical sense.

Obviously the above remarks are not all-inclusive. There are some circumstances in which reasonably

straightforward hypotheses can be formulated and subjected to more or less standard designs, both in the laboratory and in the field. One case in point is the situation where many samples can be taken over time and/or space to compare two well-defined populations. An example is the need to determine whether cooling water taken near the shore of a river contains about the same density of some small organism as is found elsewhere in the river. Also samples of organisms found in discharge water may be assessed for survival after passage through heat-exchangers or other devices without much need for involved arguments as to inferences or a need for control areas.

In those instances where a readily measured gradient of "insult" exists and persists in time one might feel reasonably comfortable with regression analyses in which various measurements on sessile (or sedentary) elements of the biota are regressed on measurements on the stressing element such as thermal gradients or salt drift from cooling towers. Various technical problems are to be expected (variability in the independent variable, non-linear responses) but there is also a body of relevant experience to draw on, such as the industrial use of regression techniques.

Perhaps the ratio approach suggested here should be known as a "pseudo-experiment" or a "pseudo-design." At this point in time, it's main value may lie in focusing attention on just how few real data points are generated by, for example, two years of "baseline" and two years of "operational" study. Much of our work with this approach has so far been devoted to assembling the necessary information (field data) and statistical machinery necessary to produce estimates of sample sizes required to be reasonably confident of detecting impacts of a certain magnitude. Details of the investigation are being included in a manuscript for publication. Generally speaking, we conclude that larger samples are needed than are ordinarily obtained on a single site.

Just what can or should be done about the problem of "sensitivity" of field studies for impact evaluation

remains an unresolved question. Our present efforts are mostly directed towards trying to document the issue and bringing it to the attention of those concerned with the problem. To that end, we participated in a 2-day workshop with Regulatory and other AEC staff on October 3 and 4, 1974, at Bethesda, Maryland. In addition to the issue presented here, we reviewed some of the existing census and survey methods and discussed questions having to do with population and community analysis (such as species diversity and productivity). These topics are also part of our present investigation and will be discussed in a subsequent report.

Sample Sizes for Population Studies
L. L. Eberhardt, R. O. Gilbert, and
J. M. Thomas

This study is mainly concerned with the important question: How large a sample should I take? To answer that question, one first has to evaluate the objectives of the study. In a detailed report now in preparation, we consider four categories of objectives in population studies (population management, population analysis, hypothesis testing, and monitoring populations). We note that these different objectives may lead to different kinds of "decision rules" from which sample size estimates may be derived. Formulation of the objectives of a study needs to be followed by development of a sampling design and a decision as to the required level of "sensitivity" in the study. In the simplest case, one can elect to have "confidence limits" or a "confidence interval" of a certain width and given a good estimate of variability in the population being sampled, estimates of the required sample size may be quite easily calculated. When a test of a hypothesis is involved somewhat more complex procedures must be followed, generally along the lines of the statistician's "power of the test."

Most of the published work on determining sample size for population studies has been concerned with those methods that provide direct estimates of population size, while most of the field work in reactor impact evaluation has been based on indirect measures of population size, which we term "indices." We have devoted part

of our efforts to an evaluation of these indices and have expended a considerable amount of effort in finding data suitable for obtaining estimates of variability for the wide range of different kinds of indices now used in aquatic and terrestrial studies (e.g., net tows, dredges, corers, visual counts, and audio counts).

The main part of the report now in preparation consists of a series of charts and tables giving sample sizes for various levels of precision and particular census methods. Four such methods, the Peterson method, Schnabel method, Change-in-ratio (or dichotomy) method, and the removal (Leslie) method have been the subject of published reports giving such charts, and our main contribution is one of extending the available results and collecting them in one report. To do so, we wrote computer programs to calculate more detailed results and produced a new set of charts and tables. No such charts have been published for two variants of the "catch-effort" (De Lury and Ricker) methods, so that we have been engaged in an attempt to develop the necessary mathematical basis for such a chart. So far we have been able to produce what we presume to be a useful approximation, but a complete solution appears to depend on evaluating two complex equations (the maximum likelihood equations).

The remaining set of charts for population estimation now being prepared have to do with index methods and depend on coefficients of variation estimated from a survey of the literature. We also plan to include a closely related set of charts intended for use in determining sample sizes in sampling for contaminants.

We have in mind several other prospective sets of charts, but these mostly depend on our being able to find a suitable rationale for estimating sample sizes, which may be a difficult task.

Problems Using Dose-Response Curves in Setting Environmental Standards J. M. Thomas and L. L. Eberhardt

Nuclear power plants are required to meet environmental standards that often are set on the basis of laboratory studies. Thus an investigation of both the quantitative aspects of

the research on which these standards are based and the mathematical and ecologic implications of the standards are an important part of our work on impact evaluation.

In general, limits for environmental toxicants are based on the results of one or more laboratory dose-response studies, using experimental animals or plants. These test organisms are often a species other than that for which "protection" is desired. For example, trials using rats, mice, and sometimes larger species are conducted to infer toxicity of suspected environmental carcinogens for human populations. The retrospective human epidemiological studies that involve historical toxicant and mortality experience (i.e., public health records) are sometimes used as a cross-check of laboratory trials.

Problems associated with cross-checking laboratory results in an ecosystem may be even more formidable because of the multiplicity of interdependencies in food webs and the difficulties of locating and assessing free-ranging and secretive individuals. One common factor in setting standards for humans or plants and animals is the need to extrapolate dose-response models based on laboratory data to levels of toxicant which produce very little, if any, effect. Extrapolations are usually necessary because the high cost of the large number of animals needed at those low doses where measurable effects can be observed.

The concept of a "safe" level usually corresponds to a value selected by a government agency, in consultation with an advisory body. Efforts are made to consider all relevant factors, including extrapolation of a dose-response curve. The curve, or its mathematical representation, can vary from linear to one of many curvilinear forms with dose usually expressed as logarithms. A large body of well established and accepted statistical methodology is available for evaluating data of this type (Finney, 1964). However, few ecologists have applied these methods to environmental toxicology problems. Moreover, in the few applications we have seen, the techniques appear to have been used incorrectly. Statistical methodology is well established

for purposes of estimating an LD₅₀. In contrast, methods for estimating "low level" effects have received little attention, and even less notice is taken of the consequences of extrapolating a mathematical model poorly founded in biological principles. To illustrate the latter case, the Panel on Safety Evaluation of Food Additives (1971) shows that for well defined data, extrapolation of three common toxicity curves gave safety factors which varied from 100 to 1,000,000 in calculating a "one in one million" effect. An alternative approach to estimating a safe level that does not involve a model was given by Mantel and Bryan (1961) but has received little attention.

Another problem associated with standards based on dose-response curves is that the experiments are usually not repeated. Repeated trials using the same standard test animals often yield differing results, including estimates of the LD₅₀. This could either be due to the difficulty in exactly repeating the environment of the trials, biological variation, or poor experimental design including randomization. Usually results from several trials lead to estimates of LD₅₀ more divergent than would be expected based on a single estimate and its associated standard error. More dramatic differences could be expected for estimates beyond the experimental observations.

Even though the problems in devising laboratory dose-response studies to assess "low dose" effects are formidable, it is nearly always impossible to check their adequacy in an environmental context (in the absence of numerous readily observable moribund or dead animals or plants). Moreover, difficulties are compounded and expenses increased when population numbers or the relative health of the age groups which constitute a population are assessed. If the animals of interest are mobile, addi-

tional problems arise. For seed-producing plants and nonmammalian species the actual effects may occur at an early life stage and not be detected until subsequent losses in readily observable life forms are recorded. For some species, size of the animal may introduce extra complications. Finally, it is quite probable that relatively short-term acute laboratory studies may not shed much light on possible environmental effects which involve long times (or latent periods) before manifestation of an effect.

Our work thus far leads us to believe that field studies employing known environmental gradients of toxicant constitute one tool which could be used effectively as an aid to assessing environmental standards. Such studies would be of a long-term nature and expensive. The information thus gathered, when evaluated with concurrent laboratory dose-response data, and fundamental ecological information, as well as more sensitive histopathological, biochemical, and behavioral changes may offer the best method of assessing environmental toxicity. Quantitative ecology and statistics are required to effectively design and appraise such studies.

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ENVIRONMENTAL CHEMISTRY

- **THE POTENTIAL FOR PLUTONIUM COMPLEXATION IN SOIL AND UPTAKE BY PLANTS**
- **CHARACTERISTICS OF HANFORD SOILS AND AQUATIC SEDIMENTS**
- **SUSPENDED PARTICLE INTERACTIONS**
- **HANFORD INTERCONTRACTOR SUPPORT**

- **Intercontractor Study**

• THE POTENTIAL FOR PLUTONIUM COMPLEXATION IN SOIL AND UPTAKE BY PLANTS

Previous studies have indicated that plutonium is largely insoluble in soil over the short-term and is not accumulated by plants. However, it is possible that naturally-occurring ligands in soil arising from organic matter decomposition processes may form complexes with plutonium increasing plutonium solubility in soil and uptake by plants. Furthermore, the possibility exists that the chemical form of plutonium may be directly altered by the soil microflora as has been demonstrated for other metals.

The present program was undertaken to determine 1) the effects of plutonium on the soil microbial population and on soil microbial processes, 2) the potential for formation of plutonium complexes in soil and the role of the soil microflora in these processes, 3) the extent of plant uptake and translocation of plutonium or its complexes, and 4) the bond types and chemical forms of plutonium or its metabolites in microbial and plant tissues and soils. Previous studies in this program involved measurement of 1) the influence of plutonium on the soil microflora and soil microfloral processes and demonstration of the role of the soil microbiota in influencing plutonium solubility in soil, 2) the subsequent availability of solubilized plutonium to plants and 3) the distribution of plutonium in plant shoots and roots. The progress of subsequent investigations to develop an understanding of the mechanisms of the microbial effects and to further elucidate the fate of different plutonium isotopes and chemical forms in soils and mature plants is summarized below.

Preparation and Chemical Characterization of Plutonium-DTPA Complexes
J. L. Swanson,* T. R. Garland, and
R. E. Wildung

The chemistry of Pu is highly complex and to avoid serious errors which

may arise in defining the fate and effects of Pu in the environment, it is essential that adequate attention be given to standard solutions, extractions, chemical characterizations and analyses. In the microbial enrichment studies, a soluble form of Pu, which would be stable in solution, was required. A Pu-DTPA complex was therefore selected as the primary Pu source

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for enrichments. The procedures utilized to prepare and characterize this complex are outlined below.

The DTPA used to prepare the required solutions was initially leached with water to remove water-soluble impurities as attempts at purification by crystallization were not successful. Three water leaches consisting of 10 ml water/g DTPA were performed, each for at least 24 hr. The leached DTPA was dried over $\text{Mg}(\text{ClO}_4)_2$ for 120 hr and the formula weight determined by titration with NaOH. The determined formula weight was 400 as compared to a calculated value of 393.

The Pu was purified by a batch anion exchange process using nitrate form, Dowex 1x3 resin. The acidity of the eluates was raised to above 1 M immediately after separation to minimize the disproportionation of Pu (IV). Spectrophotometric examination showed <0.5% Pu (VI) in the ^{239}Pu nitrate solutions. Alpha energy analyses indicated that about 2% of the alpha activity in the ^{239}Pu solutions was due to ^{241}Am and/or ^{238}Pu . The Pu used to prepare the ^{238}Pu solutions contained approximately 85% ^{238}Pu .

The ^{239}Pu -DTPA solutions were prepared by adding (dropwise with vigorous stirring) 0.3 volumes of a solution containing 0.042 M Pu in 1.3 M HNO_3 to 0.5 volumes of a solution containing 0.049 M DTPA in 0.32 M NaOH and then adding 0.2 volumes of 0.068 M DTPA in 1.9 M NaOH to bring the pH to

within the desired range. Solutions prepared in this fashion contained a small amount of a Pu-containing solid which was removed by a 0.01 μm filtration. Larger quantities of Pu-containing solids were obtained in preliminary tests when 0.3 volumes of the Pu solution were added to 0.7 volumes of solution containing all of the DTPA and NaOH or when 0.2 volumes of 1.9 M NaOH were added to 0.9 volumes containing all of the Pu and DTPA.

The ^{238}Pu -DTPA solutions were prepared by adding 0.03 volumes of a 1.1 M HNO_3 solution of ^{238}Pu to 0.925 volumes of 0.01 M DTPA and then adding 0.045 volumes of 0.068 M DTPA in 1.9 M NaOH. These solutions contained no visible precipitate.

The solutions prepared for use in soil, microbial and plant studies are outlined in Table 2. The total DTPA concentrations were selected to give uncomplexed DTPA concentrations of about 0.02 M in all of the solutions, based on the composition of the complex at this pH being $\text{Pu}_2(\text{DTPA})_3$ as determined by Hafez (1969).

A typical spectrum of one of the Pu-DTPA solutions is shown in Figure 5. Solutions have been stored for up to 4 months at room temperature (approximately 22°C) with no significant spectral changes being observed, indicating that these solutions are stable for extended periods of time.

TABLE 2. Plutonium-239 (IV) DTPA Solutions Prepared for Use in Soil, Microbial and Plant Studies.

Solution	Date of Preparation	Pu composition and concentration		Concentration of salts other than Pu		pH
		Principal Pu isotopes	Pu concentrations d/m/ml	DTPA	NaNO ₃	
				M		
TG-A1	5-30-74	239	4.5 x 10 ⁸	0.038	0.52	6.25
TG-A2	5-31-74	238	4.6 x 10 ⁸	0.018	0.086	7.00
TG-A4	6-10-74	239	4.5 x 10 ⁸	0.039	0.58	6.12
TG-A5	6-18-74	239	4.8 x 10 ⁸	0.037	0.51	6.29
TG-A6	9-04-74	239	4.5 x 10 ⁸	0.037	0.52	6.39
TG-A7	9-05-74	238	6.6 x 10 ⁸	0.018	0.10	6.42

FIGURE 5. Spectrum of Pu-DTPA Solution (TG-A6, Table 2).

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Sampling and Characterization of Surface Soils for Plutonium Studies

R. E. Wildung, R. C. Routson, T. R. Garland, and A. V. Robinson

The physical and chemical properties of soils likely play a dominant role in governing the fate of Pu in soils and plants. The complexity of soils and their wide range of physico-chemical and biological properties necessitates that investigation of the behavior of Pu in soils include these variables to ensure the broadest application of the information developed. For these reasons, studies of the influence of the physical, chemical, and biological properties of a number of soils on the uptake and distribution of Pu have been undertaken. As the first phase of this program, a range of 35 soil types from Oregon, Washington, Minnesota, Tennessee, North Carolina, and Colorado; including major soils likely to

be exposed to Pu at ORNL, SRL and Rocky Flats; have been collected, mixed and subsampled for analysis. The soils collected will form the nucleus of a soils collection that will be incrementally increased each year to include a broad spectrum of soils for research purposes. The soils were sampled in cooperation with the Soil Conservation Service at bench mark locations for which a complete soil profile description is available.

After collection of 40 to 80 kg, each soil was air-dried and screened (5 mm). After thoroughly mixing, the entire sample was quartered, and each quarter was mixed and halved into 8 individual containers for storage in a vault at constant temperature. Each primary subsample will be treated as representative of the entire sample and further subsampling will involve quartering from the primary subsamples. This technique has been previously shown to result in the most reproducible subsamples.

Analyses which will be performed on each of the collected soils include 1) detailed particle size distribution, 2) principal primary and secondary minerals in individual particle size fractions, 3) cation exchange capacity, 4) pH, 5) CaCO_3 content, 6) % ash, 7) organic C, 8) amorphous Fe and Al, and 9) water-holding capacity.

Relative Solubility of Inorganic and Complexed Forms of Plutonium-238 and Plutonium-239 in Soil

R. E. Wildung and T. R. Garland

To develop further insight into the long-term behavior of inorganic and complexed forms of ^{238}Pu and ^{239}Pu in soil, the relative water solubilities of these isotopes, initially added to soil in a soluble form, were measured as a function of incubation time.

A noncalcareous Ritzville silt loam of pH 6.8 was amended, in duplicate, with ^{238}Pu and ^{239}Pu as soluble (valence IV) nitrates and DTPA complexes (Table 3) at an activity level of 10 $\mu\text{Ci/g}$ and with starch (1%), nitrogen (0.5%), and water (22%) to provide optimum microbial activity. Subsamples of soil (approximately 1 g) were removed at intervals from 0 to 95 days of incubation (28°C) and equilibrated (10 min) with water (100 ml). This ratio of soil to water had

TABLE 3. Concentrations of ^{238}Pu and ^{239}Pu Used in Soil Incubation Studies.

Soil treatments	Levels of Pu and DTPA added to soil prior to incubation		
	Plutonium concentration	Plutonium radioactivity	DTPA ¹
	$\mu\text{g/g}$	$\mu\text{Ci/g}$	$\mu\text{g/g}$
Control, Starch and N only	0	0	0
Control, DTPA only	0	0	460
$^{238}\text{Pu}(\text{NO}_3)_4$	0.62	10	0
^{239}Pu -DTPA	1	0.07	2.7
^{239}Pu -DTPA	10	0.7	27
^{239}Pu -DTPA	144	10	400
^{238}Pu -DTPA	0.62	10	350
$^{239}\text{Pu}(\text{NO}_3)_4$	144	10	0

¹Includes excess uncomplexed DTPA required to maintain Pu-DTPA complexes in standard solutions.

been previously shown to give optimum solubility. The pH values of the suspensions were 7.0 ± 0.3 . After mixing thoroughly, the samples were allowed to settle for 48 hr. An aliquot of supernatant solution (25 ml) was removed and filtered (0.01μ). The Pu in the filtrate, operationally defined as soluble, was assayed using 2π alpha counting techniques after precipitation of solids on stainless steel planchets.

Inorganic Pu exhibits a strong tendency to hydrolyze in water solutions. The soil extraction procedure adopted thus tends to maximize the hydrolysis and soil sorption of Pu. This approach was selected because, with the exception of organic solvents, it is not possible to control hydrolysis with dilute salt solutions. It is likely that the procedure gives a minimal estimate of solubility although previous investigations have indicated that the quantity extracted still exceeds, by orders of magnitude, the quantity which is taken up by plants.

The DTPA complexes of both isotopes were water-soluble in soil and appeared to be stable over the first 40 days of incubation (Figure 6).

FIGURE 6. Solubility of ^{239}Pu and ^{238}Pu with Time After Addition to Soil in Nitrate and DTPA Forms.

After 7 days of incubation, the ^{238}Pu -DTPA appeared to be slightly less soluble than the ^{239}Pu -DTPA. After 95 days of incubation, both isotopes, initially added as the complex, appeared to be decreasing in solubility; perhaps as a result of degradation of the organic moiety.

A much lower solubility was observed for ^{238}Pu and ^{239}Pu added to the soil as nitrates. Noteworthy was the stability (48 hr in a water-soil mixture at each measurement time) of a fraction of the Pu during the initial phases of incubation. The rate of decrease in solubility of each isotope was similar. In contrast to the slightly lower solubility of the ^{238}Pu -complex compared to the ^{239}Pu complex, ^{238}Pu added as the nitrate was a consistent factor of 2-3 times more soluble than ^{239}Pu initially added as the nitrate.

The water solubility of ^{238}Pu , when incorporated in relatively large Pu oxide particles ($>1\ \mu$), would be expected to be greater than the solubility of ^{239}Pu oxide particles of similar size due to crystal damage and radiolysis arising from the greater specific activity of the ^{238}Pu (an approximate factor of 270). However, the behavior of the two isotopes is more difficult to predict on addition to soil due to the complexity of the potential physical and chemical interactions with soil colloids, chemical reaction in soil solution and biological factors. In the latter case, if the decrease in solubility of the nitrate forms of Pu was due to incorporation of the soluble fraction in microbial tissue, the solubility of ^{238}Pu would likely have been lower due to the lower mass concentration of this isotope.

In summary, the results indicate that, for up to 95 days of incubation, ^{239}Pu and ^{238}Pu -DTPA complexes may be expected to remain highly water soluble in soil, likely as the intact complex. Over longer periods, the DTPA complex may degrade resulting in reduced solubility. After 11 days equilibration, the water solubility of Pu added in nitrate forms reaches minimum levels of less than 0.1% of that initially applied. In addition, it appeared that ^{238}Pu exhibited increased solubility relative to ^{239}Pu when these isotopes were added to soil as inorganic salts.

Plutonium-Resistant Fungi and Actinomycetes in Soil. I. Mechanisms of Plutonium Toxicity

G. S. Schneiderman,* T. R. Garland, H. Drucker,* and R. E. Wildung

As a primary phase in a program to determine the potential of Pu complexation in soil by microorganisms, standard enrichment culturing was performed to isolate, in pure culture, soil microbiota capable of surviving and reproducing in the presence of Pu. The experiments dealt essentially with Pu as a chemical rather than Pu as a radiation source, and it may be assumed that the organisms isolated by enrichment would possess a high degree of radiation resistance, perhaps effected by an ability of the selected cells to repair radiation-induced damage to key cell macromolecules. Resistance to Pu as a chemical may occur by three general mechanisms including 1) inability of Pu to produce a toxic effect on cell metabolism at the levels of cytoplasmic processes or extracytoplasmic processes, 2) inability of organisms to transport Pu, or 3) ability of the organism to convert Pu to a form that is either incapable of entering the cell or is not toxic to the cell. It is the latter mechanism which is of a major concern in the present studies, for it is possible that the complexed form of Pu is more capable of entering plants, and/or is more toxic to the plants and to animals subsequently consuming plant tissue.

A noncalcareous Ritzville silt loam of pH 6.8 was separately amended (as described in a foregoing report) with ^{239}Pu -DTPA, ^{239}Pu as the soluble nitrate, ^{238}Pu -DTPA, ^{238}Pu as the soluble nitrate, and with starch, N, and water to provide optimal microbial activity. Subsamples of soil were periodically (days 0, 4, 11, 25, and 99) removed to determine changes in numbers of fungi and actinomycetes (Table 4). During this period, soil respiration was monitored by continuous collection of soil-evolved carbon dioxide.

Using CO_2 evolution as an index for microbiological activity, enrichment culturing of Pu resistant fungi and actinomycetes was performed at logarithmic growth (day 4) and stationary growth (day 25) by sampling

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TABLE 4. Effect of Plutonium and Plutonium Complexes on Population of Microorganisms in Soil.

Treatment	Replicate #	Fungal numbers at incubation time (days) of					Actinomycetes numbers at incubation time (days) of				
		0	4	11	25	95	0	4	11	25	95
		----- (numbers x 10 ⁵)-----					----- (numbers x 10 ⁶)-----				
Control (Starch and N only)	1	0.37	0.57	0.90	3.38	1.34	1.67	3.47	8.71	25.2	6.30
	2	0.37	0.57	0.98	2.71	1.01	1.68	5.28	11.60	31.4	5.36
Control, 145 µg/g DTPA only	1	0.34	0.93	2.72	3.47	1.93	2.12	4.54	8.99	31.5	7.11
	2	0.40	1.58	2.35	4.00	1.63	1.58	4.75	8.33	8.18	5.39
145 µg/g ²³⁹ Pu(NO ₃) ₄	1	0.38	0.67	0.91	0.62	0.23	1.14	4.70	7.12	6.38	0.97
	2	0.36	0.85	0.61	1.96	0.26	2.48	5.26	5.15	9.39	0.97
1 µg/g ²³⁹ Pu-DTPA	1	0.35	0.62	0.96	1.70	1.06	1.64	5.78	8.57	11.2	5.19
	2	0.31	0.65	1.07	3.11	1.06	2.11	5.06	7.90	13.9	4.44
10 µg/g ²³⁹ Pu-DTPA	1	0.38	0.66	1.05	1.19	0.09	1.78	6.38	12.0	10.7	3.97
	2	0.34	0.48	0.69	1.24	0.07	2.62	4.06	7.79	11.0	2.12
145 µg/g ²³⁹ Pu-DTPA	1	0.30	0.48	0.06	0.06	0.004	2.83	3.44	4.72	3.23	0.35
	2	0.33	0.57	0.09	0.08	0.002	2.65	4.35	5.57	3.70	0.77
0.5 µg/g ²³⁸ Pu(NO ₃) ₄	1	0.32	0.72	0.57	0.30	0.02	2.91	5.38	6.27	7.37	0.40
	2	0.39	0.42	0.31	0.37	0.01	2.54	4.88	7.36	6.43	0.25
0.5 µg/g ²³⁸ Pu-DTPA	1	0.41	0.10	0.09	0.04	0.004	3.04	3.15	6.75	2.28	0.02
	2	0.35	0.32	0.07	0.05	0.003	1.45	3.43	3.94	2.50	0.06

of duplicate soils containing ²³⁹Pu-DTPA at a level of 145 µg/g. Enrichment cultures were conducted with pH 6.0 liquid-enriched soil extract media containing ²³⁹Pu-DTPA at levels of 0, 1, 10 and 100 µg/ml. Although the 0 enrichment contained no added Pu, a level of approximately 2.5 µg/ml of ²³⁹Pu-DTPA was necessarily attained in the solution as a result of the added soil inoculum which contained Pu.

Radiation Versus Chemical Effects of Plutonium

The Pu compounds tested produced little or no effect on release of CO₂ from the soil. However, fungal numbers were observed to drop markedly as compared to the controls after 11 days incubation on soils containing ²³⁸Pu-DTPA (0.62 µg/g) and ²³⁹Pu-DTPA (145 µg/g) at the 10 µCi/g level. Since the same effect was not observed at the 10 µg/g or 1 µg/g levels of ²³⁹Pu-DTPA, the observed reduction in fungal numbers is likely a result of radiation toxicity. Furthermore, since the ²³⁸Pu(NO₃)₄ and ²³⁹Pu(NO₃)₄ at the 10 µCi/g level did

not show a corresponding reduction in fungal numbers, and since the solubility and possible availability of the nitrate form of Pu is lower by a factor of 100 to 300 (foregoing report), it would appear that toxicity is related to Pu solubility in soil.

Isolation of Plutonium Resistant Microorganisms

The enrichment procedures resulted in the isolation from soil of 14 fungal cultures and 13 cultures of actinomycetes, all distinct in colony morphology (Tables 5 and 6). Of these, 7 of the actinomycetes and 5 of the fungal isolates were capable of growth at 100 µg/ml Pu as the soluble DTPA complex. There appeared to be a succession of actinomycetes types in the soil during incubation as indicated by the different colony morphologies obtained from enrichments after 4 and 25 days incubation. Although this phenomenon may have resulted from changes in the soil arising from the production of metabolites or chemical degradation products, it may also have resulted from a response to the presence of Pu. Only one actinomycete isolate was

found which was common to both enrichment times and this organism was isolated at all Pu concentrations in the

media. In contrast, the fungal isolates exhibited 6 common morphological types at both enrichment times.

TABLE 5. Plutonium-Resistant Actinomycetes Isolated from Soil Using Enrichment Techniques.

Gross colony morphology	Conditions of isolation					
	Soil incubation time					
	4 days			25 days		
	Media Pu conc. ($\mu\text{g/ml}$)	Media Pu conc. ($\mu\text{g/ml}$)	Media Pu conc. ($\mu\text{g/ml}$)	Media Pu conc. ($\mu\text{g/ml}$)	Media Pu conc. ($\mu\text{g/ml}$)	Media Pu conc. ($\mu\text{g/ml}$)
	1	10	100	1	10	100
Diffuse, white myceliated	X	X	X		X	X
Dark green and fuzzy with white mycelia	X		X			
Flat base $\bar{C}$ lime green mycelia	X	X	X			
Diffuse, white $\bar{C}$ purple mycelia		X				
Small round yellow colony	X					
White, myceliated, sub-surface colony			X			
White myceliated, turns media salmon color	X					
Round, slightly raised, small dry looking					X	
Small round white myceliated					X	
Round, white myceliated with grey cast				X		X
Small diffuse, myceliated $\bar{C}$ dark brown spores				X		
White, diffuse, myceliated $\bar{C}$ pink center						X
White raised lacy growth						X

TABLE 6. Plutonium-Resistant Fungi Isolated from Soil Using Enrichment Techniques.

Gross colony morphology	Conditions of isolation					
	Soil incubation time					
	4 days			25 days		
	Media	Pu conc. ($\mu\text{g/ml}$)		Media	Pu conc. ($\mu\text{g/ml}$)	
	1	10	100	1	10	100
Diffuse, white mycelia	X	X				
Diffuse with tannish mycelia	X		X		X	
Yellow myceliated	X		X		X	
Purple diffuse myceliated			X			X
Diffuse, white mycelia with green center	X			X		
Round raised colony with white mycelia		X			X	
Diffuse, myceliated with tannish base		X				
Small round colony with mycelia			X			
Small round colony with green center					X	
Pink, subsurface, star-like colony		X			X	X
Blue colony with spiny swirls				X		
Yellow colony with spiny swirls				X		
Small dry looking colony				X		
Dark blue wrinkled colony					X	

Plutonium-Resistant Fungi and Actinomycetes in Soil. II. Alteration of Plutonium Form

A. V. Robinson, T. R. Garland, H. Drucker,* and R. E. Wildung

Previous studies indicating time dependent changes in ^{239}Pu solubility in soil (foregoing report) and increased plant uptake (Wildung et al., 1973), coupled with the resistance of some soil microorganisms to the toxic effects of Pu, suggest the pos-

sibility that the form of Pu in soil may be altered by microbial activity.

Selected exocellular media from Pu-resistant cultures (culture methods in foregoing report) were analyzed by thin layer chromatography (TLC) and thin layer electrophoresis (TLE) to ascertain the presence or absence of Pu compounds different from the Pu-DTPA added to the incubation media. Autoradiography was employed to visualize the position of Pu after TLC and TLE.

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Of the 6 exocellular media initially analyzed, only that derived from EM3-100, a tertiary enrichment (^{239}Pu level, 100 $\mu\text{g/ml}$) after 4 days of soil incubation, exhibited a compound differing from that present in Pu-DTPA standards (Figure 7). The unknown compound remained at the origin in buffer I or II for TLE (Table 7) followed by TLE development in solvent A (Table 7). Solvent systems B-E (Table 7) were also employed, but were not superior to solvent A.

After approximately 30 days storage of the filtered sterilized media at room temperature, the relative radioactivity of the unknown compound, as indicated by the intensity of the autoradiograph, had decreased. It appeared that the unknown compound was labile and unstable at room temperature. This was confirmed by subsequent analyses after a total of 120 days storage which indicated that the original Pu compound was no longer present in the solution at detectable levels. However, a new Pu-containing compound was visualized which had not originally been present. Further studies are underway to identify this material.

FIGURE 7. Thin-Layer Electrophoretic and Chromatographic Separations of Constituents in the Exocellular Medium of Plutonium-Resistant Fungi and Actinomycetes Isolated from Soil Using Enrichment Techniques. Plutonium initially added as the ^{239}Pu -DTPA complex (100 $\mu\text{g/ml}$).

TABLE 7. Composition of Buffers and Solvents Used in TLE and TLC Studies.

Identification	Composition	
Buffer I, pH 3.3	Redistilled pyridine	6 ml
	Acetic acid	60 ml
	Distilled water	1734 ml
Buffer II, pH 6.6	Redistilled pyridine	100 ml
	Acetic acid	3.5 ml
	Distilled water	2000 ml
Solvent A	n-butanol	600 ml
	Redistilled pyridine	400 ml
	Acetic acid	120 ml
	Distilled water	480 ml
Solvent B	n-butanol	150 ml
	Distilled water	1500 ml
Solvent C	Ethyl acetate	54 ml
	n-propanol	900 ml
	25% ammonium hydroxide	36 ml
Solvent D	Dioxane	900 ml
	Water	100 ml
Solvent E	Methanol	Pure

Several of the filtered (0.01 μ) media became contaminated with microorganisms upon storage, both at room temperature and at approximately 5°C. Selected samples were screened using the TLE-TLC system described above (solvent A, buffer I). The media was not refiltered prior to TLE-TLC. One of the samples of culture media from 2EM1-100, a primary enrichment, (^{239}Pu level, 100 $\mu\text{g/ml}$) after 25 days of soil incubation, exhibited 5 Pu-containing spots on autoradiography. It is not possible to determine with certainty whether these spots were the result of the original incubation and enrichment procedure or were formed in association with the contaminating organisms. However, the gross colonial morphology of the contaminant resembled that of several of the pure cultures of Pu-resistant organisms isolated from the soil. One spot remained largely at the origin as in the case of the EM3-100, while one of the other spots resembled that of the new spot identified in EM3-100, 120 days after the original analyses. In any event, it is likely that the formation of these spots, representing components different than Pu-DTPA, were associated with the growth of the microorganisms.

Evaluations of several TLC solvent systems and supports (utilizing 2EM1-100) are underway and resolution, under the conditions employed, of several Pu spots has been achieved. Further investigations of various TLC and TLE systems should result in suitable chemical screening methods for the cell-free media derived from growth of pure cultures of Pu-resistant microorganisms (described in the previous report). After separation of the compounds using TLC-TLE, other chemical techniques will be employed to characterize and positively identify Pu metabolites. These techniques will then be applied to extracts of intact soil and plant systems.

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Factors Affecting Uptake and Distribution of Plutonium in Barley and Soybean Plants

T. R. Garland, R. E. Wildung, J. W. Neel, and D. A. Cataldo

In an attempt to provide plant tissues of high specific activity in Pu (dpm/g) for cellular and subcellular autoradiographic studies of the localization of Pu in plant cell components, preliminary investigations were undertaken to determine the factors affecting the uptake and distribution of Pu in barley and soybean plants and to identify those tissues containing the highest Pu concentrations.

Plants were grown on soils amended with $\text{Pu}(\text{NO}_3)_4$ using the split-root technique (Wildung and Garland, 1974b). Parameters investigated included the effects of 1) soil volume and soil column height, 2) starch and N amendments to optimize microbial activity, 3) soil type, and 4) increased levels of Pu addition (100 $\mu\text{Ci/g}$), on the uptake and distribution of Pu in barley. The distribution of Pu was determined in tops and roots of soybeans (Glycine max) after 50 days of growth and

barley (Hordeum vulgare) after 27 days of growth. The results are expressed in dpm/g to facilitate use of this information in autoradiographic studies.

Although increases in the total volume of soil resulted in slight increases in total plant uptake, on both the aboveground and root tissues of barley, the height of the soil column appeared to be the most important variable (Table 8). Since the Pu was evenly mixed in the soil, increased uptake in the taller soil column was likely due to the increased contact of individual roots (increased surface area exposed) with Pu-containing soil.

Incubation of Ritzville soil (pH 6.8) with C and N to provide maximum microbial activity has been shown to increase uptake of Pu, especially in the roots (Wildung et al., 1973); and growth of plants in a Cinnebar soil, an acid (pH 4.5) forest soil sampled under pasture, has been shown to increase uptake of Pu in aerial portions of barley compared to soils of neutral pH (Cline, 1968). As previously demonstrated, incubation of the Ritzville soil markedly increased Pu uptake by the roots. Incubation of the Cinnebar soil with added C and N did not increase uptake appreciably (Table 9). However, it should be noted that this soil was stored since 1968 and likely did not exhibit optimum microbial levels.

Elevation of the soil Pu levels to 100 $\mu\text{Ci/g}$ did not markedly increase the uptake of Pu compared to the 10 $\mu\text{Ci/g}$ level of Pu amendment (Table 10). In addition, during the first week of growth, the plants showed definite toxicity symptoms, which were not apparent after the root system was established in the nutrient solution below the soil. At the time of harvest, the two treatments were indistinguishable in appearance. If the observed toxicity is concentration-dependent at the lower levels of Pu amendment, increased concentration factors ($\mu\text{g Pu plant}/\mu\text{g Pu soil}$) with decreasing Pu soil concentrations over the soil Pu concentration range of 10 $\mu\text{g/g}$ to 0.05 $\mu\text{g/g}$ reported earlier (Wildung and Garland, 1974b) may have resulted from toxic effects on the roots at higher levels.

TABLE 8. Effect of Soil Volume and Height of Soil Column on the Uptake and Distribution of ^{239}Pu in the Structural Components of Barley Plants.

Plant component	Concentration of Pu in barley ^{1,2} grown in a soil volume (height) of		
	100 g (4 cm)	200 g (6 cm)	200 g (11 cm)
	----- d/m/g ³ -----		
Young roots ⁴	100	120	620
Mature roots ⁴	490	180	1700
Outer sheath	1500	3000	11000
1st leaf blade	450	1100	4500
2nd leaf blade	350	760	3200
3rd leaf blade	330	230	2800

¹Plants grown on Ritzville soil with ^{239}Pu (10 $\mu\text{Ci/g}$) added as the nitrate and cultured using the split root method.

²Harvested 27 days from planting.

³Dry weight basis (60°C, 24 hr).

⁴Young roots defined as <5 cm in length extending below the soil in the split root system.

TABLE 9. Effect of Soil Incubation with Added Carbon and Nitrogen on the Distribution of Plutonium in the Structural Components of Barley Plants.

Plant component	Concentration of Pu in barley grown ^{1,2} on			
	Ritzville soil		Cinnebar soil	
	Non-incubated	Incubated ³	Non-incubated	Incubated ³
	----- d/m/g ⁴ -----			
Young roots ⁵	100	9900	<70	120
Mature roots ⁵	490	9900	210	250
Outer sheath	1500	5100	7600	6400
1st leaf blade	450	1200	1900	1600
2nd leaf blade	350	250	650	510
3rd leaf blade	330	300	510	350

¹Plants grown in soils (100 g) containing ^{239}Pu (10 $\mu\text{Ci/g}$), added as the nitrate and cultured using the split root method.

²Harvested at 27 days from planting.

³Incubated soils amended with starch (1%) and ammonium nitrate (0.5%). Incubation time was 4 days (maximum CO_2 evolution rate).

⁴Dry weight basis (60°C, 24 hr).

⁵Young roots defined as <5 cm in length extending below the soil in the split root system.

TABLE 10. Distribution of ^{239}Pu in Structural Components of Barley Plants Grown on Soil Containing Plutonium at Different Levels.

Plant component	Concentration of Pu in barley grown ^{1,2} on soil containing Pu at levels of	
	10 $\mu\text{Ci/g}$	100 $\mu\text{Ci/g}$
	----- d/m/g ³ -----	
Young roots ⁴	100	230
Mature roots ⁴	490	240
Outer sheath	1500	6300
1st leaf blade	450	1700
2nd leaf blade	350	580
3rd leaf blade	330	410
New growth	230	320

¹Plants grown in Ritzville soil (100 g) containing ^{239}Pu added as the nitrate and cultured using the split root method.

²Harvested at 27 days from planting.

³Dry weight basis (60°C, 24 hr).

⁴Young roots defined as <5 cm in length extending below the soil in the split root system.

Measurements of the distribution of Pu in the shoots (Table 11) and roots (Table 12) of the barley plant indicated that Pu, once in the plant, was rather mobile. When the blades were separated into three segments, base, middle, and growing tip, the older leaves showed accumulation (on weight basis) of Pu near the base of the blade, with the middle of the blade deficient, and increased levels near the tip. On younger blades, the gradient was continuous with the base of the blade exhibiting the lowest concentration of Pu and the tip containing the highest levels. In the roots, the gradient was reversed, with the higher concentrations of Pu near the top of the root and decreasing toward the growing root tip. Expression of the concentrations of Pu on a unit length basis (dpm/cm) provides information essential to autoradiographic measurements. For barley, the distribution was similar on both a unit weight or a unit length basis except the gradient in the root was more marked when Pu concentration was

calculated on a length basis. On either basis, the high concentration at the base of the first blade plus sheath, accounts for the apparent concentration of Pu in the first node region observed in autoradiographs previously described by Wildung and Garland (1974b).

The distribution of Pu in soybean tissues is, in general, similar to that found in barley (Tables 13, 14, 15). The differences are likely due to variations in plant structure and functions. In the aerial portions of the plant, the cotyledon and unifoliate have markedly higher concentrations of Pu than other plant structural components. In general, the leaf tissue contains 5 to 10 times more Pu on a unit weight basis than the stem tissue. There is a lack of a gradient in the trifoliate successive to the unifoliate and first trifoliate. A gradient is observed in the main stem tissue on both a weight and length basis. The concentrations, on a weight basis, of Pu in

TABLE 11. Distribution of ^{239}Pu in Aboveground Tissue of Barley Plants After 27 Days of Growth.

Tissue	Number of plants analyzed	Length ¹	Pu concentration ²	
		cm	d/m/g ³	d/m/cm
1st leaf blade plus sheath	9	0-3.5	5300	1500
	9	3.5-8.5	370	74
	9	8.5-15	1032	160
2nd leaf blade	13	0-8	863	110
	13	8-23	240	16
	13	23-28	610	40
3rd leaf blade	10	0-10	190	19
	10	10-25	360	24
	10	25-40	420	28
4th leaf blade	9	0-10	75	7.5
	9	10-25	236	16
	9	25-40	700	47
New growth	13	0-10	<150	<15
	8	10-25	360	24

¹Length measured from approximately 1 cm above soil.

²Plants grown in a Ritzville soil (100 g) containing ^{239}Pu (10 $\mu\text{Ci/g}$) added as the nitrate and cultured using the split root method.

³Dry weight basis (60°C, 24 hr).

the soybean root were quite similar to that observed in the leaf tissue and considerably greater than stem tissue. On a weight basis, the distribution of Pu in the soybean root is different than observed with barley in that it appeared that the top of the primary root is deficient in Pu compared to concentration levels lower (30 cm) on the root. However, on a length basis, the Pu was evenly distributed.

At about 70 days of growth, the soybean plants developed a disorder that limited production of seeds.

The barley seed, harvested after 100 days of growth, did not contain Pu in detectable quantities. The Pu concentration in the grain was less than 2 dpm/g corresponding to a concentration factor of less than 1×10^{-7} .

Freeze-substitution of plant tissues for autoradiography using dry acetone and epoxy imbedding medium has yielded tissues of good quality for study at the optical level. However, regardless of the method evaluated for increasing Pu concentration in the plant, activity levels were insufficient for subcellular autoradiographic studies although certain

TABLE 12. Distribution of ^{239}Pu in Root Tissues of Barley Plants After 27 Days of Growth.

Tissue	Number of roots analyzed	Length ¹	Pu concentration ²	
		cm	d/m/g ³	d/m/cm
Young roots ⁴	15	0-4	130	33
	15	4-8	40	10
Mature roots ⁴	9	0-5	790	160
	9	5-10	530	106
	9	10-15	200	40
	9	15-20	190	38
	9	20-30	220	22

¹Length measured from 1 cm below soil.

²Plants grown on a Ritzville soil (100 g) containing ^{239}Pu (10 $\mu\text{Ci/g}$) added as the nitrate and cultured using the split root method.

³Dry weight basis (60°C, 24 hr).

⁴Young roots defined as <5 cm in length extending below the soil in the split root system.

TABLE 13. Distribution of ^{239}Pu in Stem Tissues of Soybean Plants After 50 Days of Growth.

Tissue	Length cm	Concentration	
		d/m/g	d/m/cm
Stem	0-5	200	40
	5-8	210	70
	8-11	66	22
	11-13	120	60
	13-16	150	50
	16-19	100	33
	19-23	70	17
	23-29	55	9
	29-45	78	5
Side Branches			
To 1st trifoliolate	0-4	530	130
To 2nd trifoliolate	0-4	200	50
To 3rd trifoliolate	0-4	200	50
To 4th trifoliolate	0-4	72	18
To 5th trifoliolate	0-7	150	20
To 6th trifoliolate	0-7	85	12

TABLE 14. Distribution of ^{239}Pu in Leaf Tissues of Soybean Plants After 50 Days of Growth.

Leaf tissue	Pu concentration
	d/m/g
Cotyledon	7500
(abscised 15 days)	8600
Unifoliolate	15800
(abscised 20 days)	16300
1st trifoliolate	1600
2nd trifoliolate	980
3rd trifoliolate	900
4th trifoliolate	1200
5th trifoliolate	1100
6th trifoliolate	1000
3 immature leaves	250
Terminal bud	110

TABLE 15. Distribution of ^{239}Pu in Root Tissues of Soybean Plants After 50 Days of Growth.

Root tissue ¹	Length ²	Pu concentration ^{3,4}	
		d/m/g	d/m/cm
Primary root	0-4	510	130
	4-10	710	120
	10-20	950	95
	20-30	1200	120
	30-40	1400	140
	40-50	1200	120
	50-60	1200	120
	60-70	940	94
	70-85	1100	110
Lateral roots emanating from primary root (0-4 cm)	0-10	1200	120
	10-20	760	76
	20-30	1200	120
	30-55	780	78
Lateral roots emanating from primary root (4-10 cm)	0-10	850	85
	10-20	730	73
	20-30	960	96
	30-55	1200	120
Lateral roots emanating from primary root (10-50 cm)	0-20	2000	200
Lateral roots emanating from primary root (50-70 cm)	0-15	2400	240

¹Single root from pot containing 4 plants.

²Length measured from approximately 1 cm below the soil.

³Ritzville soil (200 g) with ^{239}Pu (10 $\mu\text{Ci/g}$) added as the nitrate cultured by split root method.

⁴Dry weight basis (60°C, 24 hr).

tissues may contain sufficient activity for cellular autoradiography with exceptionally long exposure times. As a consequence of these results, future root-shoot translocation studies will involve short-term lateral root exposures to solutions containing Pu in soluble complexes. Root tissue will be examined for transverse as well as axial translocation and localization.

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For further information on transuranium element behavior
refer to:

"Suspended Particle Interactions," Klepper, Craig, Cataldo
and Vaughan, pp. 49-55.

"Weathering and Aging of Transuranics in Soil," Rickard
et al., p. 191-203.

"Plutonium Studies on the Nevada Test Site," Eberhardt
et al., pp. 6-7.

"Ecological Behavior of Plutonium and Americium in a Fresh-
water Ecosystem: I. Limnological Analysis; II. Isotopic
Ratios," Emery et al., pp. 95-99.

"Biogeochemistry of Plutonium and Americium in Marine Sys-
tems," Robertston et al., pp. 148-150.

● CHARACTERISTICS OF HANFORD SOILS AND AQUATIC SEDIMENTS

The terrestrial aspects of these investigations were largely reoriented during the past 2 years from basic investigations dealing with carbon and nutrient cycling in the arid environment of the Hanford Project toward study of chemical and biochemical fate of nuclear wastes in surface soils and plants. The new investigations are designed to have broad application encompassing soils representing the principal soil types of the United States and plants of major agricultural significance.

In aquatic sediment studies, investigations have been directed toward measurement of the physicochemical properties of suspended matter and sediments of the lower Columbia River watershed and mercury form and distribution in water, suspended matter, sediments and fish as influenced by seasonal changes in watershed and river conditions.

The soil and sediment studies outlined herein closely interdigitate with several investigations dealing with the fate of radionuclides in terrestrial and aquatic studies outlined in the Freshwater Sciences and Terrestrial Ecology sections of this report.

Pertechnetate, Iodide and Methyl Iodide Retention by Surface Soils R. E. Wildung, R. C. Routson, R. J. Serne,* and T. R. Garland

Long-lived isotopes of Tc and I, e.g., ^{99}Tc and ^{129}I , may exist in nature as spontaneous fission products of ^{238}U and, as fission products of ^{235}U , also have the potential for entrance into the environment as emissions from nuclear fuel reprocessing plants and nuclear reactors. It is likely Tc would be present in the environment as pertechnetate, an anion which may behave in a manner similar,

in many respects, to iodide or iodate. A major portion of iodide may, initially at least, enter the environment in the form of volatile methyl iodide which likely forms in the atmosphere of the reactor vessel. These materials may enter the surface soil through rainfall or direct disposal. It was, therefore, deemed essential to evaluate the retention of pertechnetate, iodide and methyl iodide by surface soils in order to 1) determine if information on iodide, which has been extensively studied, could be used to predict the behavior of pertechnetate in soils, 2) determine if methyl iodide was sufficiently stable in soil to be sorbed in a fashion which was chemically unique with

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respect to iodide, 3) develop an insight into the chemical mechanisms involved in pertechnetate, iodide and methyl iodide sorption by soil colloids through inference from their behavior in a broad range of soils and, 4) provide a basis for evaluation and prediction of the influence of soil properties on accumulation or vertical movement of these solutes in soils. To assure that these measurements would have broad application, a number of soils were employed which differed widely in genesis and in physicochemical properties.

Using a batch equilibration technique, the distribution coefficient (K_d), defined as the ratio, at equilibrium, of the quantity of solute sorbed per gram of soil to solute per ml of equilibrating solution, was determined for pertechnetate-95m, iodide-131, and methyl iodide-131 (trace quantities in 0.01 M CaCl_2) in 22 soils representing a broad range in particle size distributions, carbonate and organic carbon contents, cation exchange capacities and soil pH values (Table 16). Anion exchange capacity was not determined.

The K_d values for pertechnetate, iodide, and methyl iodide ranged from 0.007 to 2.8, 0.8 to 52.6, and from 0.1 to 3.1, respectively. Thus, the solutes all demonstrated potential for accumulation in certain soils ($K_d > 1.0$) but iodide, an anion, was generally sorbed to a greater extent than pertechnetate, an anion which may exhibit a lower bond strength as a result of its larger radius, or methyl iodide, which is nonionic. Pertechnetate K_d values (Table 17) were positively correlated with soil

organic carbon and negatively correlated with soil pH. It appears, therefore, that the positive charge on soil organic colloids is an important factor governing Tc retention by soils. The inverse correlation of Tc- K_d values with soil pH likely resulted from an increase in total positive charge and surface positive charge density on soil organic colloids with decreased pH. As pH decreases, positive charge may be expected to increase with decreased ionization of acidic groups and increased protonation of basic groups. Pertechnetate K_d values were positively correlated with cation exchange capacity, likely because this parameter is generally related to total reactive surface area.

It was initially anticipated that soil clay and organic matter, which have the greatest surface area per unit weight and are generally considered to make the greatest contribution to positive charges in soil, would play a significant role in retention of all solutes. In the case of pertechnetate, it was evident that for this group of soils, the positive charge associated with the organic fraction was a dominant factor in retention. However, iodide K_d values, which were generally much higher, were correlated only with silt content (Table 17). A possible explanation to account for the importance of the silt fraction in retention of iodide is that amorphous iron and aluminum coatings, which may exhibit considerable positive charge, differ in the particle size fractions. The amorphous coatings likely formed as a result of the incongruent dissolution of generally unstable rock-forming minerals which predominate in the

TABLE 16. Range of Properties for Surface Soils Employed in Pertechnetate, Iodide, and Methyl Iodide Sorption Studies.¹

Cation exchange capacity me./100 g	pH	Contents of				
		Carbonate	Organic Carbon	Sand	Silt	Clay
				-----%		
5.5-90.0	3.6-8.9	0-6.5	0.23- 28.8	14.1- 73.1	17.6- 58.0	3.8- 46.6

¹Range of properties for 22 soil types collected in Oregon, Washington, and Minnesota.

TABLE 17. Simple Correlations Relating Soil Properties to Distribution Coefficients for Pertechmetate, Iodide, and Methyl Iodide.

SOIL PARAMETERS	CORRELATION COEFFICIENTS OF SOIL PARAMETERS WITH K _d VALUES ⁽¹⁾		
	PERTECHNETATE	IODIDE	METHYL IODIDE
SILT, %	0.29	0.47*	0.21
CLAY, %	0.11	0.22	0.43*
ORGANIC CARBON, %	0.77**	0.06	0.85**
CATION EXCHANGE CAPACITY, me./100 g	0.50*	0.21	0.79**
pH	-0.46*	-0.05	-0.41*

(1) SINGLE AND DOUBLE ASTERISKS DENOTE SIGNIFICANCE AT $P < 0.05$ AND $P < 0.01$, RESPECTIVELY

silt fraction. However, except for the possible effect of differences in ionic radius, no explanation is at hand to account for the apparent lack of importance of the silt fraction in pertechmetate sorption.

Methyl iodide retention by soils was positively correlated to clay, organic carbon, and cation exchange capacity and negatively correlated to pH (Table 17). These observations are consistent with the high surface area of clay and organic matter and the propensity of organic matter to serve in bonding by Van der Waals forces, including hydrogen bonding, of nonionic organic compounds. The positive correlation of methyl iodide retention with cation exchange capacity and the negative correlation with pH likely occur for reasons similar to those discussed for pertechmetate. Coulombic bonding is not likely in the case of methyl iodide, but the increase in positive charge and surface positive charge density with decreased pH may be expected to increase the potential for sorption by hydrogen bonding and charge-induced interactions.

Multiple regression techniques were employed to determine the combination of soil properties which best accounted for the retention of iodide and methyl iodide in soils. The terms included in the final regression equations were selected by the backward elimination procedure which

served to order the variables according to importance in explanation of the variation in K_d values obtained for different soils. The independent variables: silt, clay, organic carbon, and pH were selected by this technique and the final regression equations incorporating these variables are given in Table 18.

The relative importance of soil properties in sorption differed with solute. Of the variables examined, organic carbon and soil pH were the most important soil parameters influencing pertechmetate retention and together accounted for 61% ($R^2 \times 100$) of the variation between soils. Silt and organic carbon were most important in iodide retention but accounted for only 49% of the variation. Investigations are underway to determine if a greater proportion of the variation can be explained.

Methyl iodide retention in soil was largely a function of clay and organic carbon content which accounted for approximately 93% of the variation in methyl iodide retention for the soils studied. Thus, it may be concluded that 1) there is sufficient difference in the behavior of pertechmetate and iodide in soils to preclude the extrapolation of the results of previous studies on iodide to the evaluation of the fate of pertechmetate in soils and plants, 2) the behavior of methyl iodide is consistent with that predicted for either a nonionic or an anionic species,

TABLE 18. Multiple Regression Equations Relating Soil Silt (X_1), Clay (X_2), Organic Carbon (X_3), and pH (X_4) with Distribution Coefficients for Pertechnetate (Y_1), Iodide (Y_2), and Methyl Iodide (Y_3).

DEPENDENT VARIABLE	REGRESSION EQUATION ^(1,2)	CORRELATION COEFFICIENT
PERTECHNETATE DISTRIBUTION COEFFICIENT	$Y_1 = 0.08^{**}X_3 - 0.09X_4$	0.78**
IODIDE DISTRIBUTION COEFFICIENT	$Y_2 = 0.33^* X_1 + 0.09X_3$	0.71**
METHYL IODIDE DISTRIBUTION COEFFICIENT	$Y_3 = 0.027^* X_2 + 0.10^* X_3$	0.96**

(1) BASED ON MEASUREMENTS OF 22 SOILS.

(2) SINGLE AND DOUBLE ASTERISKS DENOTE SIGNIFICANCE AT THE $P < 0.05$ AND $P < 0.01$ LEVELS, RESPECTIVELY.

but it is likely that methyl iodide is stable under the conditions of the present studies and behaves as a non-ionic species since its behavior was unique compared to its most probable decomposition product, iodide, 3) in surface soils containing sufficient silt, clay, and organic matter and ideally, a pH below 6.0, pertechnetate, iodide, and methyl iodide have the potential for accumulation, and 4) it may be possible to predict the extent of sorption of pertechnetate and methyl iodide on the basis of differences in soil properties. On the basis of these conclusions, further studies of the chemical and biological fate of these materials in soils would appear to be warranted.

Nutrient Release by Mineral Weathering in an ALE Watershed

R. C. Routson, R. E. Wildung, and T. R. Garland

The initial source of the major plant nutrients K, P, S, and Fe; as well as several minor nutrients is mineral weathering in natural soil systems. Understanding and quantitating the weathering process is required to analyze the nutrient fluxes in the plant-nutrient cycle. Furthermore, the dynamics of the weathering process exerts a control on the solute composition of the soil solution which, in turn, has a major effect

upon the processes of plant uptake, fallout dissolution, and sorption of radionuclides and other pollutants by soils.

In order to determine the release of nutrients by mineral weathering in the Arid Lands Ecology (ALE) Reserve soils, it is necessary to identify the soil mineral assemblages and their rate and sequence of weathering. ALE spring water composition, which represents the net results of weathering in the watershed, was previously reported (Wildung et al., 1974). The likely stable minerals, determined from mineral stability diagrams derived from these spring water analyses, were montmorillonite and kaolinite. In addition, spring water solute data were used to determine which minerals were unstable and probably dissolving in the weathering process. These data in conjunction with a quantitative mass balance calculation involving the reconstruction of the original rock-forming minerals from spring water solute and the stable soil minerals strongly supported the dissolving of basaltic minerals: plagioclase-feldspar, biotite, and pyroxene. In recent studies, X-ray diffraction analysis was used to determine if the hypothetical soil minerals montmorillonite and kaolinite, were present in the ALE watershed.

Table 19 gives the semiquantitative X-ray diffraction analysis of mineral assemblages in three soils of the ALE watershed. Soil 1, 2, and 3 were located at different elevations in the watershed, i.e., at an elevation near the top of the watershed, approximately mid-way in the watershed, and in the lower portion of the watershed, respectively. It was apparent that montmorillonite was a major component of the coarse and fine clay fractions of all three soils. Kaolinite was present in the surface and 2 dm samples of soil No. 2. Thus, the presence of both kaolinite and montmorillonite is confirmed in the ALE watershed in good agreement with the weathering sequence postulated from spring water analyses.

Spring water analyses in conjunction with the soil mineralogical analyses and a water balance presently being developed for the ALE watershed will provide the first estimates of the release of mineral nutrients by weathering.

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TABLE 19. X-ray Diffraction Analyses of Three Soils of an ALE Watershed.

Soil	Soil depth cm	Particle size fractions									
		Silt (20-50 μ)			Clay (2-0.2 μ)					Clay (<.2 μ)	
		Quartz	Feldspar	Mica	Montmorillonite	Mica	Kaolinite	Quartz	Feldspar	Montmorillonite	Mica
1	0-20	M	P	t		M	P		P	P	
2	0-5	M	M	t		M	P	t	P	t	
2	18-20	M	P			M	P	P	P	P	
3	0-5	M	P			M	P		P	t	

M = major component
P = present
t = trace component

Preliminary Studies on the Uptake of Technetium by Soybeans

R. E. Wildung, T. R. Garland, and D. A. Cataldo

Previous studies (foregoing report) on the sorption of pertechnetate by soils representing a wide range in physicochemical properties indicated that Tc, a product of the nuclear fuel

cycle, might be accumulated by plants. Furthermore, the chemistry of pertechnetate also suggested a marked similarity to permanganate and molybdate, compounds of elements essential in plant nutrition. For these reasons a preliminary experiment was designed to 1) determine if Tc, amended to the soil as pertechnetate, was accumulated by plants and 2) obtain an estimate of

the soil concentrations required to result in detectable quantities of Tc in various structural components of the plant.

Soybeans (4/pot) were grown in duplicate using the split-root system of plant culture (Wildung and Garland, 1974), in Ritzville silt loam (200 g) amended with 0.72 and 5.1 μg ^{99}Tc and 0.001 μCi $^{95\text{m}}\text{Tc/g}$ of soil. Controls consisted of plants cultured in an identical manner except that Tc was not added.

Within 5 days of plant emergence, phenotypic responses to Tc were observed. At this time, the control plants exhibited fully-developed unifoliate leaves while plants growing in soil at both levels of ^{99}Tc exhibited only cotyledon expansion. Within 9 days of germination, the terminal buds of all plants growing on soil containing Tc were necrotic and growth appeared to have ceased.

After 10 days of growth, a single plant from each treatment level was analyzed. An additional plant from each container was harvested after 13 days, at which time plants at the lowest ^{99}Tc level of amendment exhibited adventitious growth. The remaining

plants were allowed to grow and were harvested 25 days after emergence. At the time of harvest, plants were stunted, exhibited irregular leaf blade expansion and reduced internode elongation.

The concentrations of ^{99}Tc in various plant structures harvested after 25 days of growth are shown in Table 20. Over the short growing period, the plants accumulated substantial quantities of the pertechnetate applied to soil. The calculated concentration factors (μg Tc/g dry plant tissue per μg Tc/g oven dry soil) for total uptake of Tc from soil by the above ground plant components varied depending on soil concentration and time of analyses but ranged from 65 to 100 (stems) and from 330 to 1000 (cotyledons) at the low level of Tc amendment and from 80 to 180 (stems) and from 200 to 270 (cotyledons) at the high level of Tc amendment. These values are on the order of those observed for Mo in similar (unpublished) studies with this soil (Wildung et al., 1974).

Highest levels of Tc occurred in the stems and cotyledons. The marked uptake of Tc under intensive cropping conditions removed most of the Tc from

TABLE 20. Distribution of ^{99}Tc in the Structural Components of Plants During Growth on Soil Containing Technetium at Different Levels.

Plant component	Concentrations of Tc at soil concentrations of					
	0.73 $\mu\text{g/g}$ harvested on day ¹			5.1 $\mu\text{g/g}$ harvested on day ¹		
	10	13	25	10	13	25
	----- $\mu\text{g/g}^2$ -----					
Stem	73	83	48	650	920	420
Cotyledons	240	260	670	930	1400	1300
Leaves	- ³	- ³	<5.6	- ³	- ³	- ³
Roots	<3.4	- ⁴	<1.1	- ⁴	- ⁴	25

¹Time of harvest after soil emergence.

²Oven-dry, 60°C weight.

³Leaves did not form during the growth period.

⁴Roots could not be harvested without injuring other growing plants.

the soil at the low level of amendment. The calculated quantities of Tc remaining in the soil after 10 days of plant growth and uptake of Tc at the low soil Tc concentration level amounted to less than 5% of that applied. Therefore, the low concentration of Tc in subsequently formed leaves, which appeared only at the low level of Tc amendment between 13 and 25 days of growth, likely resulted from a depletion of Tc in the soil.

At the levels of Tc in the tissues, the possibility exists that damage to the growing tip resulted from radiation effects. However, the known functions of Mo in plants are in the processes of nitrate reduction and nitrogen fixation. If Tc replaced Mo as the functional group in the enzymes responsible for nitrate reduction and led to a loss in enzyme function, or N fixation, amino acids would not be available for protein synthesis. The only source of reduced nitrogen in this case would be from that stored in the cotyledons and marked reduction in plant growth might be expected.

Due to the marked uptake of Tc from soil in quantities highly toxic to the plant, the potential implications with respect to ecosystem functions and accumulation of Tc in the food web, and the lack of published information on the distribution of Tc in the environment, further studies of the fate and biochemical effects of Tc in soils and plants have been undertaken.

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Wildung, R. E., H. Drucker, T. R. Garland and G. S. Schneiderman. 1974. Fate of Heavy Metals and Heavy Metal Complexes in Soils and Plants. Progress Report to National Institute of Environmental Health Sciences, October 1, 1973-January 31, 1974. Battelle, Pacific Northwest Laboratories, Richland, Washington.

For further information refer to:
"Radionuclides in the Environment,"
in Terrestrial Ecology section,
pp. 191-203.

Determination of Methyl Mercury in Fish

J. W. Blaylock and R. M. Bean

The Hg levels of fish in the Columbia River were previously shown to approximate 1 µg/g, a level which would be expected in more polluted waters. To determine the fraction of Hg present as the highly toxic monomethyl mercury, a procedure, based on the method of Hartung (1972), was developed.

Partially-thawed samples of fish tissue (10 g) were homogenized in a

blender with toluene (10 ml) and concentrated HCl (10 ml). The homogenized samples were transferred to a capped centrifuge tube (50 ml) with a toluene washing (5 ml) of the blender jar. Phase separation was accomplished by centrifugation and the toluene layer was transferred by pipet to a clean centrifuge tube (50 ml). The tissue homogenate was subjected to two additional extractions with toluene (10 ml and 5 ml) for a total toluene extraction volume of 30 ml. An aqueous buffer (10 ml) containing cysteine (2%) and sodium borate (5%) was added to the toluene extract and

shaken for 10 min. After centrifugation, the toluene layer was removed by pipet and discarded. The aqueous phase was then extracted, as described above, with toluene (2-5 ml) after acidification with HCl (10 ml). The toluene layers were removed and dried with sodium sulfate (approximately 0.5 g).

The analysis was conducted by gas chromatography, using a Varian Aerograph Model 2100 equipped with a tritium electron capture detector. The column consisted of a 1.8 meter x 6.4 mm OD, 2 mm ID glass columns packed with high performance Chromasorb W, 80-100 mesh, which was slurry coated with carbowax 20M (solvent to support ratio 5:1, stationary phase 10% in solvent). The carrier gas was nitrogen (35 ml/min) and the operating temperatures were injector, 100°C; column, 75°C; and detector, 200°C.

To evaluate the efficiency of this method, known quantities of methyl mercury chloride were subjected to the complete analytical scheme in the presence and absence of fish tissue. Curvina fish tissue obtained from Lake Maracaibo, Venezuela was selected for this validation work because published results indicated that it was relatively free of methyl mercury contamination and preliminary results indicated that the flesh contains less than 0.05 µg/g tissue (wet weight).

Complete recovery of added methyl mercury was obtained without fish tissue present (Table 21). In the presence of fish tissue, a reduction in recovery was encountered (Table 22). The mean recovery for four replicates was 85.6 ± 6.3%. On the basis of these results, the method was considered quantitative and is presently being applied to analysis of Columbia River fish.

TABLE 21. Recovery of Methyl Mercury in the Absence of Fish Tissue.

CH ₃ HgCl		% Recovery
µg Total Added	µg Total Recovered	
37.10	36.04	97.1
35.56	35.90	101.0

TABLE 22. Recovery of Methyl Mercury in the Presence of Fish Tissue.

CH ₃ HgCl		% Recovery
µg Total Added	µg Total Recovered	
16.47	12.28	74.6
16.47	13.06	79.3
16.47	16.32	99.1
16.47	14.69	89.2

Reference Cited

Hartung, Rolf. 1972. The determination of mono and dimethyl mercury compounds by gas chromatography, pp. 157-161. In: Proceedings of the International Conference on Mercury, University of Michigan, 1972.

Distribution of Mercury in Water, Suspended Matter, and Sediments of the Lower Columbia River Watershed
T. R. Garland, R. E. Wildung,
A. J. Scott and A. V. Robinson

Previous reports, describing the distribution of Hg in water, suspended matter, and sediments of the lower Columbia River watershed (Figure 8), had indicated that the suspended matter played a primary role in the transport of Hg in the river (Wildung and Garland, 1974). In addition, the Hg content of the suspended matter appeared to be inversely related to the total concentration of suspended matter, with the highest levels found in the winter. On the basis of these initial results, optimum sampling times were selected to conform to river conditions that appeared to be crucial in describing the seasonal behavior of Hg in the river.

There were four sampling intervals during the year which appeared to be most definitive in describing the behavior of Hg in the river. These intervals corresponded to periods encompassing 1) prefreshet, 2) freshet, 3) maximum temperature, and 4) minimum temperature (Figure 9). These periods were sampled and analyzed during 1973 through 1974. In contrast to the previous studies, the latter investigations indicated that the

FIGURE 8. Sampling Locations on the Columbia River.

FIGURE 9. Normal Columbia River Flow and Temperature at Priest Rapids Sampling Site.

maximum levels of Hg in the river corresponded to the period of maximum river temperature (Table 23). This period was not included in the earlier studies.

The effect of the tributaries on the observed levels of Hg in the Columbia River water and suspended matter was either minimal or represented a diluting effect, except during the freshet (Table 23). At all sampling periods other than the freshet, the concentration of Hg in suspended matter from the tributaries was much lower than observed in the Columbia River behind Priest Rapids Dam. In addition, considering the Hg concentration levels in the tributary waters, only the Snake River contributed sufficient water (20 to 30%) to effect a change in Columbia River water Hg levels. The sum of the con-

tributions from other tributaries generally amounted to less than 5% of the total Columbia River flow. The dilution effect of the Snake River was apparent from the general decrease in Hg concentration in the suspended matter and the increase in total suspended matter observed in the water between Priest Rapids and McNary Dams during the prefreshet, maximum temperature, and minimum temperature periods. During the freshet periods, Hg concentrations in the suspended matter of the two major tributaries (Snake and Yakima Rivers) was higher than observed at Priest Rapids Dam. This was reflected in the increase in Hg concentrations in the suspended matter at McNary Dam. However, the relative magnitude of the Hg concentrations in suspended matter during freshet (0.11 $\mu\text{g/g}$) compared to the maximum temperature period (52 $\mu\text{g/g}$) would indicate that terrestrial runoff or resuspension of sediments during the freshet were not contributing directly to the maximum observed Hg levels. However, because the levels of suspended matter were high during the freshet, these processes may be important in supplying mercury to the bottom sediments which, in turn, through chemical or biological means, may release a significant portion of the Hg to the overlying water when temperatures increase to a maximum.

In addition to the observed concentration of Hg in suspended matter on either an absolute or water volume basis, the relative flow of the river at the various time periods must be considered. To normalize the Hg levels contributed by suspended matter to the water in the Columbia River to a common base, the flow rates at each sampling period were divided by the minimum average flow rate for the year, thus obtaining a dilution factor relative to minimum flow. By multiplying this dilution factor by the observed Hg levels in water at each sampling period, the relative Hg levels can be determined. The effect of these corrections is to magnify the Hg levels in the Columbia River during the freshet and maximum temperature periods (Table 24). These values further indicate that total Hg levels during maximum river flow (freshet) were not significant in comparison to levels observed at the maximum temperature period. This further implies that, during the maximum

TABLE 23. Concentration of Suspended Matter and of Mercury in Suspended Matter in the Columbia River and its Tributaries. Time Intervals Representing Different River Conditions.

SAMPLING LOCATION	PREFRESHET			FRESHET		
	SUSPENDED MATTER CONCENTRATION ⁽¹⁾	CONCENTRATION OF Hg IN		SUSPENDED MATTER CONCENTRATION ⁽¹⁾	CONCENTRATION OF Hg IN	
		SUSPENDED MATTER ⁽¹⁾	WATER ⁽²⁾		SUSPENDED MATTER ⁽¹⁾	WATER ⁽²⁾
COLUMBIA RIVER	mg/l	µg/g	µg/l	mg/l	µg/g	µg/l
PRIEST RAPIDS DAM	16	0.67	0.010	33	0.11	0.0036
M McNARY DAM	20	0.54	0.011	98	0.78	0.027
BONNEVILLE DAM	19	0.57	0.011	18	0.43	0.0077
TRIBUTARIES						
RINGOLD WASTEWAY	25	0.03	0.001	54	0.05	0.003
ESQUATZEL WASTEWAY	77	0.10	0.008	17	0.15	0.003
YAKIMA RIVER	91	0.14	0.013	94	0.16	0.015
SNAKE RIVER	44	0.17	0.008	62	0.56	0.035
WALLA WALLA RIVER	380	0.07	0.027	32	0.08	0.003

SAMPLING LOCATION	MAXIMUM TEMPERATURE			MINIMUM TEMPERATURE		
	SUSPENDED MATTER CONCENTRATION ⁽¹⁾	CONCENTRATION OF Hg IN		SUSPENDED MATTER CONCENTRATION ⁽¹⁾	CONCENTRATION OF Hg IN	
		SUSPENDED MATTER ⁽¹⁾	WATER ⁽²⁾		SUSPENDED MATTER ⁽¹⁾	WATER ⁽²⁾
COLUMBIA RIVER	mg/l	µg/g	µg/l	mg/l	µg/g	µg/l
PRIEST RAPIDS DAM	3.0	52	0.16	1.8	43	0.077
M McNARY DAM	6.7	31	0.21	11	6.7	0.074
BONNEVILLE DAM	6.0	19	0.11	8.6	9.1	0.078
TRIBUTARIES						
RINGOLD WASTEWAY	69	6.6	0.45	37	1.6	0.058
ESQUATZEL WASTEWAY	49	12	0.59	24	4.1	0.098
YAKIMA RIVER	85	2.6	0.22	14	7.3	0.10
SNAKE RIVER	23	7.8	0.065	7.2	9.0	0.008
WALLA WALLA RIVER	19	15	0.29	700	0.22	0.15

(1) CONCENTRATION ON DRY WEIGHT BASIS (60°C, 24 hr); CONCENTRATION OF Hg DETERMINED ON FRESH WEIGHT AND CONVERTED TO DRY WEIGHT BY SEPARATE ANALYSIS OF EACH SAMPLE

(2) CONTRIBUTION OF Hg TO WATER FROM SUSPENDED MATTER ONLY

temperature period, Hg is being released from a "sink," i.e., the sediments or that sizable amounts of Hg enter the Columbia River upstream of Priest Rapids Dam.

The distribution of Hg in sediment cores for the three collection sites is outlined in Table 25. Core samples from behind Priest Rapids Dam

TABLE 24. Seasonal Concentration of Total Mercury in Columbia River Water, Normalized to Constant River Flow.

Sampling period	Temperature °C	Total mercury concentrations in suspended matter at		
		Priest Rapids	McNary	Bonneville
		----- µg/l -----		
Prefreshet	8.0	0.010	0.011	0.011
Freshet	14	0.022	0.16	0.046
Maximum temperature	18	0.60	0.79	0.41
Minimum temperature	7.0	0.077	0.074	0.078

TABLE 25. Distribution of Mercury in Columbia River Sediments.

Depth Cm	Mercury content ¹ in sediment at		
	Priest Rapids Dam	McNary Dam	Bonneville Dam
	----- µg/g -----		
0-1	0.043	0.20	0.098
1-5	0.012	0.20	0.076
5-10	0.013	0.14	0.076
10-15	0.016	0.33	0.073
15-20	0.019	0.19	0.099
20-25	0.013	0.25	0.14
25-30	0.014	0.40	0.071
30-35	0.022	0.55	0.053
35-40	-	0.50	0.068
40-45	-	0.58	0.068
45-50	-	-	0.056

¹Based on dry weight, analyzed frozen, conversion made by analyses of separate fraction.

were primarily coarse sand and contained little fine sediment. The Hg levels were low, exhibiting little variation with depth below 1 cm. Sediments behind McNary Dam (Table 25) were primarily fine sediment with nominal quantities of sand. The Hg levels in the sediments at McNary Dam increase with depth, indicating loss from the surface layers or sedimentation of lower levels of mercury in recent years. The Hg levels in previous

core samples of sediments at McNary Dam decreased below 25 cm to a minimum level of 0.03 µg/g. The discrepancy likely resulted from the differences in sampling locations. The previous core sample was taken in an area of shallow sediment and, below 25 cm, the analyses indicated an absence of fine sediment but the presence of fine sand that may have been deposited before the dam was constructed. The core samples from behind Bonneville Dam appeared to be mostly fine sand plus small amounts of fine sediment. The Hg levels were lower than at the McNary site and exhibited little variation with depth.

Preliminary assessments of the chemical forms of Hg in the suspended matter and sediments of the Columbia River and the suspended matter of the major tributaries between Priest Rapids and McNary Dam were based on the increased volatility of the Hg organic forms and Hg metal compared to inorganic Hg. Although this separation must be considered arbitrary and some overlap between organic and inorganic volatility exists, the results obtained indicated the usefulness of the method. The suspended matter from the three sampling locations on the Columbia River lost an average of 90% (range 89 to 92%) of the Hg initially present upon drying (60°C, 24 hr). In contrast, suspended matter from the tributaries lost an average of only 25% (range 7 to 58%). Similar treatment of sediment core samples with depth showed <10% loss of Hg except in the surface 0 to 1 cm. In the latter case,

drying resulted in 60, 7 and 20% losses for the Priest Rapids, McNary, and Bonneville sediments, respectively. Although at present the results serve only to indicate that the association of Hg in samples from the various locations is different, it would appear highly unlikely that volatile dimethyl Hg or metallic Hg existed in the sediment below 1 cm.

On the basis of the results to date, three avenues of research recently have been pursued. The first involved investigation of the forms of Hg in water, suspended matter, and sediments and the behavior of known organic forms of Hg in the sampling and analyses scheme employed in the present study. For example, it is doubtful that volatile dimethyl Hg was assayed to any appreciable extent using current sampling methods. Although this may not have been a serious limitation if the levels of dimethyl Hg were low, determination of the concentration of this compound in the water column would be important if this compound constituted a major portion of the Hg in water, suspended matter, and sediments. In addition, it is recognized that filtration has potential limitations because of the potential for selective adsorption of soluble species on the filter. Further definition of the magnitude of volatilization and adsorption of various model Hg compounds is now in progress.

A second research study underway involves the question of the processes responsible for the lower Hg values in sediments compared to suspended matter in the Columbia River. Previous studies (Wildung and Garland, 1974) of the Hg content of surface waters and waters immediately overlying the sediments indicated that there was not a reduction of Hg content on sedimentation. Thus, the lower sediment Hg values likely resulted from release after sedimentation or dilution on sedimentation of particles of lower Hg content at other times of the year. The concentrations of Hg in the suspended matter during the freshet are sufficiently low to have served in this latter capacity. However, it is not possible to positively determine whether this was the case until sedimentation rate and resuspension at various times of the year are quantitated. These pro-

cesses will be quantitated in the McNary Reservoir by placement of traps on the surface of the sediment, which will collect sedimenting materials, allowing comparison of the quantity of sedimented matter with losses on resuspension, measured using graduated rods placed in the sediments. Collection of the sedimented matter will also provide an integrated sampling of sedimented material with respect to time, and sufficient material for chemical characterization of Hg form.

The third avenue of research now underway involves an attempt to obtain an integrated measure of the cumulative effects of mercury levels in the Columbia River. Since recent studies by others have indicated that total Hg levels in water only partially explain the variability in methyl Hg accumulation in fish, methyl Hg levels in fish at each collection site are presently being monitored at critical time periods.

The first collection of fish was made in April 1974 (prefreshet). The reservoir behind John Day Dam was substituted as a sampling site for the reservoir behind Bonneville Dam due to the difficulty of collecting fish at the Bonneville site. The primary purpose of including a dam below McNary was to observe changes which may have occurred at the McNary site.

Comparison of the methyl Hg content in sucker, as a function of the length of the fish, indicated that two different relationships existed for the Priest Rapids and McNary sampling sites (Figure 10). No definitive relationship was apparent for the fish collected at John Day Dam, possibly due to inadequate differences in fish size. Significant levels of methyl Hg were found in the skin and bone (equal to or greater than levels from flesh) but much lower levels were found in the gut (a factor of 2 to 8 less than flesh). If the observed levels of methyl Hg in sucker were related to the presence or formation of an "available" form of Hg, then the concentration of this form was higher at McNary than at Priest Rapids.

From the preliminary information on methyl Hg in fish, it appears that similar measurements under different

FIGURE 10. Methyl Mercury Content of the Flesh of Suckers Behind McNary, Priest Rapids and John Day Dams. April 1974.

river conditions may assist in identifying those conditions important in influencing Hg availability to indigenous biota. This information, coupled with studies that will provide an estimate of the release of volatile Hg compounds from sediments and suspended matter should provide insight into the biogeochemical cycling of this element in the Columbia River system.

For further information on characteristics of Hanford Soils and Aquatic Sediments refer to:

Marine Sciences Section, "Biological Availability and Effects of Effluents from Coastal Nuclear Power Plants," pp. 122-129.

● SUSPENDED PARTICLE INTERACTIONS

The objectives of this study are to determine the factors influencing the extent of Pu deposition from airborne effluents onto foliage, the potential for the resuspension of Pu from the leaf surface and the extent of Pu uptake and translocation by the plant. Using a low-wind-speed aerosol plant-exposure chamber, polydispersed aerosols (mean AMAD and GSD of 1.13 and 1.96 μm , respectively) were generated, and parameters such as deposition rate and deposition velocity were evaluated for a plant canopy. A comparison between predicted and calculated values for deposition velocity indicates the major influencing factor to be AMAD and GSD values. Concentration factors for bean seeds using several foliarly applied Pu compounds were from 1.9×10^{-6} to 8.0×10^{-8} .

Foliar Deposition of Plutonium Aerosols

Betty Klepper and D. K. Craig*

Modifications illustrated in Figure 11 have been made to the low-wind-speed aerosol plant exposure chamber. Additional high efficiency particulate air (HEPA) filters have been installed between the transfer hatch and the working section of the glovebox to ensure that aerosols will be contained in working sections of the chamber. Also, the air intake system has been rearranged so that air pressure within the tunnel is always negative with respect to that in the glovebox. These modifications have permitted experiments with submicronic particulates containing Pu.

Ten-day-old and 5-week-old seedlings of *Phaseolus vulgaris* L. have been exposed to aerosols containing ^{238}Pu nitrate, citrate, and oxide as well as an aged or hydrated form of the oxide. Aerosol generation was

effected by nebulizing the solution or stirred suspension in a RETEC nebulizer. Aerosol concentrations (pCi/cm^3) in the wind tunnel upstream and downstream of the plants were determined with 25 mm diameter membrane filter samplers. Mercer cascade impactor samples, drawn from upstream of the plant exposure section, permitted aerodynamic equivalent size distribution characterization of the aerosols for every test run. Contaminated plants were grown for 7 days in a holding chamber and were then transferred to a hood where leaves were dissected for digestion and counted to determine the amounts of ^{238}Pu associated with each leaf. On the day of exposure, the area of each leaf was determined. Thus, the deposition rate ($\text{pCi}/\text{cm}^2/\text{sec}$) of the aerosol onto the leaf surfaces could be determined from the measured activity, the projection leaf area, and exposure time. The deposition velocity (cm/sec) of the aerosol onto leaves, V_g , was obtained by dividing this deposition rate ($\text{pCi}/\text{cm}^2/\text{sec}$) by the aerosol concentration (pCi/cm^3 of air).

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FIGURE 11. Transverse Section of Glove Box and Aerosol Exposure Wind Tunnel.

A deposition velocity from theoretical values in the literature for monodispersed aerosols was also calculated. The Mercer cascade impactor was calibrated in terms of the effective cutoff diameters of unit density spheres. The quantity of material on each stage of the impactor was used along with the average particle size represented on each stage to predict a deposition velocity for the poly-

dispersed aerosol by summing up the deposition to be expected for the size range in each stage. Details of the calculation technique will be published by Craig, Klepper and Buschbom (in press). Table 26 shows a sample set of values for the nitrate. The observed deposition velocity is slightly less than the calculated value. The ratio of observed to predicted for all runs to date (data not

TABLE 26. Deposition Velocity of Plutonium Nitrate Aerosols onto Single Leaves at Very Low Wind Velocities.

Date of Test	Particle Size Data			Deposition Rate pCi/cm ² /sec	Deposition Velocity (cm/sec)	
	AMAD μm	GSD	MCI Conc. nCi/cm ³		Observed V _g × 10 ³ cm/sec	Calculated × 10 ³ From Observed Distribution
10/15/73	0.868	1.884	2.111	7.49	4.13	6.42
10/15/73	0.895	1.752	2.015	10.60	4.91	5.68
1/15/74	0.521	2.105	4.881	21.73	5.65	2.65
1/15/74	0.575	2.303	4.600	31.26	8.29	5.59
3/25/74	1.782	2.013	3.560	33.64	11.50	25.78
3/25/74	1.740	2.116	3.114	33.08	12.62	26.88
3/25/74	1.655	1.90	3.205	40.19	14.95	20.48
4/08/74	1.040	1.790	4.234	18.50	5.25	7.97
4/08/74	1.073	1.736	3.798	14.44	4.35	7.99
Mean	1.13	1.96			8.51	12.16
SD	0.49	0.19			4.35	9.45

reported here) on all chemical forms is 0.68. The predicted deposition velocities were calculated from theoretical deposition of monodispersed particles onto smooth, horizontal surfaces. Since leaves hang down from the horizontal, the observed deposition velocities should be less than the predicted depending upon the angle of leaf orientation. Since unifoliate bean leaves show a diurnal shift in leaf angle, this orientation will change continuously during an experiment. If the leaf angle were 45°, however, a ratio of 0.707 would be expected. The observed mean, 0.68, is close to the expected ratio for an angle of inclination of 45°.

Values for canopies (not reported here) are not significantly different from the values for single leaves. Our data show that the major parameters affecting deposition velocity are the AMAD and GSD values irrespective of the Pu compound involved. Providing an adjustment is made for the inclination of leaves to the horizontal, the deposition of polydispersed aerosols onto foliage can be predicted to within a factor of two using the measured aerodynamic equivalent size distribution of the aerosols.

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Craig, D. K., Betty L. Klepper, and R. L. Buschbom. 1974. Deposition of aerosols of various plutonium compounds onto plant foliage at very low wind velocities. In: Atmospheric Surface Exchange of Particulate and Gaseous Pollutants. (G. A. Sehmel, ed.) (in press).

Biological Availability of Foliarly-Applied Pu-238 Aerosols: Factors Affecting Plant Uptake and Transport D. A. Cataldo and B. E. Vaughan

The ability to generate polydispersed and monodispersed aerosols of submicron diameter under controlled conditions is not only useful in relating physical characteristics of aerosols such as deposition rate and deposition velocity to canopy characteristics and particle interception, but serves as a useful tool in evaluating the biological availability of foliarly applied compounds such as Pu,

Mobility of Dry Aerosol Deposited Plutonium-238

Initial studies designed to evaluate the translocatability of Pu compounds following dry deposition onto bean leaves (*Phaseolus vulgaris* L.) initiated in FY 1974, have recently been completed for 10-day and 5-week-old bean plants. In these studies, either a single primary leaf of a 10-day-old plant (with the rest of the plant protected by polyethylene films) or the entire foliage of a 5-week-old plant (with the developing bean pods bagged) was exposed to aerosols. Plants were held in a holding box for 7 days following exposure; at this time, all tissues were excised, oven dried, weighed, wet ashed, and analyzed for radioactivity by liquid scintillation counting (recovery of radioactivity and counting efficiency were >95%).

Twenty-two aerosol generation and deposition experiments were performed using ²³⁸Pu, nitrate, citrate, aged oxide, and fresh oxide with six plants of each age exposed during a single aerosol run. The large number of samples generated and the time involved in sample preparation and analysis resulted in a delay in data compilation. The complete data will be published at a later date.

The relative mobility of ²³⁸Pu following dry aerosol deposition onto leaf surfaces is shown in Tables 27 and 28. The biological availability of foliar Pu deposits following their deposition onto the foliage of 10-day and 5-week-old bean plants appears to be minimal with concentration factors (CF) in unexposed tissues of 10⁻⁵ to 10⁻⁶ and transport factors of 10⁻⁴ to 10⁻⁸. For the purposes of these and subsequent foliar experiments, a new term to describe biological availability will be employed in conjunction with CF, this being the transport factor (TF). Unlike CF, TF evaluates availability on the basis of the total amount of radioactivity or material presented to foliar tissues and the proportion of the presented material that is removed from the contaminated tissues. This eliminates complications imposed by the actual mass of

TABLE 27. Transport of ^{238}Pu Compounds from the Primary Leaf of 10-day-old Bean Plants Following Exposure to Polydispersed Aerosols. The Primary Leaf Was Exposed to a Narrow Range of Polydispersed ^{238}Pu Aerosol Particles for 20 Min; the Remainder of the Plant Was Bagged in Polyethylene. Plants Were Held for 7 Days Following Exposure and then Sampled.

	pCi	pCi/g DW	CF ²	TF ³
<u>Pu 11 - nitrate</u>				
Exposed tissue	1.26×10^6	7.61×10^6	--	--
Unexposed tissue				
Primary leaf	22.3	182.2	2.4×10^{-5}	1.8×10^{-5}
Buds	2.1	30.9	2.1×10^{-6}	1.7×10^{-6}
Stem	8.5	64.4	8.5×10^{-6}	6.8×10^{-6}
Total	32.9	92.5	1.3×10^{-5}	2.7×10^{-5}
<u>Pu 13 - aged oxide</u>				
Exposed tissue	3.41×10^5	1.91×10^6	--	--
Unexposed tissue				
Primary leaf	26.7	209.8	1.1×10^{-4}	7.8×10^{-5}
Buds	1.2	15.3	8.0×10^{-6}	3.5×10^{-6}
Stem	16.9	142.1	7.5×10^{-5}	4.9×10^{-5}
Total	44.8	122.6	6.5×10^{-5}	1.3×10^{-4}
<u>Pu 14 - citrate</u>				
Exposed tissue	1.07×10^6	6.30×10^6	--	--
Unexposed tissue				
Primary leaf	10.6	98.5	1.6×10^{-5}	9.9×10^{-6}
Buds	6.6	70.8	1.1×10^{-5}	6.1×10^{-6}
Stem	24.9	161.8	2.6×10^{-5}	2.3×10^{-5}
Total	42.1	110.4	1.7×10^{-5}	3.9×10^{-5}
<u>Pu 15 - fresh oxide</u>				
Exposed tissue	6.22×10^5	3.58×10^6	--	--
Unexposed tissues				
Primary leaf	1.5	13.1	3.7×10^{-6}	2.4×10^{-6}
Buds	0.8	10.2	2.9×10^{-6}	1.2×10^{-6}
Stems	2.1	15.5	4.3×10^{-6}	3.4×10^{-6}
Total	4.4	12.9	3.6×10^{-6}	7.0×10^{-6}

¹Radioactivity data represents an average for six replicates. Concentration and transport factors are based on these data.

²CF is the concentration factor for Pu based on pCi/gDW of exposed tissue and the concentration found in specific plant parts (CF = pCi per gDW in plant part/pCi per gDW of exposed tissue).

³TF is the transport factor and equates total activity supplied to total activity transported to unexposed tissues (pCi in unexposed tissues/pCi deposited onto exposed tissues).

tissues present (stems versus buds versus seed) and is more relevant when considering the physiological and chemical parameters determining mobility patterns within the plant.

Exposures of plants of both ages suggest that little Pu is transported to growing points (sinks) such as buds and seeds. Availability of the various Pu forms studied is generally as

TABLE 28. Transport of ^{238}Pu Compounds to the Developing Bean Pod and Seed Following Exposure of the Entire Canopy of 5-week-old Bean Plants to Polydispersed ^{238}Pu Aerosol Particles. The entire Foliage, Except for Developing Bean Pods Which Were Bagged in Polyethylene, Was Exposed for 20 Min. Plants Were Held for 7 Days Following Exposure and then Sampled.¹

	pCi	pCi/g DW	CF ²	TF ³
<u>Pu 11 - nitrate</u>				
Exposed tissue	1.12×10^7	1.04×10^7	--	--
Unexposed tissue				
Bean pod	19.0	30.2	2.9×10^{-6}	1.7×10^{-6}
Seed	0.9	2.1	2.0×10^{-7}	8.0×10^{-8}
Total	19.9	16.2	1.6×10^{-6}	1.8×10^{-6}
<u>Pu 13 - aged oxide</u>				
Exposed tissue	2.05×10^6	1.67×10^6	--	--
Unexposed tissue				
Bean pod	8.5	13.3	8.0×10^{-6}	4.1×10^{-6}
Seed	2.5	32.0	1.9×10^{-5}	1.2×10^{-6}
Total	11.0	22.7	1.4×10^{-5}	5.3×10^{-6}
<u>Pu 14 - citrate</u>				
Exposed tissues	6.72×10^6	4.73×10^6	--	--
Unexposed tissue				
Bean pod	29.3	88.0	1.9×10^{-5}	4.4×10^{-6}
Seed	3.8	37.1	7.8×10^{-6}	5.6×10^{-7}
Total	33.1	62.6	1.4×10^{-5}	5.0×10^{-6}
<u>Pu 15 - fresh oxide</u>				
Exposed tissue	6.26×10^5	5.10×10^5	--	--
Unexposed tissue				
Bean pod	12.8	18.5	3.6×10^{-5}	2.0×10^{-5}
Seed	0.2	1.3	2.5×10^{-6}	3.2×10^{-7}
Total	13.0	9.9	1.9×10^{-5}	2.0×10^{-5}

¹Radioactivity data represents an average for four replicates. Concentration and transport factors are based on these data.

²CF is the concentration factor for Pu based on pCi/gDW of exposed tissue and the concentration found in specific plant parts (CF = pCi per gDW in plant part/pCi per gDW of exposed tissue).

³TF is the transport factor and equates total activity supplied to total activity transported to unexposed tissues (pCi in unexposed tissues/pCi deposited onto exposed tissues).

follows: aged oxide > citrate > nitrate > fresh oxide. Current studies indicate that final chemical form and plant availability may be a function of the method of preparation of stock solutions from the oxide, thereby affecting interpretation of data. Presently, solutions are being carefully characterized as to "soluble" compo-

nents. Until all data is processed and analyzed, further speculation on the availability of the respective Pu forms is pointless, since a small degree of variation in the data (as presented) could greatly affect interpretation. However, it should be noted that, based on the experimental design, the values presented (CF and TF) are

maximal values for biological availability following dry deposition of Pu aerosols onto foliar surfaces.

Subsequent Evaluation of Foliar Uptake

The concentration factor for the accumulation of ^{238}Pu in reproductive structures (bean pod and seed) for foliarly applied Pu compounds is of the same magnitude as found in root uptake studies. However, it should be kept in mind that experiments of this type, where relatively insoluble compounds are presented to plant roots and shoots, cannot realistically evaluate all of the factors affecting biological availability encountered in the environment. Aside from the possibility of long-term chemical modification of relatively insoluble heavy metal compounds which can occur in the environment, several immediate factors may facilitate their availability, i.e., soil vector-Pu chemical interactions, foliar surface-Pu chemical interactions, and solution chemistry occurring as a result of rainfall onto the leaf surface. The effect of such parameters may markedly alter biological availability, uptake, and mobility of Pu in plants.

The presence of a solution vector on foliarly-deposited Pu may affect biological availability directly in several ways:

1. The bathing of particulate Pu compounds in a weak ionic solution on the surface of the leaf, as would occur following a rainfall, could conceivably result in the formation of more soluble Pu compounds.
2. The presence of a solution vector on the surface of a leaf could increase the movement of soluble complexes through the epidermal layers and into the leaf interior, thereby increasing its availability for transport to edible plant parts. The simple presence of a solution vector may influence the mobility of submicron Pu particles through openings in the leaf surface (stomata, cuticular pores), and their subsequent entry into the apoplast may result in biological modification (e.g., high HCO_3^-).
3. The availability of foliar deposits for leaching and resuspension represents a further aspect influenc-

ing biological availability. Particulates impacted onto a leaf surface are subject to solubilization, wind resuspension, leaching to the soil surface, and embedment within the cuticular plates as these expand and engulf small fissures on the leaf surface. With the exception of the latter point, these processes lead to the re-entry of Pu into the environment. In the case of wind resuspension and leaching, these processes again will have an effect on biological availability as would be the case should they enter the soil and be further modified and subsequently accumulated by the plant.

These foliar interactions will provide the major thrust for the research program in the remainder of FY 1975. The basic objective will be an evaluation of the availability of foliarly applied Pu compounds for leaching and resuspension and the effect of a solution vector on the uptake and transport of Pu to the seeds of the bean plant.

A leaching chamber has been constructed that can simulate a 15 min rainfall of 0.9 to 2.5 cm. The chamber has a diameter of 25 cm and can accommodate plants 60 cm in height. Routine analysis of leachates will evaluate solubles versus particulates leached (i.e., solubles defined as passing a $0.01\ \mu$ filter). The leaching solution will consist of a synthetic "rain water" characteristic of the central Washington area (data collected by C. D. Moodie in 1963 for four locations in southcentral Washington). The type of leaf surface-Pu interaction will be studied by differential leaching (weak ionic solution, weak acid solution).

The experimental design calls for two types of leaching studies. The first is a sequential analysis of foliar leaching (1, 5, 10, 20 days) to determine the effect of successive rainfalls on foliar Pu availability (soluble and particulate). The second is a time-dependent analysis to evaluate availability at various times following aerosol deposition (1, 5, 10, 20 days).

Leaching studies will be conducted on 20-day-old bean plants; these will be subjected to contamination by Pu oxide (aged or fresh), citrate, and

hydroxide. Reproductive structures will be initiated approximately 5 days after exposure and bean development should be complete at approximately 20 days following exposure. This will enable a parallel study of the effect of a solution vector on uptake and transport to the developing seed.

Based on a better understanding of aqueous Pu chemistry and aerosol solutions, leachates will be carefully calibrated and characterized. This should enable accurate interpretation of foliar deposition and provide insights into the biologically available components of aqueous Pu solutions.

for further information on Transuranium Element Behavior refer to:

"The Potential for Plutonium Complexation in Soil and Uptake by Plants," Wildung et al., pp. 20-35.

"Weathering and Aging Effects of Transuranics in Soils," Rickard et al., pp. 191-203.

"Plutonium Studies on the Nevada Test Site," Eberhardt et al., pp. 6-7.

"Ecological Behavior of Plutonium and Americium in a Freshwater Ecosystem: I. Limnological Analysis; II. Isotopic Ratios," Emery et al., pp. 95-99.

"Biogeochemistry of Plutonium and Americium in Marine Systems," Robertson et al., pp. 148-150.

° HANFORD INTERCONTRACTOR SUPPORT *

Radioactive wastes in the Hanford environment are stored as buried liquids and solids or as sediments in ditches and ponds. Knowledge of the behavior and fate of waste radionuclides in local biota is essential to sage handling practices and sound environmental control. Work to date has emphasized the behavior of long-lived radionuclides in soil-plant systems.

The Behavior of Waste Radionuclides in Soil-Plant Systems

R. C. Routson and J. C. Draper

This program continues to provide basic plant-soil information required for environmental waste management for the Hanford waste separation areas. These investigations generally involve the interactions of Hanford wastes, soils, and plants. Effort is concentrated on the behavior of long-lived radionuclides including ^{137}Cs , ^{90}Sr , and the transuranics. Studies include:

- The effect of soil concentration on the intake of Cs and Sr by plants.

Recent studies (Wildung and Garland, 1974) have shown that relative plant uptake of some radionuclides increases as the soil concentration of radionuclides decreases. This greatly compromises much plant uptake data which has been obtained at relatively high soil radionuclide concentrations to obtain sufficiently precise and accurate uptake data. Uptake was measured using ^{134}Cs and ^{85}Sr which, due to their high specific activities, can be detected at lower molar concentrations than can ^{137}Cs and ^{90}Sr . Uptake was measured at four equivalent ^{137}Cs

and four equivalent ^{90}Sr concentrations where the equivalent ^{137}Cs or ^{90}Sr concentration was defined as total Cs or Sr added to the soil calculated as an activity of ^{137}Cs or ^{90}Sr , respectively.

The lowest equivalent ^{137}Cs concentration and equivalent ^{90}Sr concentration was made without addition of any carrier to the ^{85}Sr and ^{134}Cs . Higher equivalent concentrations of ^{137}Cs and ^{90}Sr were made by the addition of the required quantities of unlabelled Cs and Sr, respectively. Tumbleweed was grown on soil in which the activity was initially evenly distributed, harvested after 2 months, and analyzed for ^{134}Cs and ^{85}Sr uptake.

In Table 29, it can be seen that the concentration factor for Cs is independent of the soil concentration of Cs over nearly 2.5 orders of magnitude. The value of the concentration is in good agreement with reported values from the literature.

Table 30 shows that the concentration factor for Sr is independent within experimental error of the soil concentration of Sr. Values for the Sr concentration factor are approximately 300 times larger than the concentration factors for

* Work supported by Atlantic Richfield Hanford Company.

TABLE 29. Plant Uptake of ^{134}Cs by Tumbleweed as a Function of the Equivalent ^{137}Cs Concentration in the Soil.

Equivalent ^{137}Cs soil Concentration	CF ¹
$\mu\text{Ci/g}$	
3.9×10^{-2}	0.031 ± 0.008^2
1.4	0.031 ± 0.008
1.4×10^1	0.032 ± 0.005
1.4×10^2	0.039 ± 0.010
Average	0.033

¹CF = $\mu\text{Ci/g}$ in the shoot
 $\div$ $\mu\text{Ci/g}$ in the soil.
² $\pm$ standard deviation.

TABLE 30. Plant Uptake of ^{85}Sr by Tumbleweed as a Function of the Equivalent ^{90}Sr Concentration in the Soil.

Equivalent ^{90}Sr soil concentration	CF
$\mu\text{Ci/g}$	
8.5×10^{-4}	$11. \pm 1.8$
1.6×10^{-2}	9.0 ± 1.2
1.6	$10. \pm 2.1$
1.6×10^1	8.7 ± 2.0
Average	9.6

Cs. This also is in good agreement with Sr CF in the literature. The above analysis assumes that the uptake of both ^{90}Sr and ^{137}Cs are low enough to cause only insignificant metabolic damage to the plant.

- The relationship between plant age and the uptake of Cs and Sr from soil.

Tumbleweed plants were harvested at 4, 8, and 12 weeks to evaluate

the effect of plant age upon the uptake of ^{137}Cs and ^{90}Sr . In Table 31, it can be seen that both Sr and Cs follow the same general pattern of having approximately equal CF values at 4 and 8 weeks, but higher CF values at 12 weeks by approximately a factor of 2. This suggests that, as tumbleweed nears maturity, either more Sr and Cs are taken up by the plant from the soil or are remobilized from the roots. If this also occurs in the natural system, tumbleweed growing near a waste disposal site should be eradicated early in its growth cycle.

- Comparison of CF values of ^{85}Sr measured with a thin-layer technique and with a totally mixed system.

Since radionuclides are rarely evenly distributed within a soil, as is the case in most laboratory studies, the CF values for ^{85}Sr were measured in both layered and totally mixed systems. Tumbleweed plants were harvested at 4 weeks. In the totally mixed system the CF was determined to be 10.3 ± 1.2 . In the layered system the CF was determined to be 1.36 ± 0.12 if the initially wetted soil was taken as the reactive soil. If the total soil were taken as the reactive soil the CF was determined to be 109 ± 9 . By the end of the experiment all of the soil contained some activity. No method was devised to reconcile layered problems, thus totally mixed soils are preferred for measuring CF values for leachable radionuclides.

TABLE 31. Relative Plant Uptake of ^{85}Sr and ^{134}Cs as a Function Length of Growth.

Growth period weeks	CF relative to 4 weeks	
	^{134}Cs	^{85}Sr
4	1.0 ± 0.08	1.0 ± 0.10
8	1.3 ± 0.18	0.92 ± 0.15
12	2.1 ± 0.17	1.8 ± 0.19

For the Marine Sciences program, several important sediment and chemistry programs were initiated by members of the Environmental Chemistry group. These include the following studies described in the Marine Sciences section of this report:

- 1) Fate and Effects of Oil on Marine Ecosystems. "Characterization of Petroleum Hydrocarbons in the Marine Environment," R. M. Bean. pp. 113-115.
- 2) Biological Availability and Effects of Effluents from Coastal and Offshore Nuclear Power Plants. "Copper in the Marine Environment," R. L. Schmidt, R. E. Wildung and E. H. Groff. pp. 125-126.
- 3) Biological Availability and Effects of Effluents from Coastal and Offshore Nuclear Power Plants. "The Fate of Copper in Marine Sediments--An Investigative Approach," R. L. Schmidt, T. R. Garland and R. E. Wildung. pp. 126-127.
- 4) Biological Availability and Effects of Effluents from Coastal and Offshore Nuclear Power Plants. "Sediments of Sequim Bay, Washington," R. L. Schmidt, K. H. Abel and R. E. Wildung. pp. 127-129.

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- **ENVIRONMENTAL IMPACT STATEMENTS**

● ENVIRONMENTAL IMPACT STATEMENTS

The Office of Regulation's Environmental Impact Assessment program (TA-02-19) has required substantial assistance in the past from aquatic specialists employed normally full-time in DBER's Land and Freshwater Sciences program (RX-02-01). These efforts were continued on a smaller level in FY 1974. For the sake of both completeness and priority guidance in our other programs, the status of participation in the impact assessment program is summarized here.

Also during 1974, a staff member normally employed full-time in DBER's RX program has provided limited support to the Division of Controlled Thermonuclear Research. Efforts were directed at assessing, in a preliminary way, potential environmental impacts from controlled thermonuclear operations. The basis of this preliminary assessment is outlined herein.

Environmental Impact Assessment K. L. Gore and D. G. Watson

The Ecosystems Department of Battelle's Pacific Northwest Laboratory has for the past year been preparing environmental impact statements on nuclear power plants for the AEC Directorate of Licensing. These environmental impact statements are required by law (NEPA, 1969) and specifically address the potential impacts that could arise from the siting, construction and operation of nuclear power plants. During the past calendar year about one-half of the power plants evaluated were either already built and operating, partially built, or new additions to already existing power plants. The other half of the impact statement workload was addressed to new proposed nuclear power plants.

Most of the existing nuclear power plants are using once-through cooling; thus, the greatest environmental impact results from the quantity of

water required by this type of cooling system upon the aquatic biota in surface waters from which the intake and discharge water is drawn and received respectively. These nuclear plants are located over a wide geographical area with many different types of surface waters (river, estuary and marine) used for cooling (Table 32); however, the types of potential impact on the aquatic biota are quite common to many nuclear power plants. These impacts include: impingement of organisms on the intake screens, entrainment (passage of organisms through cooling systems) effects, thermal plume effects, cold shock (plant shutdown during winter conditions), gas supersaturation, thermal blockage to migrating organisms, and toxicity of chemicals discharged from the plant to the receiving water. Specific examples of these impacts have been observed at existing nuclear power plants. Impingement losses of larval and juvenile menhaden have been recorded at Indian

TABLE 32. Nuclear Power Stations Evaluated by PNL 1973 - 1974.

Name/Location	Reactor Type ¹	Type of Cooling	Type Aquatic Environment	Proposed Power Level MWt MWe		Date of Issue Final Environmental Statement	Docket No.
Barton 1-4 Verbeno AL	BWR	Towers (mechanical draft)	Freshwater (river)	3758	1159	----	----
Brunswick 1 & 2 Brunswick Co., NC	BWR	Once-through	Marine (estuary)	2436	821	01-08-74	50-324 50-325
Callaway 1 & 2 Reform, MO	PWR	Towers (natural draft)	Freshwater (river)	3579	1150	----	50-483 50-486
Crystal River 3 Crystal River, FL	PWR	Once-through	Marine	2452	855	05-22-73	50-302
Shearon Harris 1-4 Wake Co., NC	PWR	Towers (natural draft)	Freshwater (river cooling reservoir)	2785	900	03-29-74 (re-issued)	50-400- 403
Millstone 3 Waterford, CT	PWR	Once-through	Marine	3425	1150	02-07-74	50-423
Monticello Monticello, MN	BWR	Once-through (mechanical draft)	Freshwater (river)	1670	545	11-22-72	50-263
Oyster Creek Oyster Creek NJ	BWR	Once-through	Marine (estuary)	1930	670	07-05-74	50-219
Pilgrim 2 Plymouth, MA	BWR	Once-through	Marine	3629	1219	10-01-74	50-471
St. Lucie 1 Fort Pierce, FL	PWR	Once-through	Marine	2560	833	06-18-73	50-335
St. Lucie 2 Fort Pierce, FL	PWR	Once-through	Marine	2560	850	05-16-74	50-389

¹PWR - pressurized water reactor
BWR - boiling water reactor

Point Power Plant. Entrainment mortalities of fish eggs and larvae have been observed at Brayton Point and Haddam Neck Power Plants. Menhaden mortalities caused by heated water discharges and cold shock have been documented at the Oyster Creek plant in New Jersey. Gas supersaturation causing mortalities of fish have been recorded at the Pilgrim plant. Other impacts could arise from construction effects, i.e., dredging, siltation, and the alteration or destruction of habitats (both terrestrial and aquatic) for plant structures and transmission lines. The extent of the impact may depend significantly on both hydrological and water-

quality characteristics of the body of water used for cooling.

With the advent of cooling towers the potential impact on the aquatic biota is somewhat reduced due to the smaller volumes of water required for cooling as compared with once-through cooled plants, because much of the waste heat is absorbed by the atmosphere rather than the cooling water. An example would be the Shearon Harris Nuclear Power Plant Units 1-4. Originally a 10,000 acre cooling pond was designed for an open-cycle cooling system. However, design changes now call for a closed-cycle cooling tower system which requires only a 4,000 acre pond for make-up water.

Potential impacts on the terrestrial environment are usually less severe than those on the aquatic environment and are usually associated with construction effects. Such impacts include wildlife changes associated with the loss of natural vegetation and habitats which results from clearing land for construction of nuclear power plants and for maintenance of electrical transmission lines. Secondary impacts may result depending on the means used to maintain land clear from vegetation under power lines. In addition, cooling towers, especially those using saline waters, are capable of discharging large amounts of salts into the surrounding environments, with some potential for long-term adverse impact on wildlife and agricultural crops.

Historically, environmental impact statements have been centered around the impacts caused by the large quantity of water needed for the cooling system with major emphasis on temperature effects. It has become increasingly evident from the ongoing review of environmental impact statements that little information is being collected on the combined effects of pollutants on the aquatic biota. Heat, mechanical and physical stress, antifoulant compounds, corrosion products and inhibitors may combine to cause synergistic effects, which are poorly delineated at this time. Moreover, the methodology for quantifying such combined effects remains rather undeveloped with the major emphasis geared toward short-term results rather than investigating long-term single, combined, and synergistic effects. A number of the specific impacts suggested above have so far not been adequately investigated at the laboratory level with attending difficulty in forecasting potential effects in real-world operational circumstances. It seems our emphasis in environmental impacts should be heading toward these goals in the laboratory and then verifying them with field experiments. In the following sections several new programs were funded by DBER to better characterize such impacts, and the status of these programs can now be examined.

Information Requirements for Controlled Thermonuclear Reactor Environmental Statements

J. A. Strand

The Ecosystems Department, together with the Biology and the Systems Departments of the Pacific Northwest Laboratory assisted the Division of Controlled Thermonuclear Research (CTR), during FY 1974, in a review of information requirements pursuant to preparation of environmental statements for controlled thermonuclear reactor designs. The objective of the Ecosystems effort was directed to determine the most probable type and magnitude of potential environmental impact associated with the first commercial fusion reactors.

Analysis of present CTR design criteria indicated that most anticipated environmental effects to terrestrial and aquatic systems will be similar to those experienced currently by fission designs. It was concluded that suitable technology is available or would be available to provide assessment of the importance and magnitude of such effects. However, the analysis also identified three potential sources of environmental impact unique to the CTR design:

- Release of kilogram quantities of tritium
- Release of substantial quantities of lithium
- Release of substantial quantities of other chemical pollutants during mining, refining, and manufacture of CTR materials
- Possibility of metal contamination resulting from deterioration of CTR materials during reactor operation.

It was concluded that sufficient information was available to permit assessment of the radioecological consequences of potential tritium releases. The biological behavior and radiological implications are perhaps better understood for tritium than for any other radionuclide. Extensive studies over the last 30 years have

demonstrated no unique behavior for tritium in the biosphere that would not have been predicted with due consideration for physicochemical form of the principal compound type. Moreover, such experimentation has failed to produce effects data significantly different from those which might have been predicted from a general knowledge of the effects of ionizing radiation. However, this is not to say that the effort over the last 30 years has been wasted, or that further research is not required. It was suggested that in no area of radiation research was there sufficient data to conclude that experimental validation of predicted values was not required. The primary deficiencies in our knowledge of tritium include a requirement for additional information on the behavior of tritium in temperate climates and the implications of longer-term or chronic exposures. Additionally, multi-generation studies directed at assessment of potential genetic effects are needed to confirm our present knowledge of basic concepts.

Insufficient information is available presently to provide for assessment of environmental effects of large quantities of lithium released during an accident event. Research is required to determine the biological behavior and toxicological implications of lithium in both terrestrial and aquatic systems. Chronic tests extending over the entire life cycle of selected test species should be conducted to determine the highest "safe" concentration of lithium at which no adverse effects are observed. Data derived from these studies should be used to develop "application" factors that facilitate estimation of "safe" concentrations of lithium from short-term acute tests. Assessment of sublethal stress should be undertaken to permit rapid detection of responses to lithium at stressing levels as low as those found by chronic tests to be biologically "safe." Ample time should be available for obtaining the necessary in-

formation before preparation of the environmental statements for the first CTR prototypes.

The procurement of materials for construction of CTR's may entail a unique environmental impact because of the anticipated significant increase in quantities of materials required. This may potentially result in increased land alteration during mining, refining, and manufacturing of such materials and also create increased land, water and air pollution problems. However, it was concluded that no additional research was justified until the types and quantities of materials to be used in CTR construction are known.

Too little is known about operational problems at this stage of development but the deterioration of structural materials under high temperature and neutron flux raises the possibility of environmental contamination by metal elements, e.g., chromium, niobium, and vanadium.

In summary, the Ecosystems staff concluded that sufficient information was currently available to permit assessment of all environmental impacts associated with CTR's with the exception of those attributed to lithium release. However, the potential impact to biological systems can be reduced through use of large exclusion areas and proper plant design. Also, it is highly likely that additional technology will be developed as the first commercial fusion reactors are constructed and further decrease the magnitude of such potential environmental impact.

Additional studies conducted by Battelle for the Division of Controlled Thermonuclear Research are planned for FY 1975. The objective of this latter program is to prepare a draft document describing in detail the anticipated environmental effects of controlled thermonuclear reactors following adoption for commercial use.

FRESHWATER SCIENCES

- **THERMAL EFFECTS ON AQUATIC BIOTA**
- **COMBINED EFFECTS OF TEMPERATURE AND POLLUTANTS AND OF TEMPERATURE AND DISEASES ON FISH**
- **PHYSIOLOGICAL EFFECTS OF SUDDEN THERMAL EFFLUENT DISAPPEARANCE: COLD SHOCK**
- **EFFECTS OF THERMAL DISCHARGES ON FISH BEHAVIOR AND SENSORY PHYSIOLOGY**
- **FACTORS AFFECTING BIOGEOCHEMICAL CYCLING**
 - **GABLE MOUNTAIN POND STUDIES**
 - **SALMON SPAWNING STUDIES**
- **EFFECTS OF MODIFICATIONS ON AQUATIC ECOSYSTEMS**
- **ECOLOGICAL DISTRIBUTION AND FATE OF PLUTONIUM AND AMERICIUM IN A PROCESSING WASTE POND ON THE HANFORD RESERVATION**
- **EFFECTS OF TRITIUM ON AQUATIC SYSTEMS**

● THERMAL EFFECTS ON AQUATIC BIOTA

Assessment of the effects of thermal discharges and management of portions of the aquatic environment as a heat sink with minimal ecological impact require prediction of the response of populations of aquatic organisms to thermal stress. However, population effects cannot be determined without knowledge of the effect on individuals, both at lethal and sublethal levels of exposure. This project addresses mainly two specific impacts of thermal discharges and their effects: gas bubble disease and effects of fatigue on thermal tolerance. Limited effort was also expended to assess parasitism as it affects thermal resistance.

Gas Bubble Disease D. H. Fickeisen

Supersaturation of water with a dissolved gas occurs when the gas tension exceeds the gas-phase partial pressure. Man's use of water resources results in supersaturation with atmospheric gases both by increasing gas tension during heating of water used for cooling industrial facilities and by entrainment of air into water plunging over dam spillways. Aquatic organisms have evolved efficient gas exchange mechanisms and when exposed to water supersaturated with dissolved gases become supersaturated themselves. In this disequilibrium condition, a pressure gradient exists from the dissolved state to the gas phase. Therefore, gas bubbles form at nucleation sites in blood and tissue, and the physical effects of their presence cause gas bubble disease. Signs of the affliction include emphysema, petechiation, exophthalmia, and lesions in tissue. Emboli block blood vessels, causing hemostasis and congestion, and cardiac blockage by emboli results in anoxia and mortality. Fish kills have been recorded

in the thermal discharges of steam electric stations, both nuclear and fossil fueled, and in riverine environments below hydroelectric and irrigation-storage dams. The deaths were caused by gas bubble disease resulting from atmospheric gas supersaturation. This continuing portion of the project has as its major goals the identification of tolerance levels of aquatic organisms to gas supersaturation and a determination of the effect of temperature on tolerance. In addition, it addresses the ability of organisms to recover from gas bubble disease and analytical methodology for gases dissolved in water.

During the calendar year about two-thirds of a series of bioassays designed to determine the effect of temperature on tolerances of three species of fish to gas supersaturation have been completed. The testing protocol is to acclimate groups of fish from each of three species (Ictalurus melas, black bullhead; Lepomis gibbosus, pumpkinseed sunfish; and Salmo gairdneri, rainbow trout) at 4°C intervals between 8 and 32°C (except that rainbow trout will only

be tested between 8 and 20°C). The fish are held in normally saturated water during acclimation. Following this acclimation period they are exposed to a series of levels of supersaturated water for a 96-hr period to determine the median tolerance limit (TL₅₀) using death as an endpoint. Tests with black bullhead between 8 and 20°C are complete and the data have been analyzed. Tests with pumpkinseed sunfish are nearly finished in the same temperature range. Rainbow trout tests are about half completed. The relationship between temperature and tolerance is indicated in Figure 12, which shows the 96-hr TL₅₀ and fiducial limits about it for black bullhead. As can be seen there is a slight increase in tolerance at the lower test temperatures, which is statistically significant. The effect is not ecologically significant within the temperature range reported as fluctuations in gas content occur within short time periods and within short distances in the natural environment which exceed the small difference in observed tolerance. In addition, the gas-phase partial pressure is proportional to hydrostatic pressure such that depth within the water greatly affects the saturation level, and fish moving vertically in a water column are exposed to changing gas levels.

Equipment has been fabricated and installed for conducting studies of the ability of fish to recover from signs of gas bubble disease. The apparatus include a temperature-controlled supersaturation system and exposure facilities as well as modifications to a recirculating system for holding the gas bubble diseased fish in normally saturated water. A protocol for recovery experiments is in the draft stage and experiments are planned to begin in the very near future.

A preliminary study of the effect of prior exposure to excess gas supersaturation on susceptibility of trout to *C. columnaris* infection was undertaken by Mary Kujawa during a 3-month NORCUS appointment. In the short time period available, experimental techniques were developed and several exposures made. However, the project did not generate useful

FIGURE 12. Effect of Temperature Tolerance to Excess Gas Tension of Black Bullhead.

data due to lack of a sufficiently virulent strain of the columnaris organism.

A major portion of the effort in the last quarter has been the development of a modification to the standard technique of calibration of the gas chromatograph based on that reported by L. P. Atkinson, 1972. In the past, the gas chromatograph has been calibrated externally for dissolved gas analyses by injecting water samples of known gas content. To determine actual gas content, it has been necessary to perform Van Slyke manometric analysis for nitrogen and Winkler titrations for oxygen. Van Slyke manometry is time consuming and requires a skilled, meticulous operator. It also involves the use of metallic mercury which invariably results in problems of laboratory contamination. An electrolytic calibration technique has, therefore, been under development. It is based on electrolysis of hydrazine sulfate to generate nitrogen and electrolysis of water to generate oxygen. Linearity of charge versus gas chromatograph response has been demonstrated as has nearly theoretical production of nitrogen. Prior to adoption as the standard technique at PNL, theoretical production of oxygen must be demonstrated, and a permanent electrolytic stripping chamber designed as a modification of the Swinnerton chamber presently in use. The technique will be published in the next few months.

A workshop on gas bubble disease was jointly sponsored by Battelle and DBER on October 8 and 9 in Richland, Washington. It was attended by members of the Nitrogen Task Force, including 50 representatives of state and federal agencies, utilities, and consulting firms. Attendees were from a wide geographical range, including two from Canada. There were also 15 Battelle staff members present. The agenda included 18 papers describing the effects of dissolved gas supersaturation on biota, analytical methods, and a model of the Kootenai River dissolved nitrogen regime. Each of the five roundtable discussions which were also included, was co-chaired by two people and was assigned a general topic relating to gas bubble disease. The co-chairmen were successful in generating and leading informal discussions to define the state-of-the-art as well as research needs in their subject area. Proceedings of the workshop will be published and will include manuscripts of the papers presented as well as summaries of the roundtable discussions.

The Nitrogen Task Force is an interagency group meeting several times a year to coordinate both research and the operation of hydroelectric facilities such as to minimize adverse effects of dissolved gas supersaturation. Task Force meetings serve as a forum for discussion of problems and dissemination of information relating to gas bubble disease. During the year, Battelle Northwest was invited to join the Nitrogen Task Force and did so. On October 10, following the Gas Bubble Disease Workshop, the group's fall meeting was hosted by Battelle Northwest.

Three staff members attended a short course on basic gas chromatography sponsored by Occidental College and two staff members associated with gas bubble disease studies were certified by NAUI and the State of Washington as SCUBA divers during the past year.

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Effects of Water Temperature and Exercise on Blood Glucose and Lactate of Rainbow Trout

M. J. Schneider, R. G. Genoway, and S. A. Barraclough

The objective in this study is to provide information on the thermal tolerance of fish as it may be affected by the fatigue state of the organism. Most earlier laboratory studies have addressed the thermal tolerance of a wide range of fish species but the data has been gathered with the specimens in a rested state or what has been termed routine or standard metabolism. In this investigation thermal effects on fish are being studied from two aspects, the tolerance of fatigued fish to elevated temperatures and the physiology of fish performing at elevated temperatures. This report will present recent results concerning the latter topic.

The blood components, lactate and glucose, are used as indicators of the metabolic state of the fish. The effect of elevated water temperature and forced swimming, both as separate stressors and in concert, are currently being evaluated. The experimental species in this study is rainbow trout, Salmo gairdneri. The design of experiments requires three acclimation groups of 8, 12, and 16°C. Fish from each of the acclimation temperatures are exposed to a series of test temperatures above the acclimation temperature, i.e., 0, 5, 10 and 15°C ΔT . Tests run at each of these test temperatures also include three different exercise levels: static, light, and heavy. The difference between the light and heavy exercise levels is based on the maximum enforced swimming speed and the duration of the swim at that speed. The experiment has been designed to ensure that all test animals have similar thermal experiences while the temperature is changed from the acclimation temperature to the test temperature. This is done to avoid subjecting the ΔT 15°C group to a sudden thermal shock. In all cases, the fish experience the ultimate temperature increase for the last 30 min of the standard run.

At the completion of each run the fish was removed from the exercise system and all metabolic functions were terminated by plunging the animal in an alcohol-ice bath. The

blood is then sampled immediately and processed for lactate and glucose measurement. The analysis of the blood data is based on a 4 (temperature) x 3 (exercise) factorial analysis of variance with 10 observations in each cell. At this point in time all of the data for the 12°C acclimation group is complete and has been statistically treated. Some portions of the 8 and 16°C acclimation groups remain to be completed. The results of the 12°C work will be reported here.

The results of the test performed with 12°C acclimated fish are summarized in Figures 13 and 14. The figures present the values of glucose and lactate, respectively, for mg% for the various combinations of test temperature and exercise. The first point to be noted from the data is that the glucose and lactate levels for static fish at $T = 0^\circ\text{C}$ are significantly higher ($p < 0.05$ for glucose and $p < 0.10$ for lactate) than the controls. This indicates that the handling of the fish required to

FIGURE 13. Blood Glucose Means and Their 95% Confidence Intervals for Rainbow Trout Acclimated at 12°C.

FIGURE 14. Blood Lactate Means and Their 95% Confidence Intervals for Rainbow Trout Acclimated at 12°C.

place them in the exercise apparatus does influence the levels of the blood constituents.

The data for both blood constituents for static and light exercise at the 12, 17, and 22°C test temperatures is extremely interesting. Increasing the temperature 10°C from 12° to 22°C produces no apparent stress. In static and light exercised fish, the $\Delta T = 10^\circ\text{C}$ blood lactate level is lower than either $\Delta T = 0^\circ\text{C}$ or $\Delta T = 5^\circ\text{C}$. Interestingly, the Q_{10} for the thermal stress over the $\Delta T = 10^\circ\text{C}$ for glucose and lactate under all exercise levels approximates unity. Any physiological process characterized by a Q_{10} value that approximates unity and is not irreversibly thermolabile, by definition, exhibits "instantaneous temperature compensation" (Hazel and Prosser, 1974).

Javaid and Anderson, 1967, have reported that the preferred temperature of trout is related to but always

in excess of the acclimation temperature. The data presented have indicated that for rainbow trout there may be a thermal range over which their resting or standard metabolism is independent of temperature, and in this sense these fish demonstrate the capacity for instantaneous temperature compensation.

As one might expect, instantaneous temperature compensation occurs within a measurable temperature range from the acclimation temperature and the upper end of this range is demonstrated in the data in Figures 13 and 14. At all work levels the blood components are elevated significantly at 27°C ($\Delta T = 12^\circ\text{C}$). This may be due to a combined effect of exercise and heat. The effect of heat alone if tested statistically over the total experimental temperature range reveals a high significance, $p < 0.05$. The glucose and lactate measurements for fish receiving heavy exercise at $\Delta T = 10^\circ\text{C}$ indicates a combined stress of heat and work. This is especially apparent in the lactate measurement. At $\Delta T = 12^\circ\text{C}$ the lactate measurement for heavy exercise appears lower than the measurement for static or light exercise. This is explained by the fact that these fish could not cope with the exercise regime at this temperature and did not swim for the total time period called for by the experimented design.

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Thermal Resistance and Parasitism in Fish

C. D. Becker

Numerous factors are known to influence the resistance of aquatic organisms to thermal stress, including such variables as body size, season, diet, sex, water chemistry, photoperiod, and hormone levels. An additional factor, which has escaped close scrutiny, is parasitism.

Nearly all aquatic organisms in natural environments carry one or more kinds of parasites. Some cause little or no detectable physical damage to their host while others, acting alone or in synergistic combination with other parasites, implement severe epizootics. All intermediate degrees of harm occur. Indirect evidence is available that some parasitic infections can influence the susceptibility of fish to suboptimal environmental conditions such as high temperatures. This possibility has largely been overlooked during the past decade in otherwise comprehensive studies designed to evaluate ecological impacts of heated effluents discharged from nuclear power plants.

Limited effort has been extended during the past fiscal year to explore a new hypothesis: that interactions may exist between thermal resistance and parasitism in freshwater fish. The parasite considered is Posthodiplostomum minimum (Diplostomatidae), a digenetic trematode enzootic in centrarchid fish throughout the United States; the host involved is the common pumpkinseed sunfish, Lepomis gibbosus.

Six basic experiments were completed. Infected wild pumpkinseed were subjected to standard thermal bioassays that extended to lethal temperature levels. The fish were ranked in order of death. Postmortem counts were made of metacercaria in certain visceral organs--the heart, liver, kidney, and spleen--of each fish to determine infection intensities. Death times and infection intensities were then compared.

Only one bioassay, the first undertaken, indicated a possible correlation. The more heavily infected fish generally showed lower resistance and were the first to die as the water temperature increased, Figure 15. This test was conducted in June and the temperature increase was 1°C/hr from a base temperature of 20°C . The subsequent five tests revealed no correlation since infection intensities appeared to vary randomly in relation to death times.

The hypothesis requires further exploration, particularly by means of experimentally controlled infections. Pumpkinseed sunfish in nature undergo

FIGURE 15. Sequence of Pumpkinseed Sunfish Infected with Metacercariae of *Posthodiplostomum minimum* when Acclimated to 20°C and Subjected to a 1°C/hr Temperature Increase.

heavy attack from *P. minimum* cercariae in the spring when these free-swimming stages emerge from snails. The penetration and burrowing action of the parasite towards target organs at this time causes considerable trauma and exerts maximum effect on the host. The parasite eventually encysts and becomes walled off by the host's inflammatory response. The phenomenon of mutual adaptation over a period of time may explain the possible correlation between infection intensity and death time in the

June bioassay and the lack of correlation in subsequent ones.

The interaction between thermal resistance and parasitism in fish and other aquatic organisms is a relatively untapped investigational area. A review and summation of the literature dealing with the effects of temperature on the development, infectivity, and pathogenicity of parasitic and infectious diseases of aquatic organisms is also needed.

● **COMBINED EFFECTS OF TEMPERATURE AND POLLUTANTS
AND OF TEMPERATURE AND DISEASES ON FISH**

This study is intended to quantify the combined effects of thermal insult and chemical stress on the physiology of fish and fish food organisms. Chemical toxicants tested are those commonly represented as additives to nuclear power plant effluents. Acute flow-through toxicity tests are currently used to determine the combined effects on rainbow trout (Salmo gairdneri), eastern brook trout (Salvelinus fontinalis), and crayfish (Pacifastacus leniusculus). Chronic flow-through toxicity test capabilities are being developed. In addition to the acute effects, sublethal effects on growth and reproduction will be observed. Chemical accumulation in tissue and histopathological response to the toxicant will also be observed in relation to growth and reproductive capacity.

Combined Effects of Thermal and
Chemical Discharges
E. G. Wolf and T. O. Thatcher

As a result of the publication of Toxicity of Power Plant Chemicals to Aquatic Life (Becker and Thatcher, 1973) and ongoing reviews of environmental impact statements, it becomes even more evident that little information has been collected on the combined effects of pollutants and temperature on the aquatic biota. Through current discharge practices, thermal power plants daily discharge large volumes of water contaminated with antifoulants, heat and corrosion products, and inhibitors. The combined impact of the pollutants on surface waters and their biota, which often carry a burden of pollutants, is not known. Chlorine was investigated first because of its almost universal application as an antifoulant. The combined effects of chlorine and thermal stress (arising out of plant discharge or as a seasonal

occurrence of the waters of interest) on rainbow trout (Salmo gairdneri) and eastern brook trout (Salvelinus fontinalis) was of primary interest because of their wide distribution, and their culture and life cycles have been well documented.

In nature, eastern brook trout generally spawn after the second year. Fish raised at optimum temperatures in the laboratory environment may mature during the first year after hatching. Experimentation with this species enabled us to obtain mature spawners during the first year. These initial attempts produced 1247 eggs from 6 adult pairs. Modification of published spawning methods permit spawning and subsequent toxicity tests during spawning in the test aquaria of the Mount-Brungs diluter system.

To date, 32 toxicity tests have been completed on rainbow and brook trout ranging from sac fry to 175 mm

juveniles. The fish have been acclimated to four different temperatures (5, 10, 15 and 20°C) and tested at ΔT 's ranging from 0 to 15°C.

Differences in resistance have been observed between species and at different test temperatures. The eastern brook trout appear to be more resistant at all life stages tested than the rainbow trout. Ninety-six hr TL_{50} for eastern brook trout range from 0.175 to 0.065 ppm total residual chlorine; whereas, the 96-hr TL_{50} for rainbow trout only ranged from 0.082 to 0.039 ppm total residual chlorine. The eastern brook trout when tested at 20°C, no matter what the ΔT was, had a significantly lower 96-hr TL_{50} than at test temperatures of 10 and 15°C. Sensitivity changes in the rainbow trout to chlorine over the range of temperatures tested were not apparent. Likewise, the sensitivity to chlorine for both rainbow trout and eastern brook trout did not change with the increasing age and size of fish.

Early mortality was observed to vary markedly among tests with eastern

brook trout acclimated to various test temperatures. A series of toxicity tests were completed using cold-branded fish acclimated to different temperatures and tested in the same aquaria. These tests demonstrated (Table 33) that early mortality was greatest in those fish experiencing the higher thermal stress (ΔT). However, the 96-hr TL_{50} between the various ΔT 's at each test temperature was not significantly different. Comparable data has been collected for rainbow trout but has not been statistically analyzed. Since this finding was complemented by (a) the absence of delayed mortality, and (b) the observations that the greater the temperature shock, the higher the mortality during these early stages, it appears that the effects of previous temperature acclimation upon acute lethality of chlorine disappeared after about 2 days.

Additionally, crayfish were tested since they are principal food organisms for many fish including the rainbow and eastern brook trout. Crayfish from the six bioassays appear to be much more resistant than either

TABLE 33. Comparison of Early Mortality and TL_{50} Values from Bioassays in Which Fish from Two Different Acclimation Temperatures were Exposed Simultaneously in the Same Series of Chambers.

Test Groups	96-hr TL_{50}	Early Mortality	Test Temp	ΔT
A-1	0.165	5.8%	10	0
A-2	0.175	11.7%	10	5
B-1	0.160	6.7%	10	3
B-2	0.160	13.3%	10	5
C-1	0.153	0.0%	15	0
C-2	0.128	5.0%	15	5
D-1	0.110	36.7%	20	5
D-2	0.110	58.3%	20	13

fish species. Latent mortality was observed in these organisms after termination of the toxicant dosage and depuration in fresh water. Recomputation of the 96-hr TL_{50} after 6 weeks depuration reduced it from 0.96 to 0.25 ppm total residual chlorine. Such latent mortality was never observed in the fish species assayed.

The data for both improvement of eastern brook trout spawning methods and the combined effects of chlorine and temperature are being submitted to journals for publication.

The existing Mount-Brungs diluter has been modified so that duplicate analyses can be run simultaneously. A multiple Mount-Brungs system has also been constructed so that chronic bioassays may be initiated using multiple toxicants and multiple test temperatures. Parallel programs on the combined effects of temperature and power plant chemicals have been initiated between the Richland laboratories and the Marine Research Laboratory at Sequim, Washington.

Reference Cited

Becker, C. D. and T. O. Thatcher. 1973. Toxicity of Power Plant Chemicals to Aquatic Life. WASH-1249. Battelle-Northwest, Richland, Washington.

Chondrococcus columnaris Disease

M. P. Fujihara

Research during the past 30 years in the fish pathogen, Chondrococcus columnaris, has dealt with their taxonomy, pathogenesis, transmission, serological relationships, effects of radiation, and especially the effects of increased temperature on proliferation and virulence. The subtle changes being evolved in the Columbia River Basin with the construction of hydroelectric dams, power reactors and the increasing usage of river water by agriculture and industry may serve to introduce additional heat as well as other pollutants in the aquatic environment. It is necessary to more fully understand the effects of these changes and probable enhancement of C. columnaris infection on the commercially valuable anadromous species.

A monograph on Chondrococcus columnaris disease was initiated during the later part of FY 1974 and is being continued. The goal of this undertaking is to review the investigations conducted to date and to assemble pertinent information pertaining to C. columnaris infection of fish. Hopefully, when the review of published and unpublished data is completed, it will suggest where further research is indicated.

For further information on fish diseases refer to:

- 1) "Suppression of the Primary Immune Response in Rainbow Trout, Salmo gairdneri, Sublethally Irradiated During Embryogenesis," J. A. Strand et al. pp. 101-104.
- 2) "Thermal Resistance and Parasitism in Fish," C. D. Becker. pp. 69-70.

• **PHYSIOLOGICAL EFFECTS OF SUDDEN THERMAL
EFFLUENT DISAPPEARANCE: COLD SHOCK**

The objective of cold shock studies is to define the interactions between biota and the varying hydrographic regimes occurring in thermal mixing zones following cessation of heated discharges. Of direct practical interest are the physical rates of temperature decline, the rates and limits of temperature decline tolerated by poikilothermous aquatic organisms, and eventually, the physiological changes that take place within individual species. Hydrographic modeling has been employed as appropriate to the characterization of such aquatic environments.

Modeling of Temperature Declines
C. D. Becker, D. S. Trent* and
M. J. Schneider

One basic question underlying studies involving aquatic organisms and cold shock is: What rates of temperature decline prevail in thermal mixing zones when the heat source abruptly terminates? All thermal increments seldom disappear abruptly from the water mass unless there is a strong, one-directional flow. The decline rate is usually gradual, and depends upon the physical characteristics of the water mass and its movement.

Because the resistance of a poikilothermous organism to a temperature drop from an artificial high level is related to the speed that the drop occurs, reasonable approximations of temperature decline rates are necessary to develop a rational experimental program. Normally, the more rapid the decline rate, the

more severe the resulting cold shock. This is valid for an acclimation level in the upper zone of thermal tolerance for all aquatic poikilotherms.

A temperature decline model has been formulated for the time-temperature distribution of an established thermal plume following power plant shutdown. The mathematical expression was analyzed for an offshore submerged discharge, and the results are valid for either freshwater lakes and reservoirs or for coastal regions.

The model, based on prediction of thermal history, was approached by two different methods, analytical and numerical simulation. The analytical approach required several idealizations but is generally valid for the thermal decay by horizontal diffusion, vertical diffusion, and atmospheric transfer (three-dimensional model) in a quiescent or uniform moving stream. The numerical simulation used a situation involving tidal influence.

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Analytically the appropriate differential equation for three-dimensional heat transport is:

$$\frac{\partial T}{\partial t} + \frac{\partial uT}{\partial x} + \frac{\partial vT}{\partial y} + \frac{\partial wT}{\partial z} = \frac{\partial}{\partial x} \left(\epsilon_x \frac{\partial T}{\partial x} \right) + \frac{\partial}{\partial y} \left(\epsilon_y \frac{\partial T}{\partial y} \right) + \frac{\partial}{\partial z} \left(\epsilon_z \frac{\partial T}{\partial z} \right) + Q$$

where:

T = temperature

u, v, w = receiving water velocity components along x, y , and z axis, respectively

$\epsilon_x, \epsilon_y, \epsilon_z$ = diffusion coefficient along x, y and z direction, respectively

Q = heat source or sink

The analytical solution is based on the following assumptions:

- 1) Velocity field is uniform in u and v and the vertical component is zero (i.e., $w = 0$).
- 2) The diffusion coefficients are constant in space and time.
- 3) Vertical diffusion is much less than lateral diffusion hence $\epsilon_z = 0$ is assumed.
- 4) Atmospheric heat transfer is assumed to be proportional to the temperature difference, $\Delta T = T_0$.
- 5) The diffusion process takes place in a body of water of semi-infinite extent (i.e., no shoreline influence).

By adapting these assumptions, the basic heat transport equation can be reduced, modified and otherwise treated to obtain efficiency. The ultimate modification is to an equation that must be solved by numerical integration, and can be applied to computer programming.

One practical application of the model represents a situation where the flow from a large vertical port is suddenly terminated and momentum

effects push the plume outward with upwelling cold water at the center. The radial temperature distribution around this port is evaluated in terms of temperature (T), time (t), and distance in Figure 16.

FIGURE 16. Distribution of Radial Temperatures in Terms of Time and Distance Following Cessation of a Heated Point Discharge in a Simulated Lake Environment.

Effects of Cold Shock on Aquatic Organisms

C. D. Becker, M. J. Schneider,
R. G. Genoway and S. A. Barraclough

Cold shock studies are based on the need to understand, evaluate and predict the effects of reactor shutdown on aquatic biota in effluent mixing zones. In several cases of record, shutdown has unexpectedly caused severe losses of fish previously attracted into warmed areas when ambient water temperatures elsewhere were seasonally low.

In one early study phase, channel catfish (*Ictalurus punctatus*) acclimated at 30°C were shocked by immersion at 10°C, and the effects were evaluated on a physiological basis. Results indicated a slight increase in plasma osmolality and chloride ions during the first 10 min of exposure. Osmolality and chloride ions decline after 24 hr, concomitant with increased plasma glucose and tissue water. Glucose levels were elevated by 281% and plasma osmolality and chloride decreased 34% and 55%, respectively, after 45-hr exposure to

10°C. On return to 30°C, plasma concentrations of glucose, chloride and osmolarity gradually returned to control values over a 4-day period. Osmoregulatory collapse is a characteristic consequence of cold shock in channel catfish, and perhaps in other freshwater fish (Block, 1974).

The present program is based on immediate need for predictive data on the ability of selected aquatic species, when acclimated to a given temperature, to resist both gradual and abrupt exposure to cold shock. Experimental methods were developed for providing the information required to establish a suitable cold tolerance data base before the active study program began. Experimental organisms selected for preliminary work were pumpkinseed sunfish (*Lepomis gibbosus*), rainbow trout (*Salmo gairdneri*) and crayfish (*Pacifastacus leniusculus*). These test species will eventually permit quantitative comparison of a "warm-water fish", a "cold-water fish", and a decapod crustacean.

The basic experiments are essentially bioassays in which 20 test organisms of each species are exposed to cold (a) gradually by temperatures declined at rates of 1, 3, 5 and 10°C/hr, and (b) instantaneously by direct immersion in water at a fixed lower limit. Test organisms are acclimated to temperatures at 5°C intervals. Resistance to cold shock is measured directly by loss of equilibrium (LE) and death (D), and the maximum exposure period is 4 days.

The cold shock bioassays are nearing completion with over 100 separate tests conducted to date. Sunfish response to gradual temperature declines and to abrupt cold shock is illustrated in Figure 17. In both cases, response is based upon acclimation level. Sunfish are considerably more susceptible to cold than rainbow trout or crayfish when compared to the other bioassays completed.

The ability of sunfish to resist a gradual temperature decline is affected by the decline rate (Figure 17,A). Measured at the point of 50% LE, sunfish show little or no adaptation at a decline rate of 18°C/hr. Slower declines permit limited adaptation that lowers the eventual 50% LE point at least by 2°C.

FIGURE 17. A) Effect of Temperature Decline Rate on 50% LE Endpoint. B) Effect of Acclimation and Abrupt Exposure Temperature on Survival Rate (AT = Acclimation Temperature).

Gradual reduction of temperature in such cases as reactor shutdown is ecologically preferable to an abrupt reduction. A significant loss of fish is then less likely, provided the ultimate lower temperature limit is not exceeded.

The ability of sunfish to resist abrupt cold shock depends primarily on acclimation level (Figure 17,B). When acclimated to 30°C, a near maximum temperature for the species, about 50% of the fish die from instantaneous exposure to 13-14°C. When low temperatures are sustained for 4 days, however, when acclimated to 15°C, 50% mortality occurs at a sustained shock temperature of 2-3°C.

Severe cold shock at a low but sublethal level is accompanied by

temporary disorientation and LE, which has ecological significance even though the fish survive. Such temperature declines as occur from reactor shutdown are apt to be detrimental to endemic organisms at plant sites whenever the difference between temperatures of the mixing zone and the ambient water exceeds prescribed limits.

This type of information is required for the establishment of temperature standards that protect aqua-

tic populations and the ecosystem from the adverse effects of cold shock in temperate regions.

Reference Cited

Block, R. M. 1974. Effects of acute cold shock on the channel catfish. pp. 109-118. In: Thermal Ecology (J. W. Gibbons and R. R. Sharitz, eds.), Symposium proceedings. Augusta, GA., May 3-5, 1974; NTIC, Springfield, VA.

• EFFECTS OF THERMAL DISCHARGES ON FISH BEHAVIOR AND SENSORY PHYSIOLOGY

The intent of this study is to generate information on the behavioral response of fish to thermal challenges such as power plant discharge plumes. The response displayed by fish upon encountering a heated discharge could be very significant in determining the ultimate thermal experience of the organism. This study proposes to investigate the initial response of the fish.

A second portion of this program addresses the possibility that a thermal exposure of sublethal levels may have a lasting effect on the efficiency of fish sensory organs. Using behavioral conditioning methods this study will determine if a thermal insult has a lasting effect on the manner in which the whole fish responds to its environment via its sensory apparatus.

Behavioral Responses of Fish to a Simulated Thermal Discharge M. J. Schneider, R. G. Genoway and S. A. Barraclough

Knowledge of the behavioral responses of fish to thermal discharges is important to understanding the influence discharges could have on the survival and ecological success of fish populations. Earlier studies of thermal preferenda indicate that temperature does affect the responses of fish to the environment. If fish are responding in a significant manner to thermal stimuli, the resulting reaction may be significant to the total thermal experience, e.g., passage through a thermal plume. In turn, a behavioral response will have an effect on the ultimate impact of thermal exposure and population success. The most accurate predictions of thermal effects must, therefore, consider behavioral response. The objective of this study is to observe and document the behavioral responses of juvenile fish when they encounter a thermal challenge.

The experiments are conducted in a long raceway or flume constructed so that water velocity can be controlled and monitored. A jet of variable size, temperature and velocity is introduced through the flume bottom at the approximate midpoint. The raceway has a curved bottom to eliminate areas of lower current velocity in corners. It was determined early in this study that fish would take advantage of such areas, thus making it imperative that the cross-sectional velocities be as uniform as possible.

In the course of an experimental run, fish are introduced at the upstream end of the raceway and then manipulated into the discharge region by adjusting the current velocities in the raceway. The response of the fish, rainbow trout juveniles have been used thus far, is then recorded via a television camera and on a videotape system.

To date, two sets of experimental runs have been made in the raceway. In the first set a total of 36 fish

were used, 18 as single fish introduced into the system one at a time and the second 18 were placed into the flume in groups of three. The first group of fish introduced as individuals indicated that the fish prefer the side of the flume, probably due to slightly lower current velocities. For this reason the groups of three fish were used. When this was done the fish tended to distribute themselves across the flume. When the fish followed the side of the raceway they would encounter the edge of the warm-water plume downstream from the discharge point. By forcing a cross-channel distribution one or two of the fish were more likely to encounter the leading edge of the plume at the point of discharge. In this first series of tests, it was apparent that the fish were responding to the visual presence of the discharge jet (black in contrast to the white lining of the trough), or to the presence of the television lens suspended directly over the discharge point. Evidence for this was the fact that similar behavior was witnessed for several fish as they passed over the discharge point in the absence of any

discharge as for fish in the presence of both ambient and heated water discharges.

In the second set of tests fish were placed in the raceway in groups of three. For this series an attempt to reduce the visual cue of the discharge nozzle had been made. Also the ΔT of the discharge had been raised from the original 9.0 to 23.0°C. As in the first series of runs three conditions were used, no plume, ambient plume, and heated plume. A total of 24 releases were made in this series. Since the study has not reached a level of sophistication requiring qualification of data, a viewing panel has been used in observing the videotapes. The results of this second series indicate that several fish did react to the discharge jet, both when the discharge was heated and at ambient temperatures. Before qualifiable data can be gathered it will be necessary to further alter the raceway. There is still some question of the fish responding to the visual cueing by the discharge nozzle or the camera lens.

• FACTORS AFFECTING BIOGEOCHEMICAL CYCLING

The study of the fate of introduced chemical and particulate matter in freshwater ecosystems is fundamental to our understanding of the structure and function of these water bodies. These inputs can be in a variety of forms and need not be solely related to the movement of radioactive materials despite the inherent emphasis in our programs on these isotopes. Intrinsic to investigations of this nature is understanding not only where and in what quantities these materials appear in various ecosystem components, but also knowing what chemical, physical, and biological processes mediate their movement.

Our studies in this field have emphasized the Columbia River and its associated ecosystems for several years, and work on the chemical characterization of reactor effluents before and after their passage through the soil before reaching the Columbia is continuing. Emphasis in this program, however, has shifted to more detailed studies of lentic habitats receiving low-level radioactive effluents. Our goal is to provide basic data on the cycling of radionuclides within these ponds which will be useful to both ecologists and waste management personnel.

Factors Affecting Biogeochemical Cycling in Freshwater Ecosystems*

C. E. Cushing, D. G. Watson, D. E. Robertson,** and A. J. Scott

The program entitled "Factors Affecting Biogeochemical Cycling in Freshwater Ecosystems" has been one of a continuing nature with emphasis on the Columbia River and related aquatic ecosystems. Research prior to 1971 emphasized studies of the

fate of the plutonium reactor discharged radionuclides in the Columbia River and its biotic and abiotic components. Subsequent studies, after closure of the reactors in 1971, examined the decline of radioactivity in this ecosystem. Near background levels of radioactivity in the biota and burial of the sediment-associated radionuclides by uncontaminated sediments in McNary Reservoir dictated a shift of emphasis in this program.

The program is presently directed toward a detailed study of Gable Mountain Pond, an artificial lake created by the discharge of low-level radioactive wastewaters on the Hanford Reservation. This lake has been in

* Cooperative program between Ecosystems and Radiological Sciences Departments

** Radiological Sciences Department

existence for over 20 years and has developed a wide diversity of biota except for fish which are represented by one species, the goldfish (*Carassius auratus*). An abundant inventory of radionuclides is present, mainly associated with the sediments, with ^{137}Cs dominating. A thorough study of the dynamics of this wastewater pond will contribute valuable information relevant to radioactive waste management. A lesser effort is being devoted to studies of N-Reactor effluents.

Initial studies of Gable Mountain Pond will be directed toward a thorough limnological study of this ecosystem to characterize the physical, chemical, and biological components. Physical parameters to be measured include incident light, water temperature, water budgets, particle size distribution of the sediments and suspended matter, and sedimentation rates. Stable chemical analyses will be done for elements which have significant concentration levels of their radioactive forms in the pond, i.e., ^{137}Cs . Dissolved organic carbon and particulate organic carbon will be measured in the water, seston, and sediments. Qualitative and quantitative collections and analyses of biota from the various trophic levels will be done periodically to provide a seasonal picture of the fluxes of the above parameters needed to characterize the ecosystem.

In conjunction with the limnological studies described above, replicate samples of each organism plus water and sediment samples will be collected and radioanalyzed. This inventory of radionuclides in the various ecosystem components and the results of the limnological studies will provide the basis for future studies on the dynamics of radionuclide cycling within this wastewater ecosystem. The cycling studies will involve experimental manipulation of ecosystem components together with the necessary statistical interpretation to provide a model of radionuclide cycling within this lake. This research and a companion investigation of the dynamics of plutonium cycling in another waste pond will provide useful information on the formulation of waste management practices.

The studies of the N-Reactor effluents will emphasize the physicochemical speciation of the various radio-

nuclides within the discharge trench and after they percolate through the soil and emerge as seepage springs along the banks of the Columbia River. Sediment-water relationships will be examined by exposure of uncontaminated river sediments to the trench and spring water and by selective leaching studies of the radioactive sediments.

Physicochemical Characterization of N-Reactor Effluent Radionuclides in Soil and Water Systems
D. E. Robertson

Basic to our understanding of the radioecology of nuclides released to aquatic environments from nuclear installations is a knowledge of their physicochemical forms in the effluents and receiving waters. Such information is essential since various physicochemical species of the same radionuclides can exhibit very diverse environmental behavior.

Physicochemical characterization studies of reactor effluent water radionuclides at the Hanford N-Reactor are providing important information describing the mobility of radionuclides in freshwater environments. At N-Reactor, cooling water containing a wide spectrum of radionuclides in various physicochemical forms is discharged to a seepage trench located near the reactor. The effluent water migrates through a soil bank between the trench and the Columbia River, and a portion of the water emerges as seepage springs along the bank of the Columbia River near the reactor. The mobility of effluent water radionuclides during transport through soil is greatly dependent upon the physicochemical forms of the radionuclides.

Typical radionuclide concentrations in N-Reactor effluent water sampled in February 1974 at the trench and in seepage spring water sampled at the Columbia River bank are shown in Table 34. The decontamination factor afforded by the soil bank is represented by the ratio of the concentrations in the trench and in the springs (T/S ratio).

Radionuclides in particulate and cationic forms are almost completely retained in the soil bank by sorption onto mineral phases (Table 34); radionuclides in anionic and soluble nonionic forms are relatively mobile

TABLE 34. Radionuclide Concentrations in Various Physicochemical Forms in N-Reactor Effluent Water and Seepage Spring Water.

RADIONUCLIDE	CONCENTRATIONS IN D/M/liter											
	PARTICULATE			CATIONIC			ANIONIC			NONIONIC		
	TRENCH	SPRINGS	T/S RATIO	TRENCH	SPRINGS	T/S RATIO	TRENCH	SPRINGS	T/S RATIO	TRENCH	SPRINGS	T/S RATIO
²⁴ Na	3290	---	---	3.67x10 ⁵	---	---	640	---	---	49	---	---
⁴⁶ Sc	170	<0.02	>8500	<60	<0.1	>600	28	<0.3	>90	8	0.09	89
⁵¹ Cr	16,400	<0.2	>82,000	<1300	<1.0	>1300	<1500	84.1	<18	160	<0.5	>320
⁵⁴ Mn	6650	<0.02	>3.9x10 ⁵	57,200	<0.1	>5.7x10 ⁵	130	<0.3	>430	220	<0.1	>2200
⁵⁹ Fe	19,600	<0.05	>3.9x10 ⁵	140	<0.2	>700	260	<0.7	>370	70	<0.2	>350
⁵⁸ Co	1350	<0.02	>67,500	<70	<0.1	>700	<34	<0.2	>170	<2.0	<0.1	>20
⁶⁰ Co	9450	0.19	50,000	9500	12.2	780	190	51.8	3.7	160	6.4	25
⁶⁵ Zn	950	<0.05	>19,000	2990	<0.2	>15,000	<50	<0.7	>70	14	<0.2	>70
⁷⁶ As	<500	<0.06	---	<1200	<0.3	---	11,600	<2.0	>5800	3310	<0.8	>4100
⁸² Br	440	<0.09	>4900	<1300	<0.6	---	720	<0.3	>2400	26	<1.0	>26
⁹¹ Sr	<22,000	---	---	2.44x10 ⁵	---	---	<17,000	---	---	<1100	---	---
⁹⁵ Zr	7680	<0.03	>2.7x10 ⁵	540	<0.1	>5400	1060	<0.6	>1800	238	<0.1	>2400
⁹⁵ Nb	10,100	<0.02	>5.1x10 ⁵	11,700	<0.1	>1.2x10 ⁵	1990	<0.7	>2800	1120	<0.1	>11,000
⁹⁷ Zr	8630	---	---	<1000	---	---	<600	---	---	<400	---	---
⁹⁹ Mo	<700	1.0	<700	7170	2.6	2800	2.07x10 ⁵	3310	63	5310	17	320
⁹⁹ Tc	4480	1.4	3200	7290	3.7	2000	2.49x10 ⁵	4840	51	6050	20	300
¹⁰³ Ru	2320	0.36	6400	580	10.6	55	440	260	1.7	162	15	11
¹⁰⁶ Ru	280	0.44	640	<800	4.2	<190	<470	160	<2.9	76	8.6	9
¹²² Sb	420	<0.03	>14,000	<270	<0.2	---	1160	<1.0	>1200	560	<0.2	>2800
¹²⁴ Sb	74	<0.04	>1900	<90	<0.2	---	59	2.2	27	34	38.3	0.89
¹²⁵ Sb	<100	<0.05	---	<200	<0.3	---	80	3.8	21	55	86.4	0.64
¹²⁹ Ie	<400	---	---	1380	---	---	6360	---	---	89	---	---
¹³¹ I	410	5.7	72	20,200	23	880	81,700	5210	16	1770	430	4
¹³³ I	4360	0.4	10,900	2.7x10 ⁵	2.2	1.2x10 ⁵	1.02x10 ⁶	610	1700	20,000	29	700
¹³⁵ I	<2x10 ⁵	---	---	1.01x10 ⁶	---	---	1.94x10 ⁶	---	---	34,000	---	---
¹³⁴ Cs	350	<0.03	>12,000	2270	<0.1	>23,000	<36	<0.4	---	<2.0	<0.1	---
¹³⁷ Cs	1810	<0.02	>90,500	10,200	<0.1	>1.0x10 ⁵	<50	<0.6	---	<4.0	<0.1	---
¹⁴⁰ Ba	2620	<0.06	>44,000	52,700	<0.3	>1.8x10 ⁵	<270	<3.0	---	3310	<0.4	>8300
¹⁴⁰ La	2760	<0.02	>1.4x10 ⁵	40,900	<0.1	>4.1x10 ⁵	150	<0.1	>1500	180	<0.1	>1800
¹⁴¹ Ce	4770	<0.04	>1.2x10 ⁵	2210	<0.2	>11,000	460	<2.0	>230	160	<0.3	>530
¹⁴⁴ Ce	7910	<0.2	>40,000	6690	<0.9	>7400	<800	<15.0	---	470	<1.0	>4700
¹⁵¹ Pm	1070	---	---	7360	---	---	<1700	---	---	1770	---	---
¹⁸⁷ W	22,100	<0.1	>2.2x10 ⁵	<2000	<0.7	---	1530	4.8	320	420	<2.0	>210
²³⁹ Np	8110	<0.2	>41,000	21,900	<1.0	>22,000	<1000	<10.0	---	130	<2.0	>65

and are retained by the soil to a much lesser degree. Several radionuclides such as ⁶⁰Co, ¹⁰³,¹⁰⁶Ru and ¹²²,¹²⁴,¹²⁵Sb are present in reactor effluent water partitioned among particulate, cationic, anionic, and nonionic species. However, as these radionuclides migrate through the soil bank their particulate and cationic forms are retained, and predominantly

anionic and nonionic forms emerge in the seepage springs (Table 35).

The question arises as to the biological availability and geochemical reactivity of the anionic and nonionic forms of the radionuclides which are not retained by the soil bank at N-Reactor. When such radionuclides enter aquatic environments

TABLE 35. Physicochemical Forms of Various Radionuclides in N-Reactor Trench and Seepage Spring Waters.

FORM	% OF TOTAL IN VARIOUS FORMS					
	⁵⁸ Co		⁶⁰ Co			
	TRENCH	SPRINGS	TRENCH	SPRINGS		
PARTICULATE	>92	UNDETECTABLE	49	0.3		
CATIONIC	< 5	UNDETECTABLE	49	17		
ANIONIC	< 3	UNDETECTABLE	1	73		
NONIONIC	< 0.1	UNDETECTABLE	0.8	9		
	¹⁰³ Ru		¹⁰⁶ Ru			
	TRENCH	SPRINGS	TRENCH	SPRINGS		
	TRENCH	SPRINGS	TRENCH	SPRINGS		
PARTICULATE	66	0.1	UNDETECTABLE	0.3		
CATIONIC	16	4	UNDETECTABLE	2.4		
ANIONIC	13	91	UNDETECTABLE	92		
NONIONIC	5	5	UNDETECTABLE	5		
	¹²² Sb		¹²⁴ Sb		¹²⁵ Sb	
	TRENCH	SPRINGS	TRENCH	SPRINGS	TRENCH	SPRINGS
	TRENCH	SPRINGS	TRENCH	SPRINGS	TRENCH	SPRINGS
PARTICULATE	>17.4	<0.06	>29	<0.09	UNDETECTABLE	<0.06
CATIONIC	<11.2	<0.3	<35	<0.5	UNDETECTABLE	<0.3
ANIONIC	>48.1	4	>23	5.4	---	4.2
NONIONIC	>23.2	96	>13	94.5	---	95.8

do these mobile forms remain unreactive. It may be possible that the anionic and nonionic radionuclide species would be transported with only a minimal uptake and retention by the aquatic biosphere and the sedimentary mineral phases.

Studies of the behavior of these radionuclides are providing data for assessing present rad-waste treatment processes and in improving future processes for reducing environmental releases of radionuclides from nuclear installations.

For further information on specific radiocontaminants of the aquatic environment refer to:

"Radionuclides In the Environment," Rickard et al., pp. 191-203.

° GABLE MOUNTAIN POND STUDIES*

These studies represent efforts by Ecosystems Department staff in support of Hanford plant requirements for environmental surveillance. They represent small-scale efforts supported from local funding sources (Intercontractor Agreements and Service Assessment). Objectives of such tasks are in all cases subordinated to surveillance and safety needs, but the data acquired provide useful baseline information relevant to DBER program. For that reason, we have indicated their status in the footnotes.

Studies on Gable Mountain Pond were funded by the Atlantic Richfield Hanford Company (ARHCO) in support of nuclear waste management problems and are distinct from our primary research program at Gable Mountain Pond described in the section, "Factors Affecting Biogeochemical Cycling."

Gable Mountain Pond Studies**

C. E. Cushing, A. J. Scott, and J. M. Gurtisen

Two groups of experimental goldfish (obtained offsite) were placed in pens; one over an area with relatively low levels of radioactivity in the sediments and food materials and one over an area with higher levels. Fish were placed in the pens on May 25, 1974, and replicate samples collected bi-weekly for radioanalysis; results are shown in Figure 18. Native fish were collected during the winter to compare with the experimental populations. Fish in the area of higher radioactivity attained essentially stable body burdens of ^{137}Cs within 3 months. Body burdens of ^{137}Cs in the fish over the low-level sediments reached quasi-

equilibrium levels about a month earlier at levels of about one-half of those over the higher level sediments. Both populations showed evidence of declining body burdens after October and November. This probably related to the decreased metabolic rates and biological turnover as water temperatures dropped. The ^{137}Cs levels in the free-ranging native fish were similar to those in the low-level pen. It is also of interest to note that three native fish, evidently entrapped when the pen was placed in the pond, were collected on January 7, 1974 in the high-level pen. Their average body burden of ^{137}Cs was 537.3 pCi/g dry wt, about the same as that of the experimental fish in this pen. This supports the conclusion that ^{137}Cs body burdens were directly related to the concentration of this radionuclide in sediments and food. This study of differential uptake of radionuclides was terminated prior to spawning in the spring of

* ARHCO Intercontractor Service Agreement

** ARHCO Intercontractor Support

FIGURE 18. ^{137}Cs Body Burdens of Experimental Goldfish.

1974 to avoid alteration of the resident populations' gene pool by offspring of these experimental fish.

A second experiment was conducted to compare the body burdens of wild ducks and introduced mallard ducks restricted to Gable Mountain Pond. The introduced birds were rendered flightless by amputation of the manes. Table 36 presents data on body burdens in wild and experimental ducks and shows that ducks restricted to the pond attained body burdens of ^{137}Cs in the muscle and carcass many times higher than the transient waterfowl. High mortality of the experimental ducks and nonavailability of wild birds, except during migration, limited the data collected. The few results obtained show a differential uptake by these two populations. ^{137}Cs levels of wild coots, which do not fly out to feed as do wild ducks, contained ^{137}Cs concentrations equal to and often exceeding those of the experimental ducks. The coots probably had been on the pond longer than the experimental ducks.

TABLE 36. Average Body Burdens of ^{137}Cs in Experimental¹ and Wild Waterfowl on Gable Mountain Pond.

<u>Ducks, experimental</u>	<u>pCi/g dry wt</u>
9-13-73 (n=4)	
muscle	281.0
carcass	118.0
10-23-73 (n=1)	
muscle	414.4
carcass	217.8
11-5-73 (n=2)	
muscle	254.6
carcass	108.8
<u>Ducks, wild</u>	
11-5-73 (n=5)	
muscle	79.2
carcass	40.3
1-3-74 (n=6)	
muscle	44.1
carcass	8.1
<u>Coots, wild</u>	
9-13-73 (n=2)	
muscle	375.5
carcass	149.9
10-9-73 (n=4)	
muscle	537.2
carcass	401.4
1-3-74 (n=6)	
muscle	784.0
carcass	277.5

¹Placed on pond on August 8, 1973

° SALMON SPAWNING STUDIES

Results of 1974 aerial surveys of salmon spawning in the Hanford reach of the Columbia River are presented and compared with previous surveys. The relatively low number of redds observed is attributed to poor survey conditions and reduced number of salmon spawning in the area.

Fall Chinook Salmon Spawning Near Hanford - 1974* D. G. Watson

Fall chinook salmon that spawn in the Hanford reach of the Columbia River are about the only remaining main stem spawning population. Most of the suitable main stream spawning grounds have been destroyed by hydroelectric dams and their associated impoundments. Aerial surveys of the local salmon spawning have been conducted annually since 1947. The purpose of these surveys initially was to provide an index for estimating the effect of plutonium production reactor effluents on this natural fish population; and in more recent years after the closure of most of the reactors, to provide a contribution to the ecological data base of the river.

Five surveys were conducted from October 15 to November 26, 1974 in the section of the Columbia River from Richland (river mile 339) to Priest Rapids Dam (river mile 396). Survey conditions were generally very poor this year due to adverse weather conditions and higher than usual river elevations during the time of the survey. Release of water from Priest Rapids Dam during the daylight period seemed higher than usual, making observation of the river bottom uncertain, particularly in the deeper areas.

The number of salmon redds (nests) observed consequently is probably an underestimate of the actual number present.

A total of 728 redds were counted during the 1974 survey. This is far below the nearly 3000 observed last year and the 3800 in the brood year of 1970 (Table 37). Upstream passage of adult fall chinook over McNary Dam, the nearest dam downstream of Hanford, was approximately 60,000 in 1974, 73,000 in 1973, and 62,000 in 1970. Although the previously mentioned adverse weather and river conditions probably reduced the number of redds counted this year, spawning areas where visibility to the river bottom was good and that supported large numbers of spawning fish in past years, were poorly seeded. Spawning was earliest near Ringold (river mile 354), where over half of the redds observed on the October 15 survey were found. The early spawning in this area coincided with the return of fall chinook to the Washington State Department of Fisheries rearing facility at Ringold, and past years juvenile salmon releases from this station probably contributed to the early spawners in this area. Similar early spawning was noted in this area in 1973.

The relative use of the major spawning areas (expressed as "Percent of Total," Table 37) has been fairly consistent in recent years. There is some

* AEC Service Assessment Support

TABLE 37. Fall Chinook Salmon Spawning - Hanford 1947-1974.

YEAR	NUMBER OF REDDS AND PERCENT OF TOTAL ()							TOTAL
	354 ⁽¹⁾ (RINGOLD)	365 TO 368	371 (WHITE BLUFFS)	373 TO 376	383 (COYOTE RAPIDS)	393 (MIDWAY)	OTHER	
1947	0 (0)	15 (6)	25 (10)	10 (4)	0 (0)	75 (31)	115 (48)	240
1948	120 (15)	330 (42)	38 (5)	219 (28)	0 (0)	25 (3)	53 (7)	785
1949	45 (14)	50 (15)	6 (2)	195 (59)	0 (0)	1 (0.3)	33 (10)	330
1950	24 (8)	43 (14)	38 (12)	151 (48)	3 (1)	46 (15)	11 (3)	316
1951	5 (2)	10 (3)	45 (14)	151 (48)	5 (2)	95 (30)	3 (1)	314
1952	73 (14)	101 (19)	40 (7)	221 (41)	3 (0.5)	78 (14)	23 (4)	539
1953	7 (5)	5 (3)	16 (11)	38 (26)	0 (0)	83 (56)	0 (0)	149
1954	4 (3)	5 (3)	8 (5)	127 (81)	0 (0)	6 (4)	7 (4)	157
1955	0 (0)	12 (19)	0 (0)	47 (73)	0 (0)	4 (6)	1 (2)	64
1956	0 (0)	3 (3)	7 (8)	59 (64)	0 (0)	17 (18)	6 (6)	92
1957	27 (3)	173 (20)	55 (6)	440 (50)	43 (5)	132 (15)	2 (0.2)	872
1958	49 (3)	249 (17)	133 (9)	520 (35)	192 (13)	258 (17)	83 (6)	1485
1959	1 (0.3)	0 (0)	36 (13)	101 (36)	32 (11)	111 (40)	0 (0)	281
1960	0 (0)	31 (10)	22 (7)	99 (34)	38 (13)	105 (36)	0 (0)	295
1961	0 (0)	27 (3)	43 (5)	201 (21)	23 (2)	640 (68)	4 (0.4)	939
1962	6 (0.5)	195 (15)	66 (5)	456 (36)	1 (0.1)	535 (42)	2 (0.2)	1261
1963	0 (0)	283 (22)	127 (10)	506 (39)	14 (1)	370 (28)	3 (0.2)	1303
1964	5 (0.3)	163 (11)	111 (8)	510 (35)	37 (3)	624 (42)	27 (2)	1477
1965	4 (0.2)	262 (15)	211 (12)	588 (33)	54 (3)	659 (37)	11 (0.6)	1789
1966	10 (0.3)	279 (9)	267 (9)	1206 (39)	37 (1)	1300 (42)	2 (0)	3101
1967	28 (0.9)	388 (12)	273 (8)	1192 (36)	17 (0.5)	1340 (41)	29 (0.9)	3267
1968	117 (3)	595 (17)	188 (5)	1069 (30)	52 (1)	1520 (43)	39 (1)	3560
1969	265 (6)	820 (18)	427 (9)	1446 (32)	50 (1)	1500 (33)	0 (0)	4508
1970	107 (3)	615 (16)	302 (8)	1180 (31)	72 (2)	1528 (40)	9 (0.2)	3813
1971	182 (5)	560 (16)	416 (12)	1071 (30)	10 (0.3)	1361 (38)	0 (0)	3600
1972	88 (10)	247 (28)	147 (17)	259 (30)	4 (0.5)	131 (0.5)	0 (0)	876
1973	137 (5)	458 (15)	179 (6)	1273 (43)	62 (2)	856 (30)	0 (0)	2965
1974	104 (14)	156 (21)	49 (7)	238 (33)	5 (0.7)	173 (24)	3 (0.4)	728

(1) RIVER MILE

indication that the Ringold area is increasing in importance. At mile 393, an area of deep water spawning, there was a decrease in the relative numbers of salmon observed in both 1974 and

1972. Survey conditions were poor during these years, and the low percentages may be more indicative of the reduced visibility to the river bottom than to a lower rate of use of this area for spawning.

• EFFECTS OF MODIFICATIONS OF AQUATIC ECOSYSTEMS

This program is directed toward the measurement of stress in aquatic organisms and systems. Current studies are concerned with the determining of the combined effects of acute external radiation and temperature on growth and survival of trout, and the determination of basic limnological characteristics of a small desert stream at Rattlesnake Springs.

Rainbow trout were exposed to a range of temperatures and ^{60}Co gamma irradiation, including lethal or near lethal levels of each stressor to determine their interaction. Rate of breakdown of plant organic material in relation to invertebrate detritivores and season of the year were measured.

Effect of Temperature and Acute External Radiation on Trout

C. S. Abernethy and D. G. Watson

As part of a long-range study on the effects of multiple stresses on the different life stages of rainbow trout, 3 in. juveniles were exposed to four radiation doses at 13°, 16°, and 22°C acclimations. Tests at 7° and 10°C are presently in progress.

The trout were cold-branded with liquid nitrogen, acclimated at a rate of 1°C per day, and held at test temperature for 10 days prior to exposure. All experimental fish were reared together until time of exposure. The fish were then segregated by mark, weighed (total lot weight), and exposed to acute radiation doses of 250, 500, 1000, and 2000 R provided by a cobalt-60 source. Dose level was checked by tagging several fish with LiF thermoluminescent dosimeters and allowing them to swim at random with the test group during exposure. These tagged fish were removed after the exposure. Controls were sham exposed and handled in the same manner as the experimental groups.

All fish were held for an 8-week post-irradiation period. All four exposure groups and the controls were combined in the same test trough in the 13° and 22°C tests. Tests were run in duplicate. It was necessary to reduce group size from 50 to 30, and ultimately to 25 in order to accommodate all five groups in one trough.

Mortalities and temperatures were monitored daily. Temperatures were held to within $\pm 0.5^\circ\text{C}$ of the test temperature throughout the experiment. At 2-week intervals the groups were segregated by mark and total lot weights were taken. Feed schedules were adjusted to compensate for growth and mortalities. Termination of the test after the eighth week included total lot weights and individual length measurements of all fish. Lengths were used to determine length-weight relationship (condition factor) as an indication of vitality.

Preliminary analysis showed reduced mortality at the lower temperatures. The lower radiation groups (250 and 500 R) survived as well as the controls in all tests. At the

1000 R level, mortalities were slightly higher than the controls in the 13° and 22°C tests. The 2000 R groups suffered heavy mortalities in the 22°C test and moderate mortalities at 13° and 16°C (Table 38). Mortalities began to occur with regularity 10 days after exposure. In the 22°C test, the mortality increased rapidly until only two fish of 50 remained. In the 13° and 16°C tests, the majority of the mortalities occurred during the fourth and fifth weeks, but continued at a lower rate until termination of the test.

Growth in the 2000 R groups was significantly less than in the controls. Weight gains in the 13° and 16°C tests were much greater than in the 22°C test. The difference was probably due to increased catabolism and decreased appetite at 22°C. The growth data indicated that the lower-level groups (250 and 500 R) were equal to the controls, but at 1000 R growth was suppressed (Table 39). The condition factor of all 2000 R groups was lower than the controls. Although the weights of the 1000 R groups were less, the condition factor was comparable (Table 40).

It appears that temperature plays two very important roles in the growth and survival of the test fish. Its effect on metabolism and food intake causes a decrease in growth at 22°C. Although adequate food was presented, the fish did not grow as well as the 13° or 16°C test fish. The upper test temperature (22°C) is above the optimum and near the lethal thermal limit for rainbow trout. The

decreased mortality of all groups associated with lower temperatures appears to be partly the result of reduced challenge by pathogenic organisms. More pathogenic species are found in warmer water and bacterial numbers increase more rapidly, making the challenge to test fish more severe. The reduced vitality caused by the radiation exposure leaves the fish more vulnerable to attack.

Ecological Studies at Rattlesnake Springs

C. E. Cushing, J. M. Gurtisen and A. J. Scott

Most low-order streams bordered by deciduous trees and shrubs receive their dominant input of primary production in the form of leaves from the terrestrial plants. Primary production within the stream is usually low as a result of shading and these streams characteristically have a gross photosynthesis to respiration ratio of less than one, indicating heterotrophic conditions. Rattlesnake Springs is unusual in that within its relatively short length of about 5 km, it has open reaches where photosynthesis exceeds respiration and shaded sections where the reverse is true.

In the latter section, studies have been initiated to determine the fate of the allochthonous input of the peach leaf willow, *Salix amygdaloides*. These studies involve the placing into the stream of pre-weighed, air-dried leaves collected at abscission and bound loosely to

TABLE 38. Percent Survival of 2000 R Groups.

TEST TEMP	GROUP SIZE	WEEK							
		1	2	3	4	5	6	7	8
13°	30	97	97	93	93	90	80	73	67
13°	30	100	97	90	83	70	67	63	63
16°	50	100	94	84	64	54	52	48	44
16°	50	100	98	90	84	80	80	76	76
22°	25	96	8	0	0	0	0	0	0
22°	25	96	36	8	8	8	8	8	8

TABLE 39. Average Individual Weight Gains (% of Initial Weight) at Termination of Test.

TEMPERATURE	GROUP SIZE	CONTROL	250R	500R	1000R	2000R
13 ⁰	30	324	314	288	264	93
13 ⁰	30	311	323	304	271	113
16 ⁰	50	323	292	--	--	--
16 ⁰	50	327	--	308	--	--
16 ⁰	50	229	--	--	253	--
16 ⁰	50	388	--	--	--	145
16 ⁰	50	323	297	--	--	--
16 ⁰	50	312	--	303	--	--
16 ⁰	50	340	--	--	260	--
16 ⁰	50	375	--	--	--	178
22 ⁰	25	191	199	187	143	NO SURVIVORS
22 ⁰	25	179	182	180	140	120

TABLE 40. Condition Factors $\left(\frac{\text{Weight} \times 10^5 \text{ (grams)}}{\text{Length}^3 \text{ (mm)}}\right)$ of Survivors at Termination of Test.

	CONTROL	250R	500R	1000R	2000R
13 ⁰	1.44	1.44	1.42	1.41	1.30
13 ⁰	1.47	1.48	1.42	1.45	1.26
16 ⁰	1.41	1.35	--	--	--
16 ⁰	1.33	--	1.41	--	--
16 ⁰	1.36	--	--	1.33	--
16 ⁰	1.50	--	--	--	1.24
16 ⁰	1.36	1.37	--	--	--
16 ⁰	1.35	--	1.32	--	--
16 ⁰	1.37	--	--	1.26	--
16 ⁰	1.42	--	--	--	1.29
22 ⁰	1.62	1.60	1.56	1.52	NO SURVIVORS
22 ⁰	1.55	1.60	1.55	1.44	1.45

bricks to simulate the natural accumulation of leaves within the stream. Replicate packs are removed at 24-hr, and 2-, 6-, and 10-week intervals. The 24-hr sample provides an indication of the loss in weight of the leaves due to chemical leaching. The 2-, 6-, and 10-week samples are collected, the invertebrates separated, counted, and weighed, and the remaining leaf material weighed. These data provide an estimate of the rate of breakdown of the leaf packs by the combined effects of physical abrasion and insect processing and the colonization of the packs by invertebrates. Three experiments have been completed: one in spring of 1973, one in spring of 1974, and one in late summer and fall of 1974.

Table 41 presents the data from these experiments. The 24-hr leaching loss was similar in all three experiments, indicating that this loss is constant and not related to seasonal differences in other parameters. The percent of the leaf pack remaining after 2 weeks was also comparable. Leaf packs placed in the stream in March are colonized by greater numbers of organisms. This results in a more rapid processing and loss of the leaves, especially after the second week, as shown by the percent loss of dry weight for the two March experiments as compared to the August experiment. Greater numbers of chironomid larvae, the dominant organisms in the March experiments,

TABLE 41. Data from Three Leaf Pack Experiments at Rattlesnake Springs; Each Value is an Average of Three Replicates.

	% dry wt remaining	dry wt leaf packs, g	organisms per pack	dry wt organisms per pack, mg	organisms per g dry leaf	dry wt (mg) organisms per g dry wt leaf
3-6-73						
24 hr	82					
2 wk ¹	65	3.22	586	10.87	182.5	3.40
6 wk	13	0.60	176	17.25	307.3	29.62
10 wk	0	0	0	0	0	0
3-14-74						
24 hr	74					
2 wk	73	3.70	148	31.06	40.0	8.39
6 wk	31	1.54	55	4.76	35.5	3.09
10 wk	12	0.48	13	7.82	27.1	16.29
8-15-74						
24 hr	84					
2 wk	75	3.76	11	9.12	2.9	2.41
6 wk	49	2.47	13	16.38	5.7	7.25
10 wk ¹	38	1.90	4	20.35	1.9	11.24

¹average of two replicates.

Note: The dominant organisms, greater than 95% in numbers, in the two March experiments were chironomid larvae. In the August experiment, total numbers were low, but more evenly distributed among chironomids, simuliids, caddisflies, scuds, and damselflies; the latter, because of their large size, greatly influenced the weights of these samples.

are present in spring. The data in the last three columns, which have factored in the weight of the organisms, are biased for the August experiment. The presence of a few large damselfly larvae increased the weight disproportionately compared to the two March experiments where small chironomid and simuliid larvae dominated. In general, the leaf packs appear to be able to support an increased biomass of organisms per gram dry weight of leaf as the residence time of the pack increases. This may be related to the fact that some stream organisms ingest leaves to obtain nourishment from microorganisms (fungi and bacteria) which colonize the leaves rather than the leaf material itself.

The leaves placed in the stream in March 1973 completely disappeared in 10 weeks. This did not occur in either of the other experiments, and no explanation can be given for this difference other than the presence of greater numbers of organisms. Water temperatures in March 1973 were similar to those in March 1974 and only slightly lower than the August experiment.

These experiments represent initial efforts at characterizing the fauna of Rattlesnake Springs in terms of their "functional" as compared to their "trophic" components, and will be used in conjunction with proposed comparative stream studies to be carried out at several sites in the United States.

Radionuclide Cycling by Periphyton

C. E. Cushing, J. M. Thomas, and J. M. Gurtisen

Experiments were conducted in the microcosm described by Cushing and Rose (1970) this past year to investigate the cycling of ^{137}Cs by Columbia River periphyton and to compare results with similar studies of ^{65}Zn kinetics. Experimental manipulations included uptake and retention studies of living and killed communities and the addition of a second spike after quasi-equilibrium had been attained following the initial spike. Figure 19A illustrates the data obtained from ^{137}Cs experiments and Figure 19B shows the ^{65}Zn results.

FIGURE 19. A) Uptake and Retention of ^{137}Cs by Living and Killed Periphyton. B) Uptake and Retention of ^{65}Zn by Living Periphyton.

Columbia River periphyton communities attained quasi-equilibrium from an initial ambient concentration of $0.4 \text{ nCi } ^{137}\text{Cs}/\text{ml}$ in 100 hr or less as compared to about 150 hr for the same concentration of ^{65}Zn (Figure 19A and 19B). ^{137}Cs approaches equilibrium more rapidly than ^{65}Zn which usually does not exhibit as definite a plateau. The ordinates in Figure 19 are in counts per minute and seemingly show that about twice as much ^{65}Zn is accumulated than ^{137}Cs . However, we believe this is an artifact related to the counting efficiency of the detector system. Different proportions of their total energies are emitted as gamma radiation which is detectable in our system.

Periphyton showed a greater affinity for ^{65}Zn than ^{137}Cs . An effective half-life of from 70 to 170 hr was found for ^{137}Cs , whereas the half-life of ^{65}Zn exceeds 200 hr. At this time, we suspect that the uptake and retention kinetics are partially related to the different electrostatic charges of the two elements in that

the divalent cations generally possess stronger binding properties than monovalent ones.

Both formalin-killed and living communities took up similar amounts of ^{137}Cs (Figure 19A), the same as we have previously reported for ^{65}Zn (Cushing and Rose 1970). This indicates that adsorption probably plays a dominant role in the uptake of ^{137}Cs , a nonessential element for algal growth.

The upper curves in Figures 19A and 19B illustrate the response of periphyton communities to a second spike after quasi-equilibrium conditions had been reached. The second spike was equal to the first; however, ambient concentrations at the beginning of the second uptake phase were higher than the initial ambient concentration by about 65%. It is evident that the communities responded to the second spike by a rapid accumulation until near steady-state conditions were again established. The time to reach this point

was similar to the first for both isotopes. The higher absolute uptake of the communities for both isotopes in the double spike experiments was probably related to the higher biomass present when these experiments were run. Modeling and statistical evaluations of these data are in progress using the methodology presented in the 1973 Annual Report.

It is apparent from these experiments that ^{137}Cs is taken up faster and has less affinity to periphyton as compared to ^{65}Zn . Kinetics after a second spike were similar to the first for both isotopes suggesting that adsorption sites may not be saturated after one spike. The use of killed communities reflects the dominant role of adsorptive uptake for both isotopes.

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

A study concerned with the ecological behavior of Pu and Am is being carried out in a shallow waste pond (14 acres) which has existed and received Pu processing wastes for about 30 years. The system is ultra-eutrophic and the major organisms are macrophytes (mainly Potamogeton), algae (mainly Cladophora), benthic invertebrates (mainly dipteran and odonate larvae, hemipterans, amphipods, and gastropods) and goldfish. Sediments are the principal repository of Pu and Am containing about 390 pCi of $^{238,239,240}\text{Pu}(\Sigma\text{Pu})/\text{g}$ (dry) and about 83 pCi of $^{241}\text{Am}/\text{g}$. Pond water had much lower concentrations of ΣPu (0.01 pCi/l) and ^{241}Am (1.1 pCi/l). In the biota the principal concentrator of Pu and Am is decomposing algal material, the major feeding substrate for the system, containing about 2 nCi of $\Sigma\text{Pu}/\text{g}$ and 250 pCi of $^{241}\text{Am}/\text{g}$. In spite of these levels, the remaining biota have relatively lower concentrations, ranging from 0.5 to 154 pCi of $\Sigma\text{Pu}/\text{g}$ and 0.3 to 50 pCi of $^{241}\text{Am}/\text{g}$. Ratios of ^{238}Pu to $^{239,240}\text{Pu}$ in the biota are generally higher than that of the sediments (0.9), but lower than that of pond water (3.5). The same was true for ratios of $^{241}\text{Am}:\Sigma\text{Pu}$ in biota; most organisms had ratios exceeding those for sediments, but considerably less than those for water.

The Ecological Behavior of Plutonium
and Americium in a Freshwater Eco-
system: Phase I, Limnological Char-
acterization and Isotopic Distribution
R. M. Emery, D. C. Klopfer and
W. C. Weimer**

A study of the ecological behavior of plutonium (Pu) and americium (Am) in a Pu processing waste pond has been underway since July 1973. This shallow 14-acre pond has existed since 1944 and about 8.1 kg of Pu is re-

ported to have been discharged into it by way of waste trenches. Only a small fraction of this amount (21 g) has been found in the pond's sediments (down to 10 cm), water, and biota, which indicates possibilities that Pu in waste effluents has been deposited in the receiving water trenches or leached downward to depths below 10 cm in the pond's basin. The water inflow rate to the pond is about $10 \text{ m}^3/\text{min}$, and more than 95% of this inflow leaves the pond via percolation as there are no surface outflows. Evaporation from the pond is negligible. Percolation rates vary across the pond bottom with the outer edges being more permeable than the central basin.

This pond is an ultra-eutrophic system with most of the plant nutrients

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** Radiological Sciences Department

being supplied by laundry waste-waters. Enrichment from this source supports massive populations of macrophytes (mainly *Potamogeton*) and algae (mainly *Cladophora* and *Tetraspora*). The pond's food web is simple with algae, macrophytes, and, to a large extent detritus supplying the autochthonous carbon base. Predation is relatively minor in this system with dragonfly larvae (mainly *Aeschna* and *Libellula*) being the major aquatic predators. Nonpredaceous invertebrates and goldfish (*Carassius*) are major omnivorous components. Waterfowl and other birds provide some food web interaction and may serve as biological vectors for removal of Pu and Am from the pond ecosystem.

In the physical environs Pu and Am are concentrated principally in the sediments. The mean concentration of ^{238}Pu is 194 pCi/g dry weight; for $^{239,240}\text{Pu}$, 195 pCi/g dry weight; and for ^{241}Am , 83 pCi/g dry weight. The ratio of ^{238}Pu to $^{239,240}\text{Pu}$ in the sediments is about 1.0, while the ratio of ^{241}Am to ΣPu ($\Sigma\text{Pu} = ^{238}\text{Pu} + ^{239,240}\text{Pu}$) is 0.23. Only about 9% of the ΣPu and 7% of ^{241}Am in the sediments appear to be available for food web concentration. The remaining fractions of Pu and Am seem to be tightly bound to sediment particles and could only be transported ecologically in particulate form. Levels of ΣPu and ^{241}Am in interstitial water are low (between 0.5 and 13 pCi/g, expressed per gram dry weight of sediment) and appear to exist mainly in either cationic or nonionic forms. Concentrations of ΣPu in pond water are very low (about 0.01 pCi/l) and exist mainly in particulate form. Levels of ^{241}Am in the water are about 1.1 pCi/l, mainly associated with particulates. The ratio of ^{238}Pu to $^{239,240}\text{Pu}$ in the water is about 3.5, while the ratio of ^{241}Am to ΣPu is about 120.

Algal floc (decomposing algal cells) is the major concentrator of Pu and Am in the pond (about 2 nCi of ΣPu /g dry weight and 258 pCi of ^{241}Am /g dry weight, Figures 20 and 21). Watercress (*Rorippa*) has relatively high concentrations of ΣPu and ^{241}Am (532 and 125 pCi/g, respectively) ranking second to algal floc and considerably higher than any of the other biota. Dragonfly larvae, the pond's major aquatic predator, have higher concentrations of Pu and

FIGURE 20. Comparative Concentrations of ^{238}Pu and $^{239,240}\text{Pu}$ (combined ΣPu) in U Pond Biota Relative to that of the Sediments.

FIGURE 21. Comparative Concentrations of ^{241}Am in U Pond Biota Relative to that of Sediments.

Am than any of their prey. These levels are 38 to 160 pCi of Σ Pu/g and 17 to 94 pCi of ^{241}Am /g dry weight. Because of their close association with algae and sediments it is not reasonable to conclude that these higher levels of Pu and Am are entirely the result of food chain concentration.

Ratios of ^{238}Pu to $^{239,240}\text{Pu}$ in the pond biota are generally higher than those of the sediments (Figure 22). It appears that most of the pond organisms, flora and fauna alike, are selecting ^{238}Pu slightly over $^{239,240}\text{Pu}$. Plant material having the highest accumulation of Pu had ratios ranging from 1.3 to 1.6. Only goldfish muscle tissue had a ratio favoring $^{239,240}\text{Pu}$. The ratio of ^{238}Pu to $^{239,240}\text{Pu}$ in the pond water is 3.5, which may be reflected in the relatively high ratios of biota (Figure 22). Algal floc and emerging chironomids were the only biological components with ratios of ^{241}Am to Σ Pu lower than that of the sediments (0.13:1, Figure 23). Watercress had a ^{241}Am to Σ Pu ratio equal to that of the sediments (0.23), while the remaining biota sampled had ^{241}Am to Σ Pu ratios ranging from 0.4 to 2.0.

FIGURE 22. Ratios of ^{238}Pu to $^{239,240}\text{Pu}$ in Pond Biota Relative to Those of the Water and Sediments.

FIGURE 23. Ratios of ^{241}Am to Σ Pu in Pond Biota Relative to those of the Sediment and Water (Water ratio is 120:1 and does not appear in this figure).

The biological availability of ^{238}Pu appeared to be greater than that of $^{239,240}\text{Pu}$ since most of the pond organisms sampled accumulated more ^{238}Pu than $^{239,240}\text{Pu}$ on an activity basis (i.e., most ^{238}Pu to $^{239,240}\text{Pu}$ ratios exceeded 1.0). This may be somewhat of a reflection of the ^{238}Pu to $^{239,240}\text{Pu}$ ratios found in the pond water, which were about 3.5.

Although goldfish remain in the pond longer than any of the other fauna and feed most heavily on plants, algae, and organic debris, they concentrate relatively small amounts of Σ Pu and ^{241}Am (about 20 and 12 pCi/g, respectively). In situ experiments indicate that many goldfish reach a level of Σ Pu of about 20 pCi/g within a

few days after which they may remain active in the pond, possibly for many months, without further accumulation.

The Ecological Behavior of Plutonium and Americium in a Freshwater Ecosystem: Phase II, Implications of Differences in Transuranic Isotopic Ratios

R. M. Emery and T. R. Garland

The ecological behavior of Pu and Am in a freshwater processing wastewater pond has been studied since mid-1973 to characterize the pond's limnology and define the isotopic distributions in the ecosystem. Results from this study show that the history of transuranics discharged to the pond has created a complex combination of source terms and isotopic ratios. Because of this complex and cryptic discharge history special attention has been given to the relative quantities of actinides, and the ratios in which they occur, in the pond. Since the source terms and chemical forms of actinides entering and in the pond have not been clearly defined, it is concluded that a material balance for Pu and Am between levels found in the pond and those entering and leaving the pond cannot be developed.

Very large quantities of ^{238}U , approximately 1400 kg, have been discharged into waste trenches leading to the pond. These high levels of U, along with potential quantities of other isotopes such as ^{232}Th and ^{228}Th , become a matter of concern when attempting to interpret the ecological behavior of Pu and Am in this ecosystem, because of the potential toxicity of these elements.

With regard to transuranic isotopes, some unusually high ratios occur in the pond. Although weapons grade Pu processing operations should have produced wastes containing ratios of ^{238}Pu to $^{239,240}\text{Pu}$ of about 0.02, these ratios in the pond sediment are much higher (about 1.0) indicating the contribution of ^{238}Pu production processing after the early 1960's. Of even greater interest are the ratios of ^{238}Pu to $^{239,240}\text{Pu}$ and ^{241}Am to $^{239,240}\text{Pu}$ occurring in the pond biota, which are significantly higher than those in the sediments

(Figures 24 and 25). A common source of "available" Pu and Am for pond biota is suggested by the rapid establishment of ^{238}Pu to $^{239,240}\text{Pu}$, and ^{241}Am to $^{239,240}\text{Pu}$ ratios in goldfish (*Carassius*) which were experimentally introduced into the pond. Within 2 weeks after these fish had been introduced into the pond they had accumulated transuranic ratios which were similar to those of other pond biota and dissimilar to those of the pond sediment. Ratios of ^{241}Am to ^{238}Pu in the pond biota are significantly different from those of the sediments, but to a lesser degree than ratios of ^{238}Pu to $^{239,240}\text{Pu}$ and ^{241}Am to $^{239,240}\text{Pu}$ (Figure 26). In particular, ratios of ^{241}Am to ^{238}Pu in plant material were different from those of sediments, invertebrates, and goldfish in that they fall either below or above the means and medians for the other four categories of the pond ecosystem.

Sampling of the pond sediments done frequently in the same location

FIGURE 24. Ratios of ^{238}Pu : $^{239,240}\text{Pu}$ in U Pond Sediments and Biota. This Ratio is 1.91 in Interstitial Water and 3.5 in Water (n=3 in both cases).

FIGURE 25. Ratios of $^{241}\text{Am}:^{239,240}\text{Pu}$ in U Pond Sediments and Biota. This Ratio is 0.67 in Interstitial Water and 540 in Water (n=3 in both cases).

shows a substantial range of ^{238}Pu and $^{239,240}\text{Pu}$ concentrations. This may be due to a wide range in Pu particle sizes existing in the pond sediments.

Considering the rapid exchange rate of water in the pond (about 40 hr) and implications of the transuranic ratios occurring in the pond's

FIGURE 26. Ratios of $^{241}\text{Am}:^{238}\text{Pu}$ in U Pond Sediments and Biota. This Ratio is 0.40 in Interstitial Water and 154 in Water (n=3 in both cases).

ecosystem, it is possible that the source of Pu and Am which is "available" to the biota is not the pond sediments, but the sediments of a trench which supplies the pond with wastewater from Pu processing operations.

For Further Information on Transuranium Element Behavior Refer to:

- 1) "The Potential for Plutonium Complexation in Soil and Uptake by Plants," Wildung et al., pp. 20-35.
- 2) "Suspended Particle Interactions," Klepper, Craig, Cataldo and Vaughan, pp. 49-55.
- 3) "Weathering and Aging of Transuranics in Soils," Rickard et al., pp. 191-203.
- 4) "Plutonium Studies on the Nevada Test Site," Eberhardt et al., pp. 6-7.
- 5) "Biogeochemistry of Plutonium and Americium in Marine Systems," Robertson et al., pp. 148-150.

• EFFECTS OF TRITIUM ON AQUATIC SYSTEMS

In continuing studies on enhanced susceptibility to infection in fish following irradiation, antibody synthesis in response to the pathogen, Chondrococcus columnaris, was applied to investigate the effect of tritium irradiation (0, 1, 10, 100, and 1000 R during embryogenesis) on immune responsiveness in juvenile rainbow trout, Salmo gairdneri. Both serum agglutination assay and gel electrophoresis were performed following immunologic stimulation. Evidence is presented to indicate that three serum proteins were associated with antibody synthesis; and that these fractions were significantly reduced, or in the extreme case, eliminated in test fish irradiated at 10, 100, and 1000 R.

Suppression of the Primary Immune Response in Rainbow Trout, *Salmo gairdneri*, Sublethally Irradiated During Embryogenesis

J. A. Strand, M. P. Fujihara, and R. D. Burdett

Eggs of rainbow trout were spawned artificially in the laboratory, fertilized, and immediately immersed in 0, 0.01, 0.1, 1.0, and 10.0 $\mu\text{Ci/ml}$ tritium (biological grade) contaminated spring water (pathogen free). Rearing through 20 days of embryogenesis at $10.5 \pm 0.2^\circ\text{C}$ was facilitated within a recirculation drip incubation system of 150% capacity. Exposure of the embryos to 0, 0.01, 0.1, 1.0, and 10.0 $\mu\text{Ci/ml}$ tritium resulted in an estimated total dose of 0, 1, 10, 100, and 1000 R.

At 20 days (eyed stage) the embryos were transferred to a single-pass incubation system maintained on thermally controlled, $10.5 \pm 0.2^\circ\text{C}$, dechlorinated sanitary water. Hatching occurred at 28.5 ± 0.5 days.

At 14 days post hatch, the fry were transferred to 10 ft x 2 ft x

1 ft fiberglass rearing troughs also maintained on thermally controlled dechlorinated sanitary water. Rearing temperature was adjusted to $20.0 \pm 0.3^\circ\text{C}$ at a rate of $2.0 \pm 0.5^\circ\text{C}$ per week.

A final transfer of the fingerlings to permanent 10 ft x 4 ft x 3 ft concrete rearing ponds also maintained on thermally controlled, $20 \pm 0.5^\circ\text{C}$, dechlorinated sanitary water was effected 5 months post hatch. Rearing thereafter was in accordance with standard hatchery practice.

At 7 months post hatch, control and irradiated test fish were administered intraperitoneally 0.1 cc of a heat inactivated antigen (1.8×10^8 cells/ml, Chondrococcus columnaris) in 25% Freund's incomplete adjuvant. A 0.1 cc sham vaccination containing saline and 25% Freund's incomplete adjuvant was similarly administered to another group of control fish.

At 3 weeks post vaccination and at weekly intervals thereafter a standard tube agglutination test for the

specific antigen of vaccination was performed on the serum from 20 fish from each of the control and irradiated test groups.

Selected results of serum agglutination tests to assess the potential for suppressed immune competence in juvenile rainbow trout sublethally exposed to 0, 1, 10, 100, and 1000 R of tritium irradiation during embryogenesis are reported in Table 42.

As in previous experiments, the percentages of fish with agglutinins and the magnitude of titers in both

control and irradiated test groups increased following immunologic stimulation, Figure 27. However, control and 1 R treatment groups demonstrated higher agglutinin titers suggesting a suppression of immune competence at the 10, 100, and 1000 R treatment levels. A chi-square test of independence verified this finding, Table 43.

To determine the potential for qualitative and quantitative changes in serum components which would account for reduced immune competence in irradiated fish, acrylamide gel

TABLE 42. Serum Agglutinins to *Chondrococcus columnaris* in Juvenile Rainbow Trout, *Salmo gairdneri* Exposed to 0, 1, 10, 100, and 1000 R Tritium Irradiation During Embryogenesis (Pond I, Compartment A-E).

Treatment	Weeks Post Vaccination	No. Fish	% Positive Titers	Range Positive Titers	Mean Titers
Control	0	6	0	-	0
	3	22	95	1:20 -40	1:28
	4	22	100	1:20 -80	1:46
	5	22	95	1:20 -640	1:196
	6	19	100	1:320-2560	1:960
	7	21	100	1:160-1280	1:1074
1 R	0	10	0	-	0
	3	22	100	1:20 -40	1:29
	4	-	-	-	-
	5	20	100	1:20 -320	1:117
	6	-	-	-	-
	7	20	100	1:160-2560	1:1218
10 R	0	7	0	-	0
	3	25	76	1:20 -40	1:22
	4	-	-	-	-
	5	24	100	1:20 -320	1:128
	6	-	-	-	-
	7	22	100	1:320-1280	1:720
100 R	0	8	0	-	0
	3	24	63	1:20 -40	1:18
	4	-	-	-	-
	5	24	100	1:20 -640	1:132
	6	-	-	-	-
	7	21	100	1:160-1280	1:528
1000 R	0	6	0	-	0
	3	24	88	1:20 -40	1:20
	4	-	-	-	-
	5	21	100	1:20 -320	1:80
	6	22	100	1:80 -640	1:436
	7	21	100	1:80 -640	1:518

FIGURE 27. The Primary Immune Response to a Single Intraperitoneal Injection of Heat Inactivated *Chondrococcus columnaris* Antigen and 25% Freund's Incomplete Adjuvant in Control and 1, 10, 100, and 1000 R Treatment Groups. Each Datum on the Five Respective Curves Represents the Mean Value of 20 Determinations.

TABLE 43. Chi-Square Test of Independence on Titer Values from Control and Tritium Irradiated Rainbow Trout.

Treatment	Weeks Post Vaccination	D.F.	χ^2	Level
1 R	3	2	1.22	NS
	5	3	19.00	b
	7	2	0.80	NS
10 R	3	2	3.85	NS
	5	3	14.23	b
	7	2	10.74	b
100 R	3	2	8.56	a
	5	3	10.66	a
	7	2	17.75	b
1000 R	3	2	3.26	NS
	5	3	9.40	a
	7	2	21.04	b

NS - Nonsignificant chi-square at 0.05 level indicating distribution of titer values was independent of treatment.

a - Significant chi-square at 0.05 level indicating the distribution of titer values was dependent of treatment.

b - Significant chi-square at 0.01 level.

electrophoresis was performed. Additionally, total serum protein analyses were conducted.

Preliminary results with 5% acrylamide separation gel demonstrated 22 bands as reported in Figure 28. Each band is numbered following decreasing mobility. The most rapidly migrating band, No. 1, was found to be slightly more mobile than human serum albumin. All bands were generally well delimited. Mobilities did not change, but large dissimilarities in staining intensity were observed from one animal to another. A lowered total serum protein level did not influence the relative position or the number of bands and generally only affected stain intensity.

Inconstant bands were encountered in certain patterns. The main variations occurred in bands 20, 21, and 22. These bands were not observed in nonimmunized fish, either irradiated

FIGURE 28. Electrophoretic Pattern of Rainbow Trout Serum Proteins Using 5% Acrylamide Separation Gel. Bands are Numbered Following Decreasing Mobility. Inconstant Bands are Indicated by Dashed Lines. The Pattern Presented is Representative for Band Thickness and Relative Mobility, but Does Not Account Precisely for Relative Concentration.

or control; however, the presence of these bands was found to consistently coincide with detection of antibody titers of at least 1:40 in nearly all immunized test fish, either irradiated or control.

It is presumptive that bands 20, 21, and 22 represented specific humoral antibody to the immunogen, Chondrococcus columnaris. It follows that in the greater proportion of fish irradiated at 10, 100, and 1000 R, bands 20, 21, and 22 were sig-

nificantly reduced, or in the extreme case, totally absent, and undoubtedly accounted for the lowered antibody titers demonstrated in these test fish.

Total serum protein levels of all test fish throughout the test period remained the same; however, the Biuret technique here applied may not be sufficiently sensitive to detect the changes observed in bands 20, 21, and 22 by gel electrophoresis.

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MARINE SCIENCES

- **NONNUCLEAR EFFLUENTS—FATE AND EFFECTS OF OIL AND OIL COMPOUNDS ON MARINE COASTAL ECOSYSTEMS**
- **THERMAL EFFECTS OF EFFLUENT DISCHARGES FROM NUCLEAR POWER PLANTS TO COASTAL WATERS**
- **BIOLOGICAL AVAILABILITY AND EFFECTS OF EFFLUENTS FROM COASTAL NUCLEAR POWER PLANTS**
- **EFFECTS OF LOW LEVEL CHRONIC IRRADIATION ON EMBRYONIC DEVELOPMENT OF MARINE FISH AND INVERTEBRATES**
- **PHYSICAL AND RADIOLOGICAL CHEMISTRY OF OCEAN SOLUTIONS**
- **GEOCHEMICAL OCEAN SECTIONS STUDY (GEOSECS)**
- **IN-SITU POLLUTANT STUDY IN THE NEW YORK BIGHT**
- **BIOGEOCHEMISTRY OF PLUTONIUM AND AMERICIUM IN MARINE SYSTEMS**
- **^{55}Fe IN HUMAN POPULATIONS OF THE WORLD**

MARINE SCIENCES

MARINE RESEARCH LABORATORY (MRL)

B. E. Vaughan

The laboratory built by Battelle-Northwest, and located at Sequim, Washington, is a modern marine research laboratory staffed and equipped for research in the marine sciences (Figure 29). The laboratory is situated at the mouth of Sequim Bay on the straits of Juan de Fuca, about 15 miles east of Port Angeles, 1-1/2 hr by road from Seattle, and 80 min by plane from Richland. The present laboratory building was completed in 1972 and consists of 6,400 sq ft of wet laboratory, chemistry laboratory, bioenergetics laboratory, offices, shop and storage. It is presently staffed with 10 scientists, specialists and technicians. On July 1, 1974, MRL was dedicated as part of the consolidated Pacific Northwest Laboratory under terms of AEC contract AT(45-1) 1830.

The laboratory is supplied with sea water from Sequim Bay through an all PVC system. To ensure a continuous supply of sea water to experimental tanks, a second pump and diesel powered electric generator are available and automatically actuated if there is any failure in the primary system or electrical supply. A 16,000 gal storage tank provides approximately 12 hr of sea water in case of failure by both pump systems. Ambient sea water can be provided at 200 gal/min, and up to 20 gal/min of sea water heated to 40°C can be supplied through a gas-fired heat exchanger.

Untreated fresh water is supplied from an artesian well onsite and is backed up by a 15,000 gal storage tank.

A unique feature of the laboratory is the incorporation of

four separate drainage systems in the basic design. The laboratory is equipped with a standard domestic sewage system, a return system for clean sea water, and two systems for the treatment of polluted experimental waters. One of the latter systems is for oil study, and the second is for nonpetroleum based contaminants. A high-flow cleanup system is installed that makes possible the removal of oil from experimental effluents to less than 1 ppm in the discharged water.

A variety of experimental systems for exposure of organisms to pollutants are available. For example, a cascading arrangement of flow-through tanks provides for the simultaneous exposure of four replicated series of conditions after initial treatment is applied. The arrays, involving twenty exposure chambers with eight pretreatment vessels, allow statistical verification of experimental conditions applied. The exposure chambers are of a size that will allow exposure in not only single species and acute and chronic bioassays, but are also capable of supporting selected marine communities over long periods of time. Several other proven types of continuous flow exposure systems are also in operation.

In addition to a wide range of sampling gear and analytical equipment, there are two mobile aquatic laboratories which can operate as self-sufficient outstations of the main laboratory, and allow comparative or additional studies to be conducted in the same time frame at other sites. Two boats are presently available: a 26-ft craft and a Boston Whaler. Larger vessels for offshore work are available.

FIGURE 29. Marine Research Laboratory, Sequim,
Washington

• **NONNUCLEAR EFFLUENTS: FATE AND EFFECTS OF OIL AND
OIL COMPOUNDS ON MARINE COASTAL ECOSYSTEMS**

The use of petroleum fuels in steam electric generation contributes to the demand for production, transport, and refining of oil with consequent discharges into the marine environment. This year's research has examined the development of intertidal communities on oil-treated and control substrate units to determine if measurable effects of petroleum contamination are reflected in colony development. Past effort has established threshold values for acute mortality of key species, valued according to several specific criteria. Little past effort has been directed to the measurement of responses by ecologically related systems such as the organisms comprising a community. One may expect that community responses and population responses will be the more important indicators of chronic pollution, by oil and by other pollutants.

In measuring petroleum effects, two formidable technical problems arise: first, major fluctuations in the multi-phase immiscible system of oil, air, and water influence mortality and other biological end-points that are introduced by the influent flow rates and by the energy expenditure and manner of mixing; and second, the appropriate partitioning of the truly toxic fractions of oil. "Oil" is composite of thousands of compounds widely varying in volatility and solubility, and the toxic compounds represent only a small fraction of the total mixture. Research in these two areas has been aimed at controlling major sources of variability in a realistic way. Preliminary analysis of test data indicates a dramatic influence of turbulence on mortality and the need for time-stratified sampling to adequately characterize test media. Acquired data will be useful in determining optimum long-term exposure concentrations for intertidal colonies.

Effects of Petroleum on Marine Intertidal Communities

J. R. Vanderhorst, C. I. Gibson,
L. J. Moore, and P. Wilkinson.

Use of petroleum products for fuel in steam electric generating plants contributes to the demand for exploration, production, and transport of crude oils and petroleum fuels in the marine environment. Petroleum or petroleum products may enter the marine ecosystem as low-level chronic discharges from refining facilities or through accidental spillage during transport or offshore production. Marine intertidal communities, by virtue of their proximity to such activities, multi-tide level exposure opportunity, and relative confinement, are most vulnerable to the effects of discharged petroleum.

Unlike many single component and soluble contaminants, petroleum and its products consist of a complex of volatile, soluble, and insoluble compounds. Because of their subsequent distribution in the water column, they are difficult to quantify, even

in bioassay systems. Thus, our research over the past year at the MRL, Sequim, has followed three related approaches toward measuring the effects of petroleum in marine systems. First, artificial substrates were treated with Prudhoe Bay crude oil, and the influence of treatment to subsequent settling and growth of marine intertidal colonies in situ were determined. Second, the feasibility of delivering the soluble phase of crude petroleum to laboratory held communities was tested. Third, coonstripe shrimp (Pandalus danae), a predator normally associated with intertidal communities was exposed in acute bioassays. Mortality and oil in water concentrations were determined as a function of mixing.

Concrete construction blocks, 16 in. x 8 in. x 8 in. were used as colonizing substrate for in situ treatment with petroleum. To illustrate the complexity of the involved biological system, a sample list of species colonized from January 1 to April 9, 1974 is presented in Table 44.

TABLE 44. A Sample List of Organisms Colonized on Concrete Construction Blocks at Mean Low Tide in Sequim Bay.

<u>PLANTS</u>	<u>ANIMALS (CONTD)</u>
CLASS CHLOROPHYCEAE	<u>Ennoe oerstedii</u>
<u>Cladophora spp.</u>	<u>Platynereis bicanaliculata</u>
<u>Ulva spp.</u>	<u>Nereis (Neanthes) diversicolor</u>
<u>Derbesia sp.</u>	CLASS CRUSTACEA
CLASS HETEROGENERATA	<u>Idothea (Pentidotea) wosenesenskii</u>
<u>Laminaria andersonii</u>	<u>Ampithoe valida</u>
<u>Laminaria ephemera</u>	<u>Allorchestes angustus</u>
CLASS RHODOPHYCEAE	<u>Caprella laeviuscula</u>
<u>Rhodoptilum sp.</u>	<u>Ampithoe simulans</u>
<u>Polysiphonia sp.</u>	<u>Aoroides sp.</u>
<u>Porphyra sp.</u>	<u>Hyale frequens</u>
CLASS ANGIOSPERMAE	<u>Pontogenia inermis</u>
<u>Zostera marina</u>	<u>Chironesimus sp.</u>
<u>ANIMALS</u>	<u>Ampithoe spp.</u>
CLASS ENOPLA	<u>Melita oregonensis</u>
<u>Emplectonema gracile</u>	<u>Ampithoe lacertosa</u>
<u>Paranemertes peregrina</u>	<u>Aoroides colubiae</u>
CLASS POLYCHAETA	<u>Heptacarpus taylori</u>
<u>Nainereis dendritica</u>	<u>Pagurus granosimanus</u>
<u>Armandia brevis</u>	CLASS GASTROPODA
<u>Phyllodoce (Anaitides) maculata</u>	<u>Margarites spp.</u>
<u>Typosyllis sp.</u>	<u>Acmaea pelta</u>
	<u>Lacuna sp.</u>

To determine if the colonies, as established by placement of substrate in the intertidal zone, are influenced in a measurable way by contamination with petroleum, triplicate substrate units were treated by dipping them into Prudhoe Bay crude oil. After dipping, the blocks were allowed to drain and placed into the bay for colonization. The treatment is not unlike that which might be received by substrate in near-shore oil spillage. Three similar blocks not receiving the treatment served as controls. Results for primary indicator variables after 3 months of colonization are tabulated in Table 45. There are reasonably consistent differences in numbers of species, numbers of individuals, and wet weight biomass between treated and untreated substrates with apparent depressed values for each of the variables on treated units. Variability in the data, as indicated by the standard deviations, precludes attributing a specific response to the treatment. It may be concluded that with further analysis, the colonies will be units amenable to determine the effects of petroleum contamination. Investigations are being conducted on the use of smaller substrate units to increase the efficiency of data acquisition.

A laboratory system has been developed for the delivery of soluble phase of petroleum to measure long-term or chronic effects of low levels of soluble components. Problems

basic to delivery of the soluble phase are: 1) to bring the petroleum and sea water in close contact, and 2) to separate and discard insoluble residue. While such a procedure eliminates many potentially damaging components of petroleum, it offers the most promising avenue for quantitative description of biological response. This is so because a standard reproducible treatment maximizes the ability to study complex biological phenomena while minimizing the effort needed for analytical characterization.

For our studies, the intimate contact of sea water and petroleum was accomplished by passing the sea water and metered oil through a static mixer into a mixing chamber receiving vigorous agitation by electric stirring motors. Separation of soluble and insoluble phases was accomplished by flowing the mixture over a series of baffles arranged such that floating or insoluble oil passed through surface riser pipes into the laboratory waste treatment system, and water containing soluble components passed into exposure tanks through outlets placed low in the settling chambers.

The colonization of periphyton, as measured by chlorophyll *a* content on glass microscope slides, was used as the biological endpoint. Triplicate chlorophyll *a* measurements were made for each of T8 exposure tanks at 2-week intervals. At the end of the

TABLE 45. Primary Variables in Colonies Collected on Treated (Prudhoe Bay Crude Oil) versus Untreated Substrate Units.

ESTIMATOR	NUMBER OF SPECIES		NUMBER OF SPECIMENS		BIOMASS (wet-grams)	
	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED
n's	34	38	117	300	275	349
	22	32	106	212	326	375
	26	38	348	238	301	530
	—	—	—	—	—	—
MEANS	27	36	190	250	300	418
STD. DEV.	6	3	136	45	25	98

first 2-week increment there was an apparent but not statistically significant depression for chlorophyll *a* in the treated versus control tanks. In the subsequent 2-week increments, the levels of chlorophyll *a* were depressed to zero in both control and treated tanks. Difficulties were attributed to inadequate illumination.

Analysis for petroleum in the contactor discharge water during the experiment revealed that total measured oil was highly correlated ($P = 0.01$) to soluble saturate content and not correlated to soluble aromatic content. These findings are consistent with findings in earlier studies by Battelle using similar apparatus. A summary of petroleum component concentrations at the contactor discharge is presented in Table 46.

Total oil measurements in the exposure tanks indicated low and extremely variable concentrations. However, the concentrations in exposure tanks did have significant correlation

($P = 0.05$) to concentrations of total oil in the contactor discharge. This finding was different from results in earlier studies by Battelle using similar apparatus and experimental conditions.

The apparent discrepancy caused us to re-examine potential sources of variability in the contactor. Measured total oil at the contactor discharge did not have significant correlation to the volumetric concentration of petroleum introduced. The delivery of oil to the contactor was quite stable (4.41 ± 0.25 ml/min) whereas the delivery rate for water varied by 11 to 23% at four different locations within the apparatus. Water delivery rate can be stabilized by design modifications.

Potentially, an even greater source for system variability was that associated with the degree of agitation or mixing. Since the complex arrangement of water flows and mixing configuration in the contactor made monitoring the degree of agitation difficult, a series of simpler bioassays was undertaken to investigate the influence of turbulence.

Fuel oil spills have been shown to cause greater ecological damage than crude oil spills. Because of this, and the fact that only a low percentage of crude oil was solubilized in our flow-through apparatus, number 2 fuel oil was selected for three bioassays. Three replicated bioassays were completed to determine the influence of turbulence on solubilization and biological response. It is anticipated that additional bioassays will be required to adequately elucidate the problem. In each of the bioassays the coonstripe shrimp, *Pandalus danae*, was used as the test organism, and mortality served as the endpoint.

The shrimp were captured by small otter trawl from Sequim Bay and were fed freshly-cracked littleneck clams (*Protothaca staminea*) during pretest holding. Fiberglass aquaria (90 x 40 x 34 cm) receiving 2 l/min continuous flow of sea water were used in each experiment. Identical volumes of number 2 fuel (0, 5, 12.5, 25, 50, and 100 ml) were applied in each replicate in each experiment. Only the

TABLE 46. A Summary of Hydrocarbon Component Concentrations in Periphyton Bioassay at Contactor. (Concentrations in mg/l).

DATE 1974	TOTAL OIL	SOLUBLE AROMATICS	SOLUBLE SATURATES
3/11	1.09	0.50	0.26
3/12	1.00	0.89	0.31
3/13	2.04	0.90	0.47
3/14	1.53	1.00	0.38
3/15	1.53	0.49	0.43
3/18	0.47	0.32	0.18
3/19	1.03	0.99	0.34
3/20	0.94	0.95	0.31
3/20	0.96	0.85	0.30
3/21	0.98	1.04	0.42
3/25	1.21	0.88	0.37
3/26	1.03	0.99	0.40
3/27	1.41	1.06	0.46
3/28	1.24	0.92	0.33
3/29	1.51	0.90	0.37
4/1	0.92	0.56	0.36
4/3	1.93	1.20	0.57
4/5	1.83	0.90	0.41
4/8	1.87	0.47	0.39
4/10	1.12	1.10	0.53
4/12	1.96	0.81	0.38
4/15	1.60	0.79	0.37
4/22	0.77	0.63	0.44
MEAN	1.30	0.83	0.38
± 1 S. D.	0.43	0.23	0.08

method of introduction for oil and sea water differed for the separate experiments.

In the first bioassay, incoming sea water fell from a height of 20 cm to the surface of exposure tanks. Diameter of the seawater stream was 0.35 cm. Oil was introduced by pouring onto the surface of exposure tanks just beyond the radius of turbulence caused by the seawater stream. In the second bioassay, sea water was introduced through a 1/2-in. PVC pipe with the entry port 5 mm below water level in exposure tanks, and oil was introduced at 4 cm from the point of water entry. In the third experiment, oil and sea water were introduced through a static mixer with the delivery end of the mixer 1 cm above the water surface. In all experiments the point of introduction was at the opposite end of the exposure tank from the discharge port. Sampling for hydrocarbons was by fixed glass siphons placed in the exposure tanks before introduction of oil to prevent contamination.

Although data from these experiments are not completely analyzed, several generalizations can be made about them. Mortality of shrimp differed dramatically depending on the method of oil and sea water introduction. In the most highly mixed experiment both the volume and measured concentration of total oil correlate well to mortality ($r = 0.99$ for volume introduced and $r = 0.96$ for measured total oil). With respect to measured total oil standard deviations were from 66 to 79% of mean values when all samples were used to estimate oil content in the treatment. Much of the variability is removed by blocking out time as a source of variation. This finding is especially important for investigators sampling only once or a few times during the course of bioassay and is the basis for a paper now in preparation for the open literature. The data derived from the several bioassays are useful in determining appropriate sublethal concentrations to apply in chronic exposure of intertidal communities.

Characterization of Petroleum Hydrocarbons in the Marine Environment
R. M. Bean

As a potential contaminant of our environment, petroleum is a unique

material from a number of aspects. While often considered a single entity, petroleum and its refined products are actually composed of hundreds of thousands of hydrocarbon compounds having differing physical and chemical properties. Thus, the toxicity of "petroleum" is dependent upon the source of the crude oil, the type of manufacturing processes it has been exposed to, and the relative distribution of hydrocarbon compound types in the oil. In addition, the means of introduction of the oil into the marine environment can play a large role in determining both its physical state and its ultimate toxicity.

Previous petroleum programs (Vaughan, 1973) conducted by Battelle-Northwest have recognized the difficulties in defining an "exposure level," when oil was used as a toxicant. More recently, work sponsored by Battelle (Wolf and Strand, 1973) has shown the desirability of adequately characterizing the physical and chemical state of toxic materials released when oil is mixed with water. The importance of distinguishing between petroleum hydrocarbons which are suspended in droplets and those which are actually in solution has been recognized, and a procedure has been adapted for separating the soluble from the insoluble oil using a filtration technique (Templeton, 1974a, 1974b).

The experimental program, adapted for studies of the effects of petroleum hydrocarbons on marine community structures, has posed new and difficult questions concerning the physical state of the contaminant in the ecosystem under study. In addition, the biological work has increased the demand for more, rapid, and detailed information concerning the nature and concentration of hydrocarbons in water. To meet this rising need for analytical results, gas chromatographic and infrared spectrophotometric equipment has been installed at the Marine Research Laboratory, Sequim, Washington. The infrared instrument has been used extensively for routine monitoring of sea water for total oil, soluble oil, and to determine the relative proportions of aromatic and paraffinic hydrocarbons (Bean, et al. 1974). Infrared, however, gives little detailed information concerning the actual distribution of individual hydrocarbon

compounds in sea water. The gas chromatograph is the ideal instrument for use in such studies.

Figure 30 shows a gas chromatogram of a solvent extract of sea water containing appreciable quantities (greater than 10 ppm) of a petroleum product, No. 2 fuel oil. The separation column employed uses a silicone-based liquid, which permits separation of hydrocarbon compounds in the order of their boiling point. The use of this type of column in the gas chromatographic separation permits an estimate of the boiling range of the hydrocarbon sample, the total quantity of oil in the sample, and also provides a "fingerprint" against which to compare subsequent samples for qualitative changes in hydrocarbon distribution.

The gas chromatogram shown in Figure 30 also demonstrates the complexity of the petroleum sample. Although there are a few relatively sharp peaks in the chromatogram, there is also a major amount of material present as an unresolved envelope. Thus, in order to obtain information concerning the identity and distribution of hydrocarbon compound types, other techniques have been developed. The chromatogram in Figure 31 shows the compound distribution obtained

FIGURE 30. Gas Chromatogram of No. 2 Fuel Oil Using Silicone-based Separation Column. Fuel Oil Extracted from Solution in Sea Water Using Carbon Tetrachloride.

FIGURE 31. Gas Chromatogram of the Solvent Extraction of the Soluble Portion of No. 2 Fuel Oil. Column is Carbowax 20 M TPA.

when a suspension of No. 2 fuel oil in sea water was filtered through a 0.45μ filter under pressure prior to solvent extraction. Removal of insoluble oil droplets by filtration has dramatically reduced the complexity of the chromatogram sample. It is apparent that sea water is a selective solvent for certain hydrocarbon compounds in petroleum products. The use of a polar separation column (Carbowax 20 M TPA) permits the saturated (paraffinic) hydrocarbons to emerge first, while the aromatic hydrocarbons are retained and well separated, permitting identification and quantitation. The details of this procedure have been previously reported (Bean and Blaylock, 1974) using whole crude oils. No. 2 fuel oil is different from whole crude oils in that a much higher proportion of compounds with carbon numbers of 9 or greater are found in aqueous solution. This procedure for characterizing the soluble aromatic composition of seawater samples during bioassay experiments is being used routinely at MRL, Sequim.

Current methodological developments continue to center around gas chromatography as the principal analytical tool. Because the ultimate goal of the biological studies is to

provide information about the effects of chronic oil exposure to marine communities, the analytical sensitivity toward oil in water must be increased. To obtain the required sensitivity, seawater samples will be extracted with an inert gas (He) rather than with a solvent. The entire gaseous extract can then be introduced into the gas chromatograph, providing approximately 100-fold increase in the analytical sensitivity. The chromatogram in Figure 32 shows the results when a helium extract of water exposed to crude oil is injected into the instrument. The bonded carbowax column used for the separation has not only separated the aromatics well but has also provided additional separation for the paraffinic hydrocarbons. Figure 33 shows the results of a second extraction with helium of the same oil/water sample. It is apparent that the saturated hydrocarbons were almost completely removed by the first extraction but that the more soluble aromatic hydrocarbons are incompletely removed using the helium extraction technique. This phenomenon has the advantage of permitting identification of aromatic compounds in the absence of interference by saturated compounds but also results in a lower detection sensitivity for aromatics.

Analytical methods development continues to stress increased composi-

FIGURE 32. Analysis of the First Helium Extraction of Sea Water Containing Soluble Fuel Oil Hydrocarbon.

FIGURE 33. Analysis of the Second Helium Extraction. Saturated Hydrocarbons are Almost Completely Removed by the First Extraction.

tional detail at minimum cost in manpower. Both capillary columns and support-coated open-tubular columns will be investigated as a means of obtaining improved separation of hydrocarbon compound types. In addition, the gas chromatograph at MRL, Sequim, is being fitted with an automatic sample injector and integration/computation equipment to accommodate a greater sample load and reduce the manpower required per analysis.

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● THERMAL EFFECTS OF EFFLUENT DISCHARGES FROM NUCLEAR POWER PLANTS TO COASTAL WATERS

The current trend in power plant siting and outfall design has reduced the probability of large numbers of mobile organisms being exposed to sharp increases in temperature. However, the increasing size of each individual plant, and tendency to place several large plants at a single site, has increased the potential for change in the local communities caused by impingement, entrainment and an increase in the overall ambient temperature regime. Research at the Marine Research Laboratory, Sequim, Washington, has shifted emphasis from the cataloging of critical thermal maxima (CTM) to the investigation of community responses. The responses measured are those caused by increased temperature. These investigations also include the effects of sublethal temperature increases on life histories of commercially and ecologically important species.

Thermal Effects of Effluent Discharges from Nuclear Power Plants to Coastal Waters

C. T. Gibson and C. W. Apts

In this reporting period, the marine sciences thermal effects program added community response studies to its ongoing studies of the effects of temperature on single species. The current trend in power plant siting and design is reducing the probability of large numbers of mobile organisms being exposed to the shock of large changes in temperature. The problems of entrainment and impingement, and particularly the problem of ecological significance of slightly increased ambient temperatures over large areas, are still present and are of concern for large offshore and coastal power stations predicted in the future.

The current program has two tasks:
1) the study of the effects of long-

term exposure of communities to elevated temperatures; 2) the study of effects of cycling temperatures on commercially important species. This year has been spent primarily working on the methodologies to be used in the study of intertidal communities and the problems associated with the placing of intertidal communities in the laboratory for controlled exposure and observation.

The unit chosen for the community study is the assemblage of organisms that colonize an 8 in. x 8 in. x 16 in. concrete building block placed in the lower intertidal zone for a period of 6 to 12 months. This type of experimental unit was chosen because of its potential as a monitoring tool. The concrete blocks are universally available, easily placed and retrieved, inexpensive and will support a wide variety of intertidal organisms representative of the local biota.

To ensure that an assemblage representative of the local biota was established on test blocks, individual blocks that had been placed at the lower low-water elevation were collected periodically and examined. It was decided that after 5 months, a representative assemblage was established, and therefore, all blocks in subsequent tests had been in situ for at least 5 months.

The exposure system was an open system consisting of fiberglass aquaria (96 cm x 43 cm x 30 cm) provided with constant temperature sea water at a constant flow rate (5 l/min). The blocks were placed in the center of the aquaria which had the inlet near the bottom at one end, and the outfall at the surface of the opposite end. No attempt was made to prevent emigration or immigration of organisms smaller than those capable of passing through a 1/4 in. mesh screen.

To determine what effect the transfer from the natural environment to the laboratory test aquaria would have on the block communities, the first experiment was conducted at ambient temperature. Ten blocks were randomly selected from a stock population of blocks that had been in the intertidal zone for 6 months. Seven of the blocks were transferred to the test aquaria, and three were preserved for species identification and enumeration. After 6 weeks, the aquaria-held blocks were removed and preserved for species identification and enumeration. Three additional blocks in the intertidal area were also removed from the stock population and preserved.

A second test was initiated, using the same experimental design except that in addition to the blocks held at ambient temperature (10°C), three blocks each were exposed to 12°C, 15°C and 20°C. All blocks were initially placed in the laboratory at ambient temperature, and then the temperature was increased at the rate of 2°C per day until the desired exposure temperature was reached. After 5 weeks the experiment was terminated, and the blocks were preserved for later species identification and enumeration.

Because of the number of blocks involved and the complexity of the

communities on them, only preliminary data are available at this time. Table 47 lists the number of species, number of individuals, and biomass found on the three blocks examined at the initiation of the first test. The amount of variability found, particularly the number of species represented, is very low.

TABLE 47. Summary of Organism Analysis for Three Intertidal Blocks Exposed for 6 Months.

	Block 1	Block 2	Block 3	Mean	Std. Dev.
Number of Species	38	32	38	36	3.5
Number of Individuals	300	212	238	250	45.2
Biomass (g)	349	375	530	418	97.9

Table 48 lists the number of individuals in five taxonomic groups that were well represented on the blocks in both tests. The data are presented in this manner because species identifications have not been completed for all of the blocks.

Preliminary inspection of these data indicate that the Prosobranchia, i.e., snails and limpets, increased in numbers throughout the test period on all blocks kept at ambient temperatures, and that the variability between blocks for a one-exposure situation was extremely low. The block held at 12°C had a Prosobranchia population not significantly different from ambient blocks, whereas, numbers found on blocks held at 15 and 20°C were significantly lower. Other groups of organisms had a wide variability between blocks for each exposure; because of this, there is no observable pattern attributable to the increased temperature. However, there are some evident trends, and further analysis will have to be completed before their significance can be determined. Amphipods decreased in numbers throughout the test series with the lowest numbers occurring on blocks held at 15°C and 20°C. This is a pattern very similar to that shown by the Prosobranchia. Isopods were found in relatively high numbers on all field blocks and low numbers on laboratory-held blocks, suggesting that they either cannot tolerate the laboratory conditions,

TABLE 48. Number and Occurrence of Five Taxonomic Groups from Communities on Experimental and Control Concrete Blocks.

CLASSIFICATION	AMBIENT EXPOSURE			THERMAL EXPOSURES					
	PRE-TEST FIELD BLOCKS ⁽¹⁾	TEST BLOCKS ⁽¹⁾	POST-TEST FIELD BLOCKS ⁽¹⁾	PRE-TEST FIELD BLOCKS ⁽¹⁾	AMBIENT TEST BLOCK ⁽²⁾	20°C TEST BLOCK ⁽²⁾	15°C TEST BLOCK ⁽²⁾	12°C TEST BLOCK ⁽²⁾	POST-TEST FIELD BLOCK ⁽¹⁾
MOLLUSCA									
PROSOBRANCHIA (SNAILS & LIMPETS)	24	81	175	618	965	288	162	933	852
EULAMELLIBRANCHIA (CLAMS)	4	64	18	29	24	0	9	2	21
ANNELIDA									
POLYCHAETA	509	122	52	62	18	36	34	28	21
ARTHROPODA									
CRUSTACEA									
ISOPODA	89	1	323	272	28	2	3	5	229
AMPHIPODA	1045	823	642	454	553	34	62	312	267

⁽¹⁾ AN AVERAGE OF 3 REPLICATES

⁽²⁾ ONE REPLICATE; AMBIENT TEMPERATURE DURING THERMAL EXPOSURES WAS 10°C ± 1.

or that they migrated out of the system. Polychaeta numbers declined throughout the experimental period with no apparent trend attributable to increased temperature.

In conclusion, there are observable changes which occur in the block community attributable to increased temperatures. However, several changes do not appear to be related to the increased temperature but are related to other test factors. It is not apparent how the latter relate to thermal responses.

The next step will be to complete the analysis of the colonies tested to determine statistical significance of observed community response. With this information, further tests will be conducted to determine if changes caused by factors other than temperature can be identified and avoided by experimental design. If these changes cannot be eliminated by experimental design, efforts will be made to determine their possible synergism to thermal response.

The single species studies involved further examination of the effects of temperature changes on the coonstripe shrimp, *Pandalus danae*. This species is abundant throughout the Puget Sound-Juan de Fuca-Georgia Strait system, and is important as a food item for both man and marine fish. It migrates seasonally into shallow areas and is vulnerable at these times to man's coastal activi-

ties, such as entrainment in power plant cooling systems or voluntary entry into heated discharges.

To investigate the effects of rapid temperature increases such as those experienced by individuals entrained in power station circulating water systems, critical thermal maxima (CTM) tests were conducted on three size groups of shrimp. Some of these studies were reported in last year's annual report, but for continuity, all are reported in Table 49.

TABLE 49. Critical Thermal Maxima For Coonstripe Shrimp (*Pandalus danae*)

RATE OF INCREASE ⁽²⁾	SIZE OF SHRIMP ⁽¹⁾		
	LARGE CTM(n)	MEDIUM CTM(n)	SMALL CTM(n)
6.0	31.8(10)	29.6(10)	28.4(10)
2.0	23.6(10)	28.2(10)	27.7(10)
1.0	28.1(10)	27.8(10)	27.3(10)
0.5	27.3(9)	27.5(10)	26.3(10)
0.5 ⁽³⁾	29.3(8)	28.5(10)	27.7(10)

⁽¹⁾ LARGE >9 g; MEDIUM 4-7 g; SMALL 1-2 g.

⁽²⁾ RATE OF INCREASE EXPRESSED IN DEGREES C/min.

⁽³⁾ AMBIENT ACCLIMATION FOR THESE TESTS RANGED FROM 11.2 - 13.0°C AS OPPOSED TO 8.0 - 10.0°C FOR TESTS AT THE OTHER RATES OF INCREASE.

The CTM levels for the small size class (1-2 g wet weight) ranged from 26.3°C to 28.4°C depending on the acclimation temperature and rate of increase. The slightly increased CTM value at a higher acclimation temperature is a phenomenon reported by other investigators for a wide variety of marine organisms. The higher CTM levels at higher rates of temperature increase is probably caused by the time lag between the increased water temperature and the internal body temperature of the test organism.

The medium sized shrimp, 4 to 7 g wet weight, had a slightly higher CTM range, 27.5°C to 29.6°C, than the small size group. Large shrimp, >9 g wet weight, had a CTM value similar to the medium sized shrimp at the lower rate of temperature increase but a higher CTM value at the high rate of increase. In general, the CTM values for coonstripe shrimp range between 26°C and 32°C depending on the rate of temperature increase. In terms of size, the variation is about 1°C at the lower rate of increase and 3°C at the higher rate of temperature increase.

A second series of experiments was initiated to determine how well Pandalus danae survived at temperatures less than the lower CTM levels. These experiments were designed to duplicate the conditions the shrimp may experience if they exhibit preference for increased temperatures at thermal discharge sites, and to gather preliminary data on possible use of waste heat for shrimp culture.

Shrimp were placed in flow-through aquaria at ambient temperature and the temperature was increased at a rate of 4°C per day until designated holding temperatures were reached: 13°C (ambient), 16°C, 19°C, 22°C, and 25°C. The shrimp were then held at these temperatures for 2 weeks. After 2 weeks the temperature was dropped to ambient (13°C). Two test chambers were used at each exposure level. In one chamber at each exposure level, the shrimp were fed at a rate of 10% of their body weight (wet) per day, and in the other cham-

ber, no food was provided. It should be noted that the test system is supplied by unfiltered sea water so that some material was available to the tanks not receiving food. However, previous experiments have shown that the amount of organic material entering the system is small.

Table 50 lists the mortalities under each condition with time. At 25°C (2.5°C below CTM) all shrimp died within 1 day. Table 51 lists the net weight change for each group during 2 weeks of exposure to elevated temperatures and for 2 weeks following at ambient temperature. There was an increase in weight in the control group throughout both 2-week periods. At 16°C and 19°C there was a weight gain during the period of elevated temperatures but a weight loss during subsequent ambient temperature exposure. At 22°C the shrimp lost weight during both exposure periods.

Based on this single test, it appears that coonstripe shrimp will survive and grow well at 16°C, a temperature approximately 3°C above normal summer maximums for the shallows of the Straits of Juan de Fuca. At temperatures above 16°C the growth rate is reduced, and a sudden decrease in temperature will cause a further weight loss. However, shrimp held at 16°C for 2 weeks, and then at 13°C for 2 weeks, gained approximately twice as much as shrimp held for 4 weeks at 13°C.

Further studies are being conducted to determine how initial size affects growth rate and to determine how long the increased rate of growth will continue. Other single species groups are being given long-term thermal exposure to help understand community response to increased temperature. If we can isolate which species or groups of marine organisms do well at specific temperatures, we can better understand changes observed in community studies. In addition, community studies allow examination of interspecific as opposed to intraspecific competitive factors.

TABLE 50. Daily Record of Number of Shrimp Remaining Alive in Each Test Aquaria.

DATE	OCTOBER																															NOVEMBER						
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7					
FEED	(1) TANKS AT TEST TEMPERATURE																COLD SHOCK (TANKS RETURNED TO 13°C)																					
TEMP. °C	(1) TANKS AT TEST TEMPERATURE																COLD SHOCK (TANKS RETURNED TO 13°C)																					
16	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10						
19	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10					
22	10	10	10	10	10	10	9	9	9	9	9	9	9	9	9	9	9	7 ⁽²⁾	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3					
25	10	10	10	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
AMBIENT	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10					
NO FEED	(1) TANKS AT TEST TEMPERATURE																COLD SHOCK (TANKS RETURNED TO 13°C)																					
TEMP. °C	(1) TANKS AT TEST TEMPERATURE																COLD SHOCK (TANKS RETURNED TO 13°C)																					
16	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9						
19	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10					
22	10	10	10	10	10	10	10	10	10	10	10	10	9 ⁽³⁾	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9					
25	10	10	10	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
AMBIENT	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10					

(1) TEMPERATURE WAS RAISED AT A RATE OF 4°C PER DAY UNTIL TEST TEMPERATURE WAS REACHED

(2) TEMPERATURE ROSE BEYOND NORMAL (22°C) DUE TO SYSTEM MALFUNCTION

(3) SHRIMP ESCAPED AND SHOULD NOT BE CONSIDERED A MORTALITY

TABLE 51. Weight Changes for Coonstripe Shrimp (*Pandalus danae*) Exposed to Elevated Temperatures Followed by Cold Shock¹.

TEMPERATURE	WEIGHT CHANGES AFTER TWO WEEKS AT ELEVATED TEMPERATURES		WEIGHT CHANGES TWO WEEKS AFTER TEMPERATURE WAS RETURNED TO AMBIENT		NET WEIGHT CHANGES AFTER FOUR WEEKS	
	FEED	NO FEED	FEED	NO FEED	FEED	NO FEED
AMBIENT ⁽²⁾	0.21	-0.07	0.04	-0.17	0.25	-0.24
16°C	0.65	0.15	-0.17	-0.46	0.48	-0.21
19°C	0.17	0.07	-0.08	-0.34	0.09	-0.27
22°C	-0.17	-0.49	-0.34	---	-0.46	---
25°C ⁽⁴⁾	---	---	---	---	---	---

(1) ALL DATA REPRESENTS THE MEAN VALUE FOR 10 SHRIMP IN g

(2) AMBIENT TEMPERATURE WAS 13°C ± 5

(3) SHRIMP DIED DUE TO MALFUNCTION OF SYSTEM

(4) ALL SHRIMP AT 25°C DIED AFTER 3 DAYS OF EXPOSURE

• BIOLOGICAL AVAILABILITY AND EFFECTS OF EFFLUENTS FROM COASTAL NUCLEAR POWER PLANTS

A major new multi-disciplinary study of the long-term effects of effluents from coastal and offshore nuclear power plants was initiated in July 1974. The program combines the expertise of the Ecosystems, Radiological Sciences, and Water and Land Resources Departments to produce a research effort that will be integrated at the working level. Personnel from both Ecosystems Department and Radiological Sciences Department are now in residence at Marine Research Laboratory, Sequim, Washington, where the primary activities of the program are being conducted. The program is presently in its detailed planning stage, with the investigators now defining the major inter-discipline efforts required. The intent of the program will be to better integrate current approaches in source term chemistry, ocean transport and biological uptake, so that biological availability of effluent materials can be measured in terms of the major variables in the natural environment.

An important effort, on the environmental chemistry of copper and its fate, has also been initiated under this program. Copper has been identified as a potentially significant chemical pollutant resulting from both reactor operation and coal combustion, based on our impact assessment experience, both in the AEC Regulatory program and the Battelle Energy Program (coal). Copper, also, is of major marine and estuarine concern as to its well-recognized role in multiple factor toxicity (synergistic effects).

Research Plan and Initial Efforts

C. I. Gibson, K. H. Abel,
D. E. Robertson, R. L. Schmidt,
T. O. Thatcher and D. S. Trent

The coastal siting of nuclear power plants and the use of the coastal waters for cooling raises a number of potential long-term problems. Most sites have plants that

are designed with an effective life of 30 to 40 years, and have plans for the addition of new units that are to be added about every 10 years. So in effect, any one site can be expected to be in use for 100 years or more. This long-term use of a single site means that the coastal environment will be receiving a continuous input

of pollutants from the plant's effluent. Present plant design and operating procedures limit the effluent material to quantities that are not considered harmful in the short term, but there is no information on how these effluent materials may effect the ecosystems over the long term.

To investigate the potential effect of these effluents on the marine ecosystem, a new program was initiated by DBER at the MRL, Sequim, Washington, in July 1974. The objectives of the program are to: 1) increase our understanding of abiotic and biotic factors which influence transport, transfer and accumulation of coastal and offshore nuclear power station effluents; 2) develop an insight as to how the effluents may effect the coastal marine community; and 3) develop realistic environmental criteria for marine-based nuclear power plant discharges.

To fulfill the objectives of the program the integrated field and laboratory program established will address the following long-term projects:

- Identify the source terms and characterize the physiochemical forms of the pollutants, including radionuclides in selected nuclear power plant effluents.
- Determine transport functions for selected pollutants including sediment transport.
- Determine the relationships of ambient concentrations of these pollutants between water, sediment and biota as a function of physical and chemical forms.
- Determine the role of suspended materials and sediments in redistribution and transformation of pollutants, and the influence on biological availability.
- Determine the accumulation and effects of pollutants on coastal water biotic communities as related to physiochemical forms and biological availability from water, food and sediments.
- Initiate studies to evaluate state-of-the-art methods for assessments of biological impact.

These programs involve scientists from several different research sections and are coordinated through the research team at MRL, Sequim. The major biological and chemical programs will be carried out at Sequim; however, part of the program requires state-of-the-art techniques and equipment that exist in the Pacific Northwest Laboratory (PNL), Richland. Our plan is to use PNL as a resource for studies that can not practicably be done at Sequim because of equipment or expertise requirements. Where possible and practicable, expertise and equipment will be transferred to Sequim as the program develops in order to encourage closer cooperation between the disciplines involved in the program.

Since July 1974, effort has commenced in six task areas planned for 1975:

1) Physical, Chemical, and Quantitative Nature of Effluent Materials.

Under this task the discharges from Southern California Edison's San Onofre Unit One nuclear power plant will be characterized. Samples will be collected from the primary and secondary coolant loop, the radiological waste treatment system, the circulating water system and the sediments and biota in the region of the plant's discharge for radioactive and stable element characterization. This information will be used in the design of biological accumulation and affects laboratory studies to ensure that "real world" levels and forms are presented to the test organisms.

Contact has been made with Southern California Edison, and negotiations are underway for permission to sample within their facility. It is anticipated that the initial sampling will be conducted in January, and a second sampling will take place in the spring.

2) Factors Affecting the Exchange of Metals Between the Sediments and Water Phases.

See subsequent article.

3) Toxicity of Chlorine to Pacific Ocean Coastal Organisms and the Identification of Chemical Products Resulting from Chlorination.

The objectives of this portion of the Biological Availability program are to compare species sensitivity to biocides and metallic effluents associated with power plants, and then to examine the effects of sublethal concentrations of these materials during chronic exposures. The combined effects (which may be additive, synergistic or antagonistic) of several components of these effluents (i.e., heat, biocides, metals, etc.) will be determined.

The toxicity studies are being conducted in an 8 x 50 ft mobile laboratory that has heated and ambient salt- and freshwater systems. The experimental area is temperature controlled, and contains numerous small organism rearing chambers, two continuous-flow diluters which can supply four 6-chamber toxicity bioassay systems, chlorine analysis equipment, and activated charcoal waste treatment systems.

Much of the first quarter of FY-75 has been spent in preparing the mobile lab and investigating the problems associated with chlorine bioassay research. Stocks of experimental organisms, including fishes, clams, shrimp and crabs have been collected and are being maintained for acute and chronic exposures to chlorine. A series of [acute (96-hr) toxicity bioassays] studies will be initiated in November. These include about 15 estuarine species and involve approximately 60 separate experiments. These are planned for completion by the end of the fiscal year. A report will be prepared at that time for publication in the open literature.

In addition, chronic studies will be initiated on several species for determination of the effect of chlorine on growth and reproduction. The species will be selected for these studies on the basis of their relative sensitivity during the acute exposures. Preliminary examination into the identification of forms of chlorine available to and incorporated into tissues of fish and fish food organisms is also being initiated.

4) Effects of Gas Supersaturation on Pacific Ocean Coastal Organisms.

Preliminary design and construction of a gas supersaturation exposure system has begun. The system is scheduled for completion in early January,

and bioassay studies using shiner perch (*Cymatogaster aggregata*) will begin in early 1975. The system design and bioassay procedures are being coordinated with the Aquatic Physiology group that has been conducting gas supersaturation studies on freshwater organisms for 2 years.

5) Characterization of Sequim Bay, Washington.

The study of community response to pollution is a very difficult task when one considers that the behavior of a community to natural occurring physical and chemical changes is not well understood. Sequim Bay, Washington, is a marine embayment that is large enough to be biologically representative of most Northwest protected waters but still small enough that a good characterization of its chemical and physical processes can be made.

The physical, chemical and biological characteristics of Sequim Bay are being investigated to determine the extent to which the systems in the bay can be used for studies under the Biological Availability Program. The first of these studies was an investigation of the sediments which is reported in the subsequent article.

Biological studies will be initiated in January 1975 and will include regular sampling of plankton, bottom fish, benthos and intertidal fauna. These studies will be coordinated with regular collection of physical and chemical data. Hydrological characterization of Sequim Bay will be initiated in the late spring or early summer of 1975.

6) Transport Model Development.

Two subtasks are outlined for FY-1975 work:

a) Ocean Currents Data Base. Using existing data, a discharge decoupled current field for the San Onofre area consisting of discharge and regional current fields, will be constructed. Because it is a substantial and difficult task to construct a mass conserving and time dependent velocity field appropriate for transport analysis, the initial regional velocity field will be crude. It will, however, encompass with more detail the local San Onofre area. This

subtask is critical to reliable transport analysis, therefore the methods and techniques of storing data and resolving data on a grid system will be laid out so that the data base can be continuously expanded and updated. The data base format will be valid for other areas and be capable of expansion to much larger oceanographic regions.

b) Transport Computer Program. This task will be conducted simultaneously with subtask a. For the purposes of numerical experimentation at the onset of the program, velocity fields, which have been constructed for previous work at San Onofre, will be used. Modeling will be based on techniques, which have been used in the past, dealing with oil spill drift forecasting and dispersion of heat and other constituents. This model will include the following:

1. Time dependency
2. Two-dimensional advection (lateral plane)
3. Three-dimensional dispersion
4. Constituent sources/sinks (point or distributed)

Copper in the Marine Environment - Preliminary Literature Review

R. L. Schmidt,* R. E. Wildung,* and E. H. Groff**

A knowledge of the fate and effects, in the marine environment, of Cu, a corrosion product of nuclear reactor operation, is an important part of a total assessment of the impact of coastal nuclear power plants. A review of the literature on environmental Cu was initiated, preliminary to establishment of laboratory investigations, to determine the state-of-the-art of research on the physicochemical forms, transformations, accumulation, and transport of Cu in marine ecosystems.

The literature can be generally categorized by the ecological substrate in which Cu is found, e.g., sea water, marine sediments, fresh water, freshwater aquatic sediments, soil, and biota. A portion of the

literature can also be subcategorized under organic complexes of Cu and analytical techniques used for determination of Cu concentrations in various substrates.

Copper exists in sea water primarily as soluble metallo-organic complexes with little in ionic or particulate forms. It is apparently toxic only in the ionic form but is readily complexed by organic moieties such as polypeptides. Natural concentrations of Cu in the sea, i.e., those not measurably influenced by anthropocentric activities, most often range between 1.0 and 50 $\mu\text{g/l}$. The highest concentrations of extractable Cu are found at depths where decaying detritus is likely to release organic matter containing complexed Cu. Enrichment of trace metals, including Cu, has also been observed in the particulate and organic fraction of the microlayer at the sea-air interface. Concentration factors up to 50 for Cu have been measured. Although Cu is a nonconservative element in sea water, very little seasonal variation in its concentration has been detected.

Marine sediments exhibit highest concentrations of Cu in the organic fraction concentrated at the sediment-water interface. This fraction arises from accumulation of organic detritus after the death and deposition of organisms in the overlying waters. Copper is also associated, but to a lesser extent, with montmorillonite and, particularly, illite clay minerals. Oxidation of organic matter in sediments due to increased dissolved oxygen concentrations and the presence of free Cl from industrial cooling waters was credited for increasing the release of Cu from marine sediments. In Atlantic offshore sediments, 44% of the Cu was estimated to originate from lithogenous sources, a value which decreased with distance from the continental shelf.

The organic matter in sea water and marine sediments contains humic acids which are generally felt to be formed in situ from degradation products of plankton and not necessarily transported from continental sources. The major oxygen-containing groups of humic acid, carbonyl being the most prevalent, are concentrated in the low molecular weight

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fractions; the size fraction found to be most capable of complexing Cu.

Copper in soil solutions is largely present (up to 99%) as organic complexes and complexing is more predominant in solutions from calcareous soils. Copper in soil is bound primarily to acidic groups, likely carboxyls, in 1:1 molar metal: organic ratio at pH 3 and 2:1 ratios at pH 5. The water solubility of the organo-cupric complexes increases as this ratio increases. It is postulated that Cu is absorbed by bentonite clay through two mechanisms: by lattice hydroxyl groups and by functional groups of organic matter adsorbed by the clay.

In freshwater systems, most of the Cu in rivers is associated with suspended solids while the soluble fraction consists mostly of organic complexed forms. Copper binding to sediments can be related to the carbonate content of the sediment but Cu is generally immobilized by the organic fraction of sediment. Accumulation of Cu in sediment cores taken from lakes have been related, in several cases, to historical applications of CuSO_4 algicide.

Copper has been found to accumulate in oyster shells at concentrations sufficient to cause "greening" of the shell. Since Cu forms strong organic complexes, it also tends to concentrate in the internal organs of organisms and was found in the highest concentrations in the digestive organs and gills of mollusks. Concentration factors of 5×10^3 to 1×10^4 were observed for Cu from sea water to organisms. Copper introduced to oysters in sea water of low salinity was more toxic to the organisms than when introduced in water of higher salinity.

The sensitivity of atomic absorption spectrophotometry for Cu was enhanced when Cu in environmental samples was complexed with ammonium pyrrolidine dithiocarbamate (APDC) and then extracted into methyl isobutyl ketone (MIBK). Solvent extraction is described as a crude method of differentiation between total and extractable forms of Cu. Chelating ion-exchange resins are used to concentrate Cu from sea water with ca. 100% efficiency. Copper in particulate matter is determined by col-

lecting the suspended solids on cellulose acetate membrane filters and dissolving in acetone-HCl mixtures. Recent studies in this laboratory indicate that difficulties may be experienced in investigations dealing with trace quantities of Cu due to contamination from containers, glassware, and filters as well as from chemical reagents. Unfortunately, use of radiotracers to circumvent contamination problems in these studies is limited by short (< 13 hr) half-lives for Cu isotopes.

The Fate of Copper in Marine Sediments - An Investigative Approach

R. L. Schmidt,* T. R. Garland* and R. E. Wildung*

The fate of copper entering marine sediments as a corrosion product from coastal nuclear power plants will be investigated using both field and laboratory approaches. The field approach will include characterization of Cu in sediment cores taken near existing coastal power plants and the study of Cu release from these sediments under various environmental conditions. The laboratory approach will involve the exposure of different sediment types in various environmental regimes to concentrated source material from a nuclear power plant.

Sediments which have a history of exposure to reactor effluents will be sampled and characterized mechanically, mineralogically, and chemically. X-ray photographs of sediment cores will be used to delineate between different horizons. Selected horizons will be examined for Cu content in the mineral and organic fractions separated by sequential chemical extractions. Characterization of the mineral and organic fractions and determination of the quantity of Cu associated with these fractions in each horizon should aid in the elucidation of the fate of Cu in undisturbed sediments. Chemical characterization of the metal species in sea water, interstitial water, and chemical extracts of the sediments will be attempted with emphasis on molecular weight fractionation by gel filtration and thin-layer electrophoresis of the fractions.

Mechanical, physical, or chemical perturbations of sediments near power

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plants may increase the likelihood of release of contaminants to the biota. The fate in marine sediments of freshly released Cu from coastal power plants will be investigated using local sediments equilibrated with concentrated effluent material. Sediments having different mineralogical and organic characteristics will be studied under a factorial design which will include the variables: sediment Cu concentration, pH, salinity, redox potential, temperature, and time of equilibration. After equilibration, the interstitial water and sediment will be examined for Cu content and characterization of the chemical species of Cu will be undertaken to determine both the short- and long-term fate of this metal in marine sediments.

Sediments of Sequim Bay, Washington
R. L. Schmidt,** K. H. Abel* and
R. E. Wildung**

The properties of suspended materials and sediments are important factors in the transport, biological availability, accumulation, and effects of effluent material from coastal nuclear power plants. Sediments having a range of mineral types, organic matter, particle size, and redox conditions are exposed to reactor effluents, and research assessing the impact of coastal power plants should include studies of the influence of sediment parameters on the fate and effects of discharged material on the marine environment. An investigation of the properties of sediments from Sequim Bay, Washington, was initiated to determine the suitability of these readily obtainable sediments in the immediate vicinity of the Battelle Marine Research Laboratory for use in this research.

Seven sampling transects were established and sampled on September 24-25, 1974. These transects, shown in Figure 34, provide a preliminary observation of the distribution of sediment in Sequim Bay. The data listed in Table 52 include an estimation of sediment type based on visual and tactual observation and indicate the presence of anoxic conditions as determined by the presence of H_2S emanating from the freshly sampled

FIGURE 34. Sediment Sampling Locations, Sequim Bay, Washington.

sediment. The water contents of the sediments after drying at $60^{\circ}C$ are also given.

The sediment types vary with location and are apparently well-correlated with the influence of the tidal currents, for example, samples taken in the narrow entrance channel to the bay are apparently void of fine material. The largest quantities of sand, i.e., the lowest levels of silt and clay, were formed on the west shore. The central, deeper portion of the bay is a depository of fine material ranging from fine sands and silts to silty clays. The detection of H_2S in sediments taken along the east shore of the south end of Sequim Bay is evidence that little tidal mixing occurs in the bottom waters of this area (Figure 35).

This preliminary survey indicates that the sediments taken from different areas of Sequim Bay may have sufficiently varied properties to be used as sources of material for investigations of the influence of sediment parameters on the fate and effects of pollutants in the marine environment.

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TABLE 52. Sampling Depth, Estimated Sediment Type, Sediment Water Content, and Presence of H₂S Odor for Sediments Sampled in Sequim Bay, Washington.

SAMPLE LOCATION	SAMPLING DEPTH, feet	ESTIMATED SEDIMENT TYPE	PERCENT WATER (60°C)	H ₂ O ODOR DETECTED
1-1	20	SANDY SILT	26.0	-
1-2	72	SILT	60.7	-
1-3	72	SILTY CLAY	59.7	-
1-4	72	SILTY CLAY	63.5	-
1-5	81	SILTY CLAY	63.1	-
1-6	93	SILTY CLAY	64.2	-
1-7	39	SAND	39.6	-
2-1	12	SILTY SAND	23.4	+
2-2	20	SILTY SAND	31.0	+
2-3	40	SILTY CLAY	58.0	-
2-4	46	SILTY CLAY	61.5	-
2-5	51	SILTY CLAY	62.7	-
2-6	56	SANDY, SILTY CLAY	60.8	-
2-7	57	SANDY, SILTY CLAY	58.0	-
3-1	57	SANDY SILT	52.0	-
3-2	65	SANDY, SILTY CLAY	63.4	-
3-3	65	SILTY CLAY	62.2	-
3-4	64	SILTY CLAY	64.1	-
3-5	64	SILTY CLAY	64.1	-
3-6	58	SILTY CLAY	64.8	-
3-7	51	SILTY CLAY	71.3	+
4-1	30	SANDY GRAVEL	-	-
4-2	132	SILTY SAND	45.7	-
4-3	114	SANDY SILT	53.5	-
4-4	102	SANDY SILT	-	-
4-5	95	SANDY SILT	62.6	-
4-6	102	SILT	63.5	-
4-7	114	SILT	71.1	-
5-1	50	SILTY SAND	34.9	-
5-2	72	CLAYEY SILT	58.1	-
5-3	78	SILTY CLAY	58.7	-
5-4	87	SILT	55.2	-
5-5	96	SANDY SILT	50.3	-
5-6	99	SANDY SILT	47.3	-
5-7	111	SAND, GRAVEL, ROCK	-	-
5-8	50	SAND	27.4	-
5-9	56	SAND	29.8	-
5-10	42	COBBLES	-	-
5-11	18	GRAVEL	-	-
5-12	14	ROCK	-	-
5-13	30	COBBLES	-	-
6-1	25	SILTY SAND, GRAVEL	18.2	-
6-2	102	SILT	61.8	+
6-3	90	SILT	64.9	-
6-4	81	SILT	62.8	-
6-5	78	SILT	62.4	-
6-6	84	CLAYEY SILT	60.1	-
6-7	102	CLAYEY SILT	67.7	-
6-8	58	SANDY SILT	60.9	-
7-1	18	ROCK	-	-
7-2	8	ROCK	-	-
7-3	37	SAND	-	-
7-4	99	SAND	24.1	-
7-5	90	SANDY SILT	38.8	-
7-6	87	SANDY SILT	59.7	-
7-7	84	SILTY CLAY	59.4	-
7-8	90	SILTY CLAY	63.3	-

FIGURE 35. Sediment Characteristics Estimated from Sampling Locations Shown in Figure 34 and Physicochemical Analyses Summarized in Table 52.

For further information on Sediment Analysis refer to:
Environmental Chemistry section, "Characteristics of
Hanford Soils," pp. 36-48.

• **EFFECTS OF LOW LEVEL CHRONIC IRRADIATION ON EMBRYONIC
DEVELOPMENT OF MARINE FISH AND INVERTEBRATES**

Studies of the effects of low level chronic irradiation on embryonic development of marine fish and invertebrates was transferred to the Marine Research Laboratory, Sequim in July 1974. Work has centered on the design and construction of facilities for the holding and exposure of marine organisms to radioactive isotopes. Work will be initiated in the early part of 1975 on the effects of tritium on the eggs and larvae of the English sole Parophrys vetulus, the Dungeness crab Cancer magister, and coon-stripe shrimp Pandalus danae.

Effects of Low Level Chronic Irradiation on Embryonic Development of Marine Fish and Invertebrates
C. I. Gibson and C. W. Apts

Under the present expansion in the development of nuclear energy, it is inevitable that the marine environment will continue to receive an increasing burden of radiation. Since tritium will be the major contributor to the radioactivity in the effluent from nuclear power plants, some concern has been expressed as to the ultimate concentration in and the effect upon segments of the aquatic food web.

The majority of studies on accumulation of tritium have involved acute exposures when turnover times are relatively rapid. However, under chronic exposure conditions, there may be greater organic binding resulting in an overall slower turnover rate. Under equilibrium conditions, this could result in the concentration in seafoods being higher than would be predicted from concentrations of water alone.

Prior to July 1974, work on the effects of low-level chronic irradiation

on marine organisms had been conducted at PNL using a closed seawater system. Numerous problems were encountered in maintaining healthy stocks of organisms for use in exposure work and the maintenance of the organisms during and subsequent to exposure. These problems made the identification of effects caused by irradiation difficult to separate from effects due to holding experience.

To eliminate problems associated with holding and maintaining marine organisms in closed systems, the program was moved to MRL, Sequim, where facilities are available for holding and maintaining marine organisms in open seawater systems. In addition, there is a wider selection of species available for exposure studies, and organisms can be obtained more efficiently.

Since July, work has centered mainly on preparing the laboratory to handle radioactive materials and the designing of special holding and exposure systems. Stocks of organisms are being obtained, and exposure work will be initiated in late winter and early spring, when most of the local

species spawn. It is anticipated that eggs and larvae of the English sole *Parophrys vetulus*, the Dungeness crab *Cancer magister* and coonstripe shrimp *Pandalus danae* will be exposed to tritium solutions with radioactivity levels between 10^{-5} and 10^{-12} Ci/l.

In establishing radiation effects, eggs and embryos will be exposed throughout embryogenesis. Damage will be assessed by the proportion of fertile embryos failing to reach larval stage, and by proportions of morphologically abnormal embryos (relative to total reaching larvae stage). Degree of delay, if any, will also be quantitated, for definite, recognizable developmental stages. Because small changes in salinity, temperature, or CO_2 tension can alter rate of development and proportion of hatching, parallel controls will be run, as necessary, to assess effects of such spurious variables. Exposure will be carried out at different stages of development to determine if there is a stage of maximum radio-

sensitivity. Also, since radiation damage remains latent in some cases, development would be followed to the larval stage and beyond where possible.

With respect to the calculation of dose rates from radionuclides in the aqueous phase, three distributions of activity need to be considered; namely 1) uniformly distributed on the egg-shell surface; 2) uniformly distributed throughout the egg volume; and 3) uniformly distributed within the aqueous medium surrounding the egg.

Work will be initiated on the distribution of radioelement in the egg, by separating the outer membrane from the yolk region and comparing radioactivities. We also plan to examine distribution of radioelements in the marine eggs and larvae by radioautography. Rate of accumulation, degree of incorporation and degree of binding for a radioelement of concern will be determined by lyophilization.

• PHYSICAL AND RADIOLOGICAL CHEMISTRY OF OCEAN SOLUTIONS

The rates and mechanisms of air-sea exchange and mixing processes in the ocean are being investigated from measurements of the oceanic distributions of cosmogenic ^7Be , fission products and plutonium isotopes. Surface water sampling by the NOAA fleet has continued to provide substantial areal coverage of the world's oceans. These studies provide the basis for predicting the dispersion and transport of materials injected into the oceans.

Latitudinal Constancy of Oceanic Fallout W. B. Silker

To effectively use ^7Be as a tracer of air-to-sea exchange and ocean mixing, it is necessary to determine its temporal and geographical variability in the oceans. During the past 5 years data has been accumulated which show that concentrations of ^7Be in surface waters of the North Pacific Ocean exhibit a remarkable latitudinal constancy for a given season (Figure 36).

At first glance, it appears that there is a uniformity in the surface concentrations throughout the entire year. As we have shown in the past, the surface concentration is proportional to the total amount of ^7Be in the water column, thus the aforementioned constancy also applies to the total delivery of ^7Be to the ocean from its atmospheric source. The surface water concentration of ^7Be is basically dependent on two factors: 1) the depth of the surface mixed layer, for the material is uniformly distributed within this stratum; and 2) its concentration in the overlying air mass, as the amount delivered to the sea is proportional to its air concentration.

The expected concentration for the 40 to 50° N. latitude band was calculated using New York City air concentrations reported by HASL and Bathens' values for the monthly average of mixed layer depths, and accommodating for radioactive decay and changes in the mixed layer depth. The generated curve was normalized to pass through the data points of the May samples. The measured and calculated values actually fit quite well. The measured values in March and early April were collected northeast of Hawaii, where the actual mixed layer was shallower than the value used for calculation; hence the resultant high values. Conversely, the measured depth of the mixed layer in October 1973 was considerably deeper than the "average" literature value, resulting in dilution of the ^7Be .

At low latitudes the deviation of ^7Be concentrations from year-to-year is low, while there is considerably more variation in more northern locations. This is believed to result from increased frequency and magnitude of frontal systems and meteorological activity in the northern sampling regions, which can drastically affect the rate and magnitude of air-to-sea transfer of the atmospheric aerosol to which the ^7Be is attached.

FIGURE 36. Measured and Predicted Concentrations of ^7Be in North Pacific Surface Waters.

Depth Distribution of ^7Be in the Gulf of Alaska

W. B. Silker

The application of the cosmic-ray produced radionuclide, ^7Be ($t_{1/2} = 50$ days), for characterizing mixing rates in the upper several hundred meters of the ocean was demonstrated under two diverse conditions of water column thermal structure. This was accomplished during two cruises, in May and August 1974, traversing the Gulf of Alaska from Seattle to Bowie Seamount ($53^\circ 22'\text{N}$, $136^\circ 16'\text{W}$), to Patton Seamount ($54^\circ 45'\text{N}$, $150^\circ 32'\text{W}$), and then to Cook Inlet.

In addition to underway sampling of surface waters for contained radionuclides, vertical profiles were sampled in the vicinity of the seamounts on both cruises. During May, the thermal structure of the water column at both seamounts was such that no isothermal mixed layer was present, no distinct thermocline region could be identified, and the temperature differential between the surface and 90 m depth was only 1.75°C . An apparent

vertical eddy diffusion coefficient of $39 \text{ cm}^2/\text{sec}$ was calculated for the 0-30 m depth interval, and $0.7 \text{ cm}^2/\text{sec}$ for the 75-100 m interval. The value for K_z for the deeper interval is reasonable, but for the surface layers the observed K_z , which is too high, simply reflects increased mixing at the surface by wind-driven turbulence.

In August, both an isothermal mixed layer and a well-defined thermocline had been established at both sampling locations. Uniform ^7Be concentrations were found within the mixed layers, and diffusive mixing dominated within the thermocline with an eddy diffusion coefficient of $0.7 \text{ cm}^2/\text{sec}$ calculated for the upper boundary of this region at Patton Seamount and about $2 \text{ cm}^2/\text{sec}$ at Bowie Seamount. Concentrations of ^7Be in deeper waters were somewhat higher than would be predicted, and were probably an artifact of the larger amounts of material that were present prior to establishment of the seasonal thermocline.

Plutonium Concentrations in the Pacific Ocean
W. B. Silker

Interest in determining the geographical, vertical and temporal distribution of plutonium (Pu) isotopes in the marine environment has increased in recent years in recognition of 1) the large concentration factors exhibited by certain marine plants and invertebrates, and 2) the potential applicability of these isotopes as tracers of geochemical processes. During the past 6 years, ocean studies conducted under this program have provided hundreds of preconcentrated seawater samples from which accurate Pu concentrations can be determined. Samples are in the form of filters and activated alumina, through which several thousand liters of sea water have been passed. The filters and alumina collect particulate and soluble species of radionuclides, and they have previously been nondestructively analyzed for gamma-emitting radionuclides by high-sensitivity gamma-ray spectrometry. Thus, a library of samples collected from many parts of the world's oceans is available for measurement of the temporal and spatial distribution of Pu isotopes.

The efficiency with which the alumina in the large volume water samplers removes non-filterable Pu from sea water was determined by a multiple bed technique. It was assumed that the adsorption of Pu by the alumina obeyed the Langmuir isotherm, as has been shown to be the case for ^7Be and fission products. This being the case, if a 1/4-in. thick bed were to remove 50% of the material, then a second bed of the same thickness would remove 50% of the residual, or 25% of the amount initially present, and the retention efficiency is easily calculable. It was determined from several such dual bed experiments, under field conditions, that $55 \pm 7\%$ of the "soluble" Pu was retained by the alumina.

Owing to the low concentrations of the Pu isotopes measured, and a limitation of the counting interval, the proportionate standard deviation (± 1) of measured values for $^{239,240}\text{Pu}$ and for ^{238}Pu usually ranges between 10-20% and 30-70%, respectively. This deviation severely limits inter-

pretation of the oceanic distribution of ^{238}Pu in particular, and puts considerable restraint on evaluation of the $^{238}/^{239}\text{Pu}$ ratios at the various sampling sites.

Measurements on vertical profile samples collected in 1968 at 41°N , $143^\circ 30'\text{W}$ in the North Pacific Ocean revealed ^{239}Pu concentrations of 0.72 dpm/m^3 in the mixed surface layer, decreasing to 0.31 to 0.42 dpm/m^3 within the thermocline to a depth of 100 m. One sample taken from 200 m contained 2.4 dpm/m^3 , which seems to be an inordinately large amount. It is felt that this might represent a situation in which small particulates sink to an isopycnic layer and become hydrolyzed to a colloid, which was collected.

Analysis of selected surface samples collected on a 1971 cruise from Seattle to Samoa showed ^{239}Pu concentrations which averaged 0.30 dpm/m^3 in the 30° to 45°N latitude band, increased to 1.0 dpm/m^3 at 20°N and then decreased to 0.52 to 0.67 dpm/m^3 at the equator. The $^{239,240}/^{238}\text{Pu}$ ratios in the Northern Hemisphere were 0.11 ± 0.1 , and increased to nearly 0.3 south of the equator, reflecting the introduction of ^{238}Pu to the Southern Hemisphere by failure of SNAP-9A. In the same year, samples were collected along the eastern boundary of the Pacific Ocean. From the equator to 10°S , the ^{239}Pu concentrations averaged 0.18 dpm/m^3 and north of the equator the concentration was quite constant, averaging 0.31 dpm/m^3 . This is interesting from two standpoints. First, no concentration maximum was observed in the 8 to 10°N latitude region, where drastically increased concentrations of ^7Be and shorter-lived fission products were seen. Secondly, there was no concentration gradient with increasing latitude. Both of these conditions indicate that the water mass in this region was well-homogenized as a result of years of advective and diffusive processes existent within the oceans.

Air-to-Sea Transfer of Marine Aerosol
W. B. Silker

Beryllium-7 is a radionuclide produced by spallation reactions of cosmic rays with atmospheric oxygen and nitrogen. Owing to its relatively

constant production rate and short half-life ($t_{1/2} = 53$ days), it provided an excellent tracer for measuring transfer rates of the marine aerosol across the air-sea interface. The average flux of ^7Be across this boundary appears to be latitudinally dependent, averaging $4-5 \times 10^{-2}$ atoms/cm²/sec⁻¹ north of 30°N, and 7×10^{-3} atoms/cm²/sec⁻¹ in the equatorial region. Respective deposition velocities of 1.5 to 2.2 and 0.7 to 1.0 cm/sec⁻¹ were calculated for these regions.

The application of ^7Be tracers for determination of the rates and magnitude of transfer of other atmospheric pollutants associated with the marine aerosol is also discussed.

Plasma Excitation Emission Spectrometry Applied to Marine Chemistry E. A. Crecelius

The chemical behavior and toxicity of an element depends to a large degree on its chemical species. Very few techniques are available for the determination of the chemical speciation of trace metals in solutions and biological samples at low concentrations. Instrumentation and technique development for this type of analyses are now in progress.

The concentration and chemical speciation of several trace metals in

solutions and biological matrices can be determined by plasma excitation emission spectrometry combined with the appropriate chemistry. This analytical technique is sensitive to nanogram amounts of arsenic, antimony, mercury, selenium, germanium and bismuth. Further developments will undoubtedly extend the number of elements which can be measured.

The instrumentation now in use is equipped with a direct current discharge excitation source (Figure 37). The electrical arc creates a helium plasma capable of producing characteristic spectral emission lines from all elements in the helium carrier gas. Chemical procedures are developed to selectively volatilize from solution, and transport into the arc, gaseous compounds and volatile derivative compounds. Chemical species that can be determined include As^{+5} , As^{+3} , methylarsonic acid, dimethylarsenic acid; Sb^{+5} , Sb^{+3} , dimethylmercury, and elemental mercury. Technique development is underway for other chemical species.

These chemical speciation techniques will be applied to marine chemistry to determine the speciation of trace metals in solutions and in organisms. These studies should indicate chemical transformations which take place in sea water, interstitial water and marine organisms.

FIGURE 37. Schematic Diagram of the Reaction System and Excitation Source of the Plasma Excitation Emission Spectrometer.

• GEOCHEMICAL OCEAN SECTIONS STUDY (GEOSECS)

The GEOSECS program 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-Pacific Northwest Laboratory participation in GEOSECS involves 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. These studies are designed to:

- Determine the geographical and vertical oceanic distribution of trace element and radionuclide constituents
- Identify sources, sinks and reservoirs for these stable and radio elements
- Characterize the rates and mechanisms of the marine aerosol deposition and subsequent mixing in the upper layers of the ocean
- Provide important baseline data for trace metal and fallout radionuclide contaminants in the oceans.

Geographical Distribution of ^7Be at Pacific GEOSECS Stations W. B. Silker

During the GEOSECS Pacific cruise of the R/V Melville, air and water samples were collected for analysis of ^7Be and the low concentrations of fission products resulting from nuclear weapons testing. Sample analysis provided information describing geographical variations in the atmospheric and surface water concentrations of ^7Be . These data provide the input for determining air-to-sea exchange rates and deposition velocities which are useful in characterizing the input of aerosol materials into the ocean.

Air samples were collected with a 350 CFM Roots-Connerville blower, which drew the maritime air through an IPC filter at a velocity sufficient to ensure collection of sub-micron aerosol particles. All water samples were processed through Battelle large-volume water samplers.

The concentrations of ^7Be in air and surface waters are presented graphically in Figures 38 and 39. It should be emphasized that the air samples are point source samples in reality, as the sampling periods were of 2- to 6-day durations. Thus, the existing radionuclide concentrations are highly dependent on the atmospheric conditions to which the particular air mass was subjected prior

FIGURE 38. Surface Water Concentrations of ^7Be During Pacific GEOSECS.

FIGURE 39. Air Concentrations of ^7Be During Pacific GEOSECS.

to sampling. For example, if precipitation scavenging of the air had occurred, a low concentration of the ^7Be would be expected. On the other hand, the ^7Be content of the water represents the integration of the total amount of material that was delivered to the water column during several previous months. Owing to the relatively short half-life of ^7Be , modifications of its distribution by advective transport are minimized.

If just the general trends of both the air and water concentrations of ^7Be are considered, it is obvious that minimum values are present in the equatorial region, highest concentrations are found at mid-latitudes, and the amounts decrease at higher latitudes in both hemispheres. Exceptions to this general trend are present, particularly the very low air concentrations in two samples north-east of the Hawaiian Islands and the one low value east of Japan. These were probably the result of precipitation scavenging of the atmospheric aerosol from the particular air masses prior to sampling. The one high value just east of Hawaii is unexplainable. The two regions of high airborne ^7Be concentrations east of Japan are mirrored by correspondingly high concentrations of ^7Be in the surface waters, thus these values probably result from the local meteorological conditions imposed on the air mass as it is transported from the Asian continent over the maritime regime. This could result from reduced precipitation over the land mass, or a source enhancement resulting from injection of stratospheric air of higher ^7Be concentration through the tropopause discontinuity at this latitude.

From the measured concentrations of ^7Be in surface water, its total flux across the air-sea interface was calculated to be 3.6×10^{-2} , 3.3×10^{-2} and 6.9×10^{-3} atoms/cm 2 /sec within the respective latitude belts of 50 to 30°N, 30 to 5°N, and 5°N to 5°S. These compare with Lal's worldwide yearly average flux of 2.2×10^{-2} atoms/cm 2 /sec. Using these values of the flux and the measured air concentrations, deposition velocities of the atmospheric aerosol of 2.2, 3.0 and 0.7 cm/sec were

calculated for the aforementioned latitude belts. These deposition velocities are valid for any materials associated with the marine aerosol, and can be used, together with measured air concentrations, to estimate the rate of addition of airborne trace elements and pollutants to the oceans.

Two vertical profiles were sampled in the North Pacific at 37°27'N, 177°22'W and 50°27'N, 176°26'W. From the measured concentration gradients of ^7Be , vertical eddy diffusion coefficients of 2.9 and 2.4 cm 2 /sec were calculated for the thermocline region of the two respective sampling locations. Some vertical profile sampling was attempted in the Southern Hemisphere; however, the volumes sampled were too low to provide statistically meaningful counting results.

The fission products $^{95}\text{ZrNb}$, ^{106}Ru and ^{144}Ce , resulting mainly from the Chinese nuclear weapons test of June 1973, were also measured in the air and water samples collected. In many samples, the concentrations of these radionuclides were too low to provide useful information. Samples from the two stations east of Japan, where high levels of ^7Be were observed, also contained the highest levels of fission products; which supports the premise that the air at this location contained material of stratospheric origin.

Distribution of Mercury in the Atlantic and Pacific Oceans

D. E. Robertson

A knowledge of the natural geographical and vertical distributions of trace elements in oceanic waters is essential for identifying the major biogeochemical and physical processes which control their apportionment in the oceans. This data is also necessary for providing baseline information for evaluating the impact of anthropogenic pollutants entering the oceans. To provide this information, a large group of trace elements are being measured during the GEOSECS program to characterize their oceanic distribution over entire north-south transects of the Atlantic and Pacific Oceans in a detail never before achieved.

Mercury

One of the trace elements of environmental concern that has received recent emphasis in our work is mercury. We have analyzed over 1400 GEOSECS seawater samples from the Atlantic Ocean to provide the most comprehensive set of measurements describing the oceanic distribution of Hg. The Hg distribution in the western basin of the Atlantic Ocean is shown in Figure 40. By measuring Hg in the detail provided by the GEOSECS samples we have identified several very important features of the mercury distribution in the Atlantic and Pacific Oceans. The Hg concentrations in the western Atlantic Ocean are highly variable. The Hg distribution is characterized by extremely low concentrations (20 to 40 ng/l) over most of the ocean between about 60°S to 15°N latitude. At approximately 15°N a very sharp concentration gradient exists. South of 15°N the Hg concentrations range between 2 to 20 ng/l from the surface to the ocean floor, whereas north of 15°N the concentrations range between 20 to 80 ng/l. Between about 20°N to 45°N a large core of water located at depths between 1500 to 3400 m was observed to contain highly elevated levels of Hg (160 to 1370 ng/l). No systematic concentration gradients of Hg extending from the ocean surface or from the ocean floor were observed

at this location suggesting that the core of high Hg water had not originated from the surface or bottom waters at 20°N to 45°N. The water masses located between 1500 to 3400 m at this location have been identified from salinity-temperature-density data as Labrador Sea water (1500-2000 m), Iceland-Scotland overflow water (2800-3200 m) and Denmark Straits overflow water (3700-4000 m). Mediterranean Sea water flows into the western basin of the Atlantic Ocean at this latitude between a depth of 1000 to 1400 m. The observed core of high Hg sea water thus originated in the north polar regions by chilling and sinking of surface waters, followed by their subsequent southward transport at mid-depths. Since the north polar regions have been shown to be relatively pristine and uncontaminated by man's industrial wastes, the high Hg concentrations in the North Atlantic mid-depth water masses must have resulted from natural, large-scale inputs of Hg to the oceans in the high northern latitudes. The only mechanism which can be conceived to mobilize such enormous amounts of Hg into the oceans is degassing of Hg from the earth's mantle during submarine volcanism and tectonic activity. The Iceland area is the most volcanic region of Earth. Iceland is actually a surface manifestation of the great

FIGURE 40. Mercury Distribution in the Western Basin of the Atlantic Ocean.

Mid-Atlantic Ridge, the gigantic submarine mountain range which transects the entire Atlantic Ocean floor in a north-south direction. Near Iceland the Mid-Atlantic Ridge is extremely active volcanically, with both surface and submarine eruptions occurring at frequent intervals due to fresh magma being extruded at the ridge crest.

It appears rather certain that these enormous geophysical volcanic and tectonic processes are the sources of Hg in the deep ocean. Extremely high concentrations of Hg are associated with volcanic gases and liquids released to the environment during volcanism and geothermal processes. For example, by making some rather conservative assumptions, it has been estimated that 1000 to 10,000 tons of mercury could have been degassed to the oceans during the 1963 to 1967 submarine and surface eruptions of Surtsey volcano off the southern coast of Iceland.

Another mechanism by which high levels of Hg could be transported into the western basin of the Atlantic Ocean between 20°N and 45°N is by the lateral, east-to-west flow of Mediterranean Sea water across the volcanically active seamounts in the region of the Azores. This water enters the western basin of the Atlantic between depths of about 1000 to 1400 m.

This knowledge is extremely important since it places the global mercury pollution problem into a proper perspective. While localized coastal pollution of the oceans with mercury can present serious problems, the open oceans are apparently not significantly increased in Hg concentrations by industrially mobilized mercury. These findings emphasize the fact that we must understand the natural distributions and behavior of heavy metal pollutants in the environment before we can intelligently assess man-made impacts.

We are now in the process of analyzing our Pacific Ocean GEOSECS seawater samples for Hg. The stations that have thus far been analyzed are shown in Figure 41. We are also finding evidence of Hg injections into the Pacific Ocean, presumably by degassing during submarine volcanism. Much of the North Pacific Ocean water contains very low Hg concentrations, normally ranging between 15 to 40 ng/l. However, several stations contain definite Hg maxima at various depths which contain extremely high Hg concentrations. At Station 212 an Hg maximum exists between 400 and 600 m, in which the concentration ranges as high as 340 to 1000 ng/l. At Station 227 moderate Hg maxima exist at depths of about 550, 980 and 1470 m, as well as in the bottom water at 5800 m. Station 241 contains the highest Hg concentrations yet

FIGURE 41. North Pacific Ocean GEOSECS Stations Used for Mercury Analysis.

observed in the oceans. These high concentrations, ranging between 340 to 3120 ng/l are observed in the bottom waters between a depth of 3900 m and the bottom, which is at 5700 m. It is too early to piece together a comprehensive distribution pattern, such as the Atlantic Hg data, but it is rather certain that we detected the results of mercury mobilization to the Pacific Ocean from submarine volcanism.

Evaluation of Sampling, Storage and Analysis Techniques for Measurement of Mercury in Natural Waters
D. E. Robertson

The oceanic mercury data being generated during the GEOSECS program is of great importance to the scientific community since it has identified major, natural inputs of Hg to the oceans, and is providing detailed environmental quality baseline information. It is therefore imperative that the accuracy and precision of these data be evaluated to ensure their validity. The following parameter evaluation has been carefully and systematically conducted to authenticate our mercury data:

- Sampling Techniques
- Storage Methods
- Consistent and Known Yields of Chemical Manipulations
- Consistently Low Procedural Blanks
- Verification by Another Analytical Method
- Analysis of Standard Reference Solutions
- Determine Precision at Representative Concentrations
- Chemical and Instrumental Interferences

After evaluation of the above parameters, no systematic or random errors have been identified within our GEOSECS sampling, storage and analysis procedures which are large enough to seriously obviate the data obtained.

However, we have identified numerous potential pitfalls in the determination of mercury in natural waters

which could seriously invalidate results, if the investigators are not aware of them. The most serious potential errors are associated with sampling and storage techniques. Most water sampling devices can lead to serious contamination (either positive or negative) if the sea water is allowed to stand in the samplers for any extended length of time. If sea water is not acidified immediately after collection both organic and inorganic forms of mercury are eventually quantitatively removed from solution by adsorption onto container walls.

A serious positive contamination mechanism that was discovered when acidified sea water is stored in polyethylene bottles can occur by diffusion of airborne Hg vapor through the polyethylene bottle walls resulting in the accumulation of Hg in the acidified sea water. This mechanism became apparent during a series of tests to evaluate the seawater storage methods used during the GEOSECS program. When polyethylene bottles containing acidified sea water were stored on a laboratory floor or on an open laboratory bench top, the Hg levels greatly increased with time (see Figure 42). However, when identically prepared bottles were stored in a sealed plywood box (the GEOSECS storage procedure) no significant increase in the Hg levels in the sea water was observed. The Hg increases in the bottles stored on the floor and on the bench top were undoubtedly due to diffusion of airborne Hg through the polyethylene bottles where it accumulated in the acidified

FIGURE 42. Evaluation of Storage Conditions of Sea Water in Polyethylene Bottles.

sea water. This contamination mechanism was demonstrated in a test in which acidified sea water contained in a polyethylene bottle was exposed under controlled conditions to an airborne Hg concentration of about 20 mg/m³. After 5 days the Hg concentration in the sea water had increased from 2 ng/l to greater than 2000 ng/l, an increase of over 1000-fold. Thus, when natural waters are acidified and stored in polyethylene bottles great care must be taken to store the samples in a place where they are protected from airborne Hg vapors.

Determination of Selenium in Sea Water by Gas Chromatography with Electron Capture Detection

M. R. Petersen and D. E. Robertson

Selenium is a trace element of considerable biological and geochemical interest in the marine environment. To aid in the characterization of the marine biogeochemistry of selenium, methods to provide a fast and simple procedure for measuring selenium in sea water have been evaluated using gas chromatography and electron capture detection. This technique will be further refined and used for analysis of seawater and marine biological samples.

High concentrations of selenium coexist with high mercury, cadmium and silver concentrations in some marine organisms, and recent evidence suggests that selenium is accumulated as a detoxifying agent for mercury and other heavy metals. In the geochemical cycle, selenium is a rather volatile element and enormous amounts may be mobilized to the ocean by degassing during submarine volcanism, the same mechanism postulated for the injection of mercury into the oceans.

The technique evaluated consists of forming a stable complex with Se(IV) using 4-nitro-o-phenylenediamine as a reagent. The complex, 5-nitropiaselenol, is removed by extracting with toluene, separated by gas chromatography with a column packed with 1.5% OV-17 on 80-100 mesh Chromosorb W, and determined with an electron capture detector. Some difficulties were encountered in obtaining good extraction efficiency of the complex, resolving the desired peak on the gas chromatogram, and in long-

term stability of the standard solutions. Further improvements are possible by optimizing the experimental conditions already used. When the procedure was run under the best conditions in distilled water, the lowest detection level obtained (50 ng/l) is comparable to the reported concentrations of selenium in sea water. Further improvement in sensitivity is being investigated so that this technique, which is comparably fast and simple, can serve as a working procedure for determination of selenium in sea water. This procedure will be applied to the analysis of GEOSECS seawater samples to determine if the distribution of selenium in the oceans is related to that of mercury and other volatile elements.

Trace Element Distribution at the GEOSECS Atlantic Ocean Stations

D. E. Robertson

The trace element measurements of GEOSECS seawater samples are providing the first reliable data for understanding the distribution, origin and fate of many of the ocean's trace element constituents. These data are revolutionizing much of the early, erroneous information contained in the literature. The accurate trace element measurements performed during the GEOSECS study are needed to correctly assess radiological predictions based on the specific activity approach to critical pathway analysis. These data will also provide a basis for more intelligently assessing oceanic contamination resulting from man-made, industrial impacts.

Utilizing neutron activation techniques we have analyzed a large number of Atlantic Ocean GEOSEC seawater samples for Co, Zn, Ag, As, Sb, U, Cs and Rb. Analytical methods have been developed for Cd and Se.

Our recent measurements of Ag in the western Atlantic Ocean range in concentration from less than 0.1 to 8 ng/l, and average about 2 ng/l. These extremely low concentrations are about 100 times lower than present literature values. This knowledge will require a reassessment of the magnitude by which certain marine organisms accumulate Ag from sea water. For example, the accumulation of Ag by marine organisms such as crustacea, squid and certain finfish which have Ag concentrations of 0.1

to 5 ppm in various tissues, must represent concentration factors from sea water ranging between 10^5 to 10^6 . This would explain why the Ag radionuclides ^{108m}Ag and ^{110m}Ag have frequently been observed in marine organisms, but never directly in sea water. Thus, Ag radionuclides released to the marine environment from coastal nuclear power reactors, can be greatly amplified in concentration by the biosphere.

The same story is true for Co in the oceans. Our GEOSECS data show that the Co concentrations in the oceans normally range from about 5 to 20 ng/l, and average about 10 ng/l. These values are at least 10 times lower than previous estimates. Thus, marine organisms will have Co and radiocobalt concentration factors from sea water which are 10 times higher than earlier calculations had indicated.

The trace element showing the greatest systematic variability in vertical distribution (other than Hg) is zinc. At essentially all GEOSECS stations at least one and often several Zn maxima exist in the vertical distribution. These appear as well-defined peaks in which the Zn concentrations increase to 5-10 $\mu\text{g/l}$. As more data are accumulated, we hope to piece together a picture which will elucidate the biogeochemical processes which create these concentration variations.

Comprehensive analyses of U, Cs, Sb, As and Rb in GEOSECS seawater samples are documenting the very conservative nature of these trace elements in the marine environment. The distributions of these trace elements in oceanic waters normally do not vary outside of the analytical uncertainty of the analysis, usually about 5 to 10%.

• IN SITU POLLUTANT STUDY IN THE NEW YORK BIGHT

The objective of this study is to develop the capability for the real-time measurement of trace element and radionuclide pollutants in the vicinity of the New York Bight. This technology permits the rapid mapping of the distribution of these pollutants in the surficial and bulk sediments.

Sedimentary Pollutant Analysis by X-ray Fluorescence

N. A. Wogman, H. G. Rieck and
J. R. Kosorok

Measuring the concentrations of marine pollutants on a real-time basis would allow environmental scientists to rapidly map the distribution of elemental contamination in marine sediments and at the sediment/sea-water interface. Such real-time measurements were recently accomplished in Long Island Sound, New York, using an energy dispersive X-ray fluorescence analyzer (EDXRA).

The EDXRA is composed of a 10 mc ^{109}Cd excitation source, an Ag collimator, an 80 mm² Si(Li) detector, and a Nuclear Data 4420 mini computer. This instrumentation was installed on the submersible BEAVER MARK IV. A typical in situ-measured X-ray spectrum of Long Island Sound sediment is shown at the top of Figure 43. In addition, samples of bottom sediments were obtained, and elemental concentrations were measured with a laboratory X-ray fluorescence analyzer. This latter data, shown in Figure 43 and Table 53, is compared with the in situ spectrum. This comparison indicates that the in situ EDXRA system can detect the elements Cr, Fe, Ni, Cu, Br, As, Pb, Zn, Zr, Sr, and Rb at their ambient concentrations in polluted Long Island Sound sediments. Instrument resolution problems, which decreased the undersea probe resolu-

tion to 400 eV, did occur due to the electronic requirements imposed by submarine utilization. In addition, the high temperatures of the submarine interior (94°F) interfered with the electronics, allowing only low dead-time analyses to be used; thus long counting periods of 48 min were necessary. In general, the data illustrate a fairly uniform concentration of pollutants, although the areas of analyses were only 1/4 mile apart. As is shown in Figure 43, measurements of the environmental pollutant elements can be accomplished nearly as well with the in situ system as with a laboratory-based EDXRA system. The in situ data shown in Figure 43 were used to calculate the limits of sensitivity for measuring pollutants, and this was determined to be the 20 to 50 ppm range. Although the sedimentary material in Long Island Sound varied from colloidal suspensions at a water depth of 25 ft, to a thick, pudding-type pollution sediment at 100 ft, analyses were possible with the in situ instrument. In these polluted waters, the elemental concentrations could be quantified by using the calibrated efficiency of the system and by ratios of the peak area for a specific element to that of iron. By normalizing the data to iron, the effect of water dilution can be taken into account. With the in situ system it was proven that 10 samples per hour could be analyzed. If a bottom time of 8 hr per day were used, 80 sample

FIGURE 43. X-ray Spectra. Long Island Sound Sediments, New York. July 25, 1974.

TABLE 53. Elemental Composition of Selected Long Island Sound Muds as Determined by EDXRA.

ELEMENT	DATE COLLECTED (PPM)*		
	7/24	7/25	7/26
Si	28%	27%	18.5%
Cl	3.1%	8.9%	3.5%
K	2.3%	1.9%	1.8%
Ca	0.50%	0.83%	1.2%
Ti	0.40%	0.44%	0.49%
Cr	190	230	450
Mn	760	1190	820
Fe	4.7%	4.7%	6.9%
Ni	100	94	100
Cu	300	290	200
Zn	530	430	315
Ga	20	<3	-
As	24	10	18
Br	150	300	150
Rb	160	160	120
Sr	150	165	245
Y	50	45	53
Zr	230	190	215
Nb	23	18	27
Hg	<9	<9	<9
Pb	210	245	175

* PPM UNLESS OTHERWISE NOTED

points per day could be obtained for contour mapping of pollutant elements on the sea floor.

Sedimentary Pollutant Analysis by Neutron Activation

N. A. Wogman, H. G. Rieck,
J. R. Kosorok

Although a system of in situ neutron activation has been shown to allow the measurement of some 33 elements at concentrations of economic interest (percent levels to ppm), it has not been evaluated to determine the concentrations of pollutants in marine sediments. During the recent study in Long Island Sound a newly developed system, using a 15% Ge(Li) detector and a 570 μg ^{252}Cf neutron source, was evaluated for this purpose. The study illustrated that the system can measure the elements Ti, V, Al, Mn, Mg, Cu, Na and Cl in the deposited pollution layer.

Sediment samples were irradiated for 300 sec with the ^{252}Cf neutron source and counted for 480 sec with the Ge(Li) detector. Counts were taken after successive decay periods of 34, 720, and 1380 sec, and the spectra are shown in Figure 44. It was determined that the pollutant elements were measured at their envi-

ronmental levels ranging from 6.2% Al to a low of 70 ppm V. The size of the sample analyzed by the system is a hemisphere of 15 cm radius. The study illustrated that the system can be utilized either from a boat or from a submersible if radiation safeguard shielding can be achieved for the pilot and crew of the submarine. The neutron activation unit utilized the same electronics and hull penetration system used with the in situ X-ray fluorescence analyzer.

Radionuclide Pollutant Analysis with NaI(Tl) or Ge(Li) Detectors

N. A. Wogman, J. R. Kosorok,
H. G. Rieck

Concern exists over the degree of radioactive pollution which results from radioactive fallout, operation of nuclear power plants and their associated chemical separations facilities, and radionuclides used in research and medical applications. The study of Long Island Sound pollution provided a vehicle to allow the use of a NaI(Tl) and a Ge(Li) detector system for the measurement of this type of pollution.

Detectors were lowered to the ocean floor on a support member attached to the anchor of the BEAVER

FIGURE 44. In Situ Spectra of Neutron Activated Sediments-Long Island Sound, New York.

MARK IV submersible, and spectra were recorded for time periods ranging from 15 to 60 min. Potassium-40, the major gamma-emitting radionuclide in the sediments, was measured with a 6-in. by 4-in. thick NaI(Tl) crystal. A concentration of 36,000 dpm ^{40}K /kg of sediments was observed. Using

the Ge(Li) detector, the concentrations of ^{40}K and of ^{232}Th and ^{238}U as calculated through their daughter radioactivities were of the order of 2000 dpm/kg for ^{208}Tl (^{232}Th) and ^{214}Bi (^{226}Ra). No fission or activation product radionuclides were detected.

• BIOGEOCHEMISTRY OF PLUTONIUM AND AMERICIUM IN MARINE SYSTEMS

The projected growth of the nuclear power industry and the use of coastal and offshore nuclear installations necessitates an adequate understanding of the biogeochemical behavior of the transuranium elements in the marine environment. Two marine locations, containing elevated levels of plutonium and americium, that can serve as natural laboratories for conducting detailed studies of these radioelements are the former Pacific test site at Enewetak Atoll and Bylot Sound off Thule, Greenland. Studies of the plutonium and americium in sea water, sediments, and marine organisms at these locations are helping to characterize their behavior and fate in the oceans.

Environmental Studies at Thule, Greenland D. E. Robertson

Following the crash of a USAF B-52 airplane at Thule, Greenland, which contaminated the marine environment with ^{239}Pu , radioecological surveys of Bylot Sound were conducted in 1968 and 1970 by the Danish and U.S. Atomic Energy Commissions. Measurements of the concentrations and distribution of ^{239}Pu in the sea water, sediments and marine organisms documented above-background ^{239}Pu concentrations in the biotic and abiotic compartments of the marine ecosystem near the point of impact. This contamination was shown to have spread along the sea floor as far as 30 km from the impact area during the 2 years following the crash.

In August 1974, a third Danish-U.S. radiological expedition was organized to follow up on the 1968 and 1970 surveys. Battelle-Northwest participation was to complement the monitoring survey of the Danes by developing information on the physicochemical speciation and mobilization of Pu in the

Thule marine environment. The inventory of samples collected for analysis and the sampling locations are shown in Figure 45 and Table 54. The basic objective of our study was to determine what factors are important in mobilizing ^{239}Pu contained in the sediments. To determine if ^{239}Pu is being solubilized during in situ leaching of the sediments by sea water, large volume water sampling was conducted at four locations in Bylot Sound at the surface and near the bottom to determine the concentration of ^{239}Pu in soluble and particulate phases. To determine if significant amounts of ^{239}Pu are being redissolved from the sediments, four undisturbed sediment cores were collected, sectioned into 3-cm segments, and the interstitial waters contained in the sediments were extracted by pressure squeezing. The pore waters are being analyzed for ^{239}Pu . The squeezed sediments were then leached for 1 hr with a 0.1 N HCl solution containing a small amount of H_2O_2 to remove hydrous oxide coatings from the sediment particles. The leachate and residual sediments are being analyzed for Pu. It is possible that Pu is

FIGURE 45. Sediment Core and Seawater Sampling Locations in Bylot Sound, Thule, Greenland. August 1974.

TABLE 54. Battelle-Northwest Participation in Third Radioecological Investigation at Thule, Greenland. August 1974.

APPROX. NO. OF SAMPLES	SEAWATER ANALYSIS
16	<ul style="list-style-type: none"> • OCCUPY 4 STATIONS; SAMPLE AT SURFACE AND NEAR BOTTOM • BLVWS TO PROCESS 420 TO 2200 LITERS/SAMPLE • COLLECT AND ANALYZE Pu, FALLOUT AND COSMIC-RAY RADIONUCLIDES IN SOLUBLE AND PARTICULATE FORMS
24	<p data-bbox="889 1440 1073 1465"><u>SEDIMENT STUDIES</u></p> <ul style="list-style-type: none"> • 4 CORE SAMPLES PROCESSED AS FOLLOWS: <ol style="list-style-type: none"> 1. SECTION INTO 3 cm LENGTHS 2. SQUEEZE PORE WATERS - ANALYZE FOR Pu 3. LEACH SQUEEZED SEDIMENTS WITH DILUTE HCl-H₂O₂ - ANALYZE Pu IN LEACHATE AND RESIDUE TO DETERMINE DEGREE OF CHEMICAL BINDING TO SEDIMENTS
6	<ul style="list-style-type: none"> • PARTICLE SIZE SEPARATION OF SEDIMENTS AND Pu ANALYSIS OF VARIOUS SIZE FRACTIONS
2	<p data-bbox="899 1766 1049 1791"><u>BIOTA STUDIES</u></p> <ul style="list-style-type: none"> • DETERMINE Pu DISTRIBUTION IN VARIOUS TISSUES AND SHELL OF BIVALVES
TOTAL 48	

being dissolved from the sediments by the leaching action of sea water and then eventually reattaching to sediments during coprecipitation of hydrous iron and manganese oxide coatings onto sediment particle surfaces. The leaching studies will give an indication of the importance of this potential mechanism for mobilizing sediment-attached Pu from the impact area.

If physical transport of Pu-bearing sediments from the impact area by tidal currents is the main mobilization mechanism, the particle size distribution of the sediments is an important consideration for such sediment transport processes. Selected segments of the sediment cores are being subjected to particle size separations and the Pu contents of each size fraction are being determined. This information will relate the effect of particle size to the observed areal distribution of ^{239}Pu in the bottom sediments.

The bivalve, Macoma calcareous, is the most abundant benthic organism in Bylot Sound and the highest accumulator of ^{239}Pu from the sediments. We are, therefore, examining several collections of these bivalves to determine the specific sites of ^{239}Pu accumulation in the soft tissues and shell.

Biogeochemical Studies of Plutonium and Americium in the Enewetak Marine Environment

W. C. Weimer, K. H. Abel and C. I. Gibson

This new program will emphasize investigation of the geochemical and biological processes controlling the dynamic release of Pu and Am from the Enewetak Atoll sediments into the water and uptake by bottom-dwelling members of the marine food web. An initial site visit to Enewetak Atoll is scheduled for early in 1975. This trip will permit the collection of sediments, water, and biota samples from preselected sites; these samples will be transferred to our laboratory for analysis.

We will be conducting studies of the chemical forms of Pu and Am in Enewetak sediments, sediment interstitial waters, and Atoll waters. Laboratory studies of the rates of release of Pu and Am from sediments into sea water under conditions simulating the Enewetak environment will be conducted. Selected organisms will be collected together with associated sediments and water and the radionuclide content of each will be determined to obtain estimates of sediment:organism concentration factors. Pilot studies to determine the feasibility of holding organisms in laboratory aquaria for long periods of time will be initiated using organisms known to concentrate the radionuclides of interest.

For further information on transuranium element behavior refer to:

- 1) "The Potential for Plutonium Complexation in Soil and Uptake by Plants," Wildung et al., pp. 20-35.
- 2) "Suspended Particle Interactions," Klepper, Craig, Cataldo and Vaughan, pp. 49-55.
- 3) "Weathering and Aging of Transuranics in Soil," Rickard et al., pp. 191-203.
- 4) "Plutonium Studies on the Nevada Test Site," Eberhardt et al., pp. 6-7.
- 5) "Ecological Behavior of Plutonium and Americium in a Freshwater Ecosystem: I. Limnological Analysis; II. Isotopic Ratios," Emery et al., pp. 95-99.

• **^{55}Fe IN HUMAN POPULATIONS OF THE WORLD**

The preferential concentration of ^{55}Fe over stable iron by marine organisms is explained by the difference in physico-chemical forms between stable iron and freshly-deposited ^{55}Fe in sea water. Investigations to identify the different forms in both sea water and oceanic aerosols are continuing.

Physicochemical Forms of ^{55}Fe and Stable Fe in Sea Water and Oceanic Aerosols

W. C. Weimer and J. C. Langford

The emphasis of this program has shifted from analysis of ^{55}Fe specific activities in marine organisms to a determination of the differences in the physicochemical forms of both stable and radio iron in ocean waters and in the atmospheric source of these materials to the marine environment. This information is being developed through analyses of both stable iron and ^{55}Fe in North Pacific Ocean surface waters and in atmospheric particulates.

The preferential uptake of ^{55}Fe over stable Fe by some marine organisms and the transfer of this higher $^{55}\text{Fe}/\text{Fe}$ specific activity to man has been well documented. Some marine organisms preferentially concentrate ^{55}Fe by a factor of 100 to 1000 over stable Fe. The latitudinal effect of different animal and human populations and their varying body burdens has also been shown. The total body ^{55}Fe burden of adult Alaskan Eskimos is approximately 10-30 times greater than the body burdens of residents of Richland, Washington. Although the greater biological availability of

^{55}Fe to marine organisms in comparison with stable Fe availability is well known, there is little evidence to identify the causes for these different availabilities.

Large volume air samples have been collected from several locations in the North Pacific Ocean; water samples were collected from the surface layer at the same locations. The specific activities of ^{55}Fe on these air filters are being compared to the specific activities in the surface water samples. An investigation of the leaching characteristics of stable and radioactive Fe from these air filters and from filters collected at the Baseline Station at Quillayute, Washington, is in progress. These tests will give an indication of the relative availability of the ^{55}Fe and stable Fe in the atmospheric source term.

Because of the low concentrations of Fe in sea water and in the leaching solutions from air filters, an extremely sensitive technique to rapidly measure Fe concentrations is necessary. A procedure to measure $< \mu\text{g}/\text{l}$ quantities of stable Fe in sea water and in chemical eluant solutions has been evaluated. This technique allows the routine determination of less than 0.2 μg of Fe/l.

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Young, J. A., and W. B. Silker. 1974. The determination of air-sea exchange and oceanic mixing rates during the BOMEX experiment. J. Geophys. Res. 79: 4481.

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- **RADIOANALYTICAL PROCEDURES DEVELOPMENT**

• RADIOANALYTICAL PROCEDURES DEVELOPMENT

Although developments in instrumental analysis have greatly improved the sensitivity and selectivity for measurement of multi-constituents in environmental media, rapid and simplified chemical separations are still necessary to provide the sensitivity required for measuring specific pollutants in the environment. Because radionuclides, trace elements, and other environmental pollutants are often present at very low concentrations in environmental materials, it is necessary to continue to develop procedures for specific elements using up-to-date, state-of-the-art methods and instruments.

Procedure for Measuring Uranium, Thorium, Radium, Plutonium, and Americium in Soil Samples C. W. Thomas

To help assess the impact of nuclear plant operations on the environs, a procedure was developed to determine naturally occurring radionuclides of uranium (U), thorium (Th), and radium (Ra) as well as pollutant radionuclides of plutonium (Pu) and americium (Am) in soils. This method allows accurate measurement of low concentrations of radionuclides through chemical separation and alpha energy analysis.

The procedure begins by thoroughly drying the soil sample and then a 30g aliquot is weighed and placed in a container of suitable counting geometry. The gamma emitting radionuclides including ^{241}Am , ^{226}Ra , and ^{228}Ac are measured using a low background anticoincident shielded Ge(Li) diode that has the capability of separating the radionuclides with coincidence gamma photons from those radionuclides that emit non-coincidence gamma photons. By using this method of counting, a much lower background can be achieved than by conventional

methods of gamma counting. Background levels for the ^{241}Am , ^{226}Ra , and ^{228}Ac gamma photon regions are 0.134, 0.109, and 0.018 counts per min. The soil sample is removed from the counting container and placed into a 1000 ml beaker. Plutonium-236 and ^{243}Am , as tracers for chemical yield, are added and the sample is thoroughly mixed and dried. The soil sample is leached twice with a mixture of 30 ml of concentrated HNO_3 and 100 ml of concentrated HCl with contact times of 1 hr each. The hot leachate is filtered through a fritted glass filter and evaporated to dryness. The residue is dissolved in 7.7 M HNO_3 and passed through a column of 200-400 mesh Dowex 1 x 8 anion exchange resin. The resin is pre-equilibrated with 7.7 M HNO_3 . The column is washed with 7.7 M HNO_3 and the filtrate and washings are combined for Am-U separations. Thorium is eluted from the column with several column volumes of 12 M HCl followed by elution of Pu with several column volumes of a mixture of 12 M HCl :0.1 M HI .

The Am-U fraction is evaporated to dryness and the residue dissolved in 2N HCl . Americium and U are carried

on a precipitate of ferric hydroxide formed by making the solution basic with NH_4OH . The precipitate is dissolved in 10 M HCl and passed through a column of 200-400 mesh Dowex 1 x 8 anion exchange resin that has been equilibrated with 10 M HCl . After washing with 10 M HCl , the filtrate and the washings are combined for the Am determination if the concentration of ^{241}Am was below the detection limit afforded by the direct gamma counting. Uranium is eluted from the column with several column volumes of 7.7 M HNO_3 and the elutant is taken to dryness. The residue is dissolved in 2 N HCl and the U is coprecipitated with ferric hydroxide using NH_4OH for pH adjustment. The precipitate is washed with water, dissolved in 1 M acetic acid, and passed through a column of 200-400 mesh Dowex 1 x 8 resin, previously equilibrated with 1 M acetic acid. The filtrate containing iron is discarded. Uranium is eluted from the column with 4 M HNO_3 and the solution is saved for electroplating.

Plutonium, Th, and U are prepared for electroplating by evaporating the final solutions to dryness with 12 M HNO_3 . The residues are dissolved in H_2SO_4 , and NH_4OH is added to obtain the acid end point of thymol blue indicator. The Pu, Th, and U are electroplated onto stainless steel discs at 1.1 amps for 2 hr. Individual isotopes are measured using alpha energy analysis.

Rapid Radiochemical Separations for High Sensitivity Neutron Activation Analysis

J. C. Langford, C. W. Thomas,
R. L. Brodzinski and L. A. Rancitelli

An important aspect of the Controlled Thermonuclear Reactor (CTR) Program is the possibility that 14 MeV neutrons from the fusion reaction will have a high probability of interacting with the containment vessel, resulting in recoil losses of stable atoms from the containment material. Metals considered for use in containment vessels include stainless steel, Nb, V, Mo and Ti. This study provides specific information on the sputtering yields of these materials to aid in evaluating their applicability.

Initial measurements by other laboratories have indicated that these fast neutron sputtering yields, i.e., the number of atoms knocked out of a

surface per incident 14 MeV neutrons, may be as high as 10^{-1} . If these sputtering yields are correct, they would not only cause rapid erosion of the fusion reactor containment vessel but would also inject stable atoms into the plasma which might poison the reaction. Hence, it is of paramount importance that the sputtering rates be accurately and firmly established. While not among the candidate materials for this study, neutron sputtering studies of Au and Cu have been conducted with questionable success in other laboratories.

In an effort to establish sputtering yields from metal surfaces it is necessary to bombard thin foils of these materials with 14 MeV neutrons and to collect the sputtered atoms on ultra-pure, contamination-free surfaces. Once collected, these sputtered atoms can be measured by high sensitivity thermal neutron activation analysis. With the highest fluxes presently available from a 14 MeV neutron generator, one can expect to sputter only about 10^{10} - 10^{12} atoms on a collection surface within a reasonable period of time. Hence, the measurement of these sputtered atoms by neutron activation analysis requires not only high sensitivity but freedom from interfering thermal and fast neutron-induced radioactivities. Thus, it is necessary to develop radiochemical separation procedures of neutron activation products which will isolate the radionuclides of interest from possible interferences. These separation procedures, as in the case of Nb, must be rather rapid since the radionuclides to be measured have half-lives of only a few minutes. Several chemical procedures have recently been developed which meet the above criteria and are being applied to measure neutron sputtering yields.

Separation of ^{94m}Nb

The ^{94m}Nb is removed from the surface of the thermal neutron activated silicon collector foil using a mixture of hot HNO_3 - HF . Niobium-95 for yield determination and perchloric and boric acids are added, and the solution is heated to fumes of HClO_4 . One-half mg of Fe^{+++} and 100 mg Al^{+++} carrier are added, and the solution is then transferred to a 100 ml centrifuge cone using 6 N H_2SO_4 . The solution is adjusted to pH >10 with

8 N NaOH and is filtered through a 1-in. diameter, 5 μ Millipore filter. The precipitate is then counted on an intrinsic germanium diode to measure ^{94m}Nb ($t_{1/2} = 6.3\text{m}$) X-rays. The procedure is completed in under 5 min with yields of $90 \pm 10\%$.

Separation of ^{52}V

The ^{52}V is removed from the surface of the thermal neutron activated collector using 10 ml of 10 N HNO_3 , and the solution is decanted to a pyrex centrifuge cone. Vanadium-48 for yield determination, 100 mg V^{+++} carrier, and 1 ml of 70% HClO_3 acid are added, and the solution is heated to fumes of HClO_4 . Twenty ml of water and a few mg of Na_2SO_4 are added, and the solution is heated in a water bath. One ml of 5% $\text{Na}_3\text{Fe}(\text{CN})_6$ and approximately 100 mg of ascorbic acid are added, and the resulting precipitate is filtered through a 2-in. diameter, 5 μ Millipore filter. The precipitate is then counted on a $\text{Ge}(\text{Li})$ diode to measure the ^{52}V activity ($t_{1/2} = 3.77\text{ min.}$, $E = 1.43\text{ MeV}$). The chemical yield is determined by measuring the ^{48}V in a $\text{NaI}(\text{Tl})$ scintillation well crystal after decay of ^{52}V . The procedure can be completed in under 3 min with a yield of $94 \pm 3\%$.

Separation of ^{64}Cu and ^{198}Au

Sputtered gold is removed from thermal neutron activated Si discs using hot aqua regia. The gold is then electroplated with carrier onto copper discs. Yields are in the range of $98 \pm 2\%$. Sputtered copper is removed from the discs using hot concentrated HNO_3 , then electroplated with carrier from a cyanide solution onto stainless steel discs. Yields are in the range of $95 \pm 5\%$.

Separation of ^{99}Mo

The ^{99}Mo is removed from the surface of the thermal neutron activated silicon collectors with hot concentrated nitric acid. The acid is decanted into a pyrex beaker, and the solution is evaporated to near dryness and transferred with 2 ml of 0.5 N H_2SO_4 to a separatory funnel. Molybdenum-99 is extracted with 2 ml of 0.5% benzoin-oxime into chloroform. The benzoin-oxime phase is separated and ^{99m}Tc is allowed to build into the sample for 24 hr. The 142 Kev gamma from ^{99m}Tc decay is counted on a $\text{Ge}(\text{Li})$ diode. Yields are about 80%.

For further information on cooperating Radiological-Ecological Programs refer to:

- 1) Marine Sciences section, "Physical and Radiological Chemistry of Ocean Solutions," pp. 132-135.
- 2) Marine Sciences section, "GEOSECS," pp. 136-143.
- 3) Marine Sciences section, "In Situ Pollutant Study in the New York Bight," pp. 144-147.
- 4) Marine Sciences section, "Biogeochemistry of Plutonium and Americium in Marine Systems," pp. 148-150.
- 5) Marine Sciences section, " ^{55}Fe in Human Populations of the World," p. 151.

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TERRESTRIAL ECOLOGY

HANFORD RESERVATION SUPPORT SERVICES

- **ECOLOGICAL MONITORING OF THE HANFORD RESERVATION**
- **ECOLOGICAL CHARACTERIZATION OF THE 200-AREA PLATEAU**

• TERRESTRIAL ECOLOGY

- **GRASSLAND BIOME**
- **ANIMAL ECOLOGY**
- **ECOLOGICAL MICROMETEOROLOGY AND CLIMATOLOGY**

RADIONUCLIDES IN THE ENVIRONMENT

- **WEATHERING AND AGING OF TRANSURANICS IN SOIL**
- **RADIOECOLOGY OF IODINE-129 AND TECHNETIUM**
- **RADIONUCLIDES IN ALASKA**

- **Local Funding Through Services Assessment or ARHCO Intercontractor Agreements**

TERRESTRIAL ECOLOGY

HANFORD RESERVATION SUPPORT SERVICES

- **ECOLOGICAL MONITORING OF THE HANFORD RESERVATION**
- **ECOLOGICAL CHARACTERIZATION OF THE 200-AREA PLATEAU**

Following practice in previous annual reports, we have included in this section pertinent descriptions of efforts the staff provides to RLOO and other Hanford contractors supported by AEC programs. Apart from the DBER-funded schedule 189's, the objectives for these small-scale tasks are concerned in a proximate sense with the monitoring and surveillance program supporting nuclear fuel reprocessing activities. Since information and data useful to the DBER terrestrial program is gained through this sense as well, we include a summary here.

Ecological monitoring provides a factual record of wildlife populations, abundance, seasonal distribution and dispersion in relation to the operation of experimental and production nuclear facilities, unusual weather conditions, wildfire, predation, and changing land use patterns both on and adjoining the Hanford Reservation.

Historically, the isolation of the Hanford Reservation and the protection afforded by islands in the Columbia River have provided a suitable environment for the successful nesting of the Canada Goose. A survey of the entire nesting population during the spring of 1974 provided the 25th consecutive year of observation.

Fuel reprocessing facilities located in the 200 Areas have a fairly extensive series of ponds and ditches. In several cases riparian habitats attractive to wildlife have become well-established, with a resulting flora and fauna differing from that of the ALE Reserve. Ecological characterization of these areas has been completed with respect to avifauna and mammals, together with a preliminary assessment of their radioecological importance.

Ecological Monitoring of the Hanford Reservation*

W. H. Rickard and J. D. Hedlund

Technical Assistance:

D. T. McCullugh and H. A. Sweany

Canada Goose Nesting

During spring of 1974, a survey of the entire nesting population was concluded (Table 55). Of 20 islands surveyed, 9 did not support nests. Four islands--1, 2, 12, and 17--supported 74% of the nests (Figure 46). Although 137 nests were found, only 75 (55%) were successful. Unsuccessful nests were destroyed by predators, especially the coyote, and by flooding of the Columbia River. Twenty nests were deserted by parent birds for unknown reasons. All nests on island number 1 were destroyed by predators. It was determined that 406 goslings were hatched. Specially designed elevated nesting platforms erected in 1973 as a shelter from mammalian predators were not used by geese during the 1974 nesting season.

Radionuclides in Eggs

Six eggs taken from deserted nests were weighed individually. The average egg weighed 153 g and nearly 15% of the weight was comprised of egg shell (Table 56). Eleven gamma-emitting radionuclides were identified and quantitatively measured in egg contents (Table 57). The most abundant radionuclide was the naturally occurring isotope ^{40}K . Fission products such as ^{95}Zr , ^{106}Ru , and ^{137}Cs were measured in egg contents but not ^{144}Ce . Cerium-144 was measured in one sample of egg shells which probably represents external contamination derived from fallout. One of the egg content samples had 35 times more ^{137}Cs than the other. Because several eggs were pooled for radiochemical analyses, it is not known if the high cesium value is due to a single egg or to several eggs from a single female parent or eggs from more than one parent bird. This suggests that future sampling for radiocesium should be performed on individual eggs or clutches. Neutron activation products, i.e., ^{22}Na , ^{54}Mn , ^{60}Co , and ^{65}Zn were measured in the egg contents but not in egg shells. The biologically active ele-

ments were probably derived from the female parent which accumulated these isotopes from available foods and water.

Although surveys have been conducted over a long time period, eggs have not been regularly analyzed for radionuclides. The data in Table 57 shows that eggs from deserted nests can be used for evaluation of environmental uptake of radionuclides by an important wildlife species without imposing additional mortality on the goose population.

Mule Deer Tagging

The mule deer (*Odocoileus hemionus*) is the only large game mammal on the Hanford Reservation. The population has benefited from the refuge from human influences offered by the Hanford Reservation since the early 1940's. Although mule deer can be expected anywhere on the Reservation, most of the population is more or less confined to a narrow strip bordering the Columbia River. Fawns have been tagged each June since 1969 by hand capture after locating them by use of a helicopter. Thirty-four fawns were tagged in June 1974 (Table 58). Seventeen were females and 16 were males. One fawn was tagged and released and the sex inadvertently not recorded. The locations of captured fawns are shown in Figure 47. The most productive fawning area was the thick riparian willow vegetation between the abandoned townsite of Hanford and the 100-F Area. The barren but isolated sand dune area near island number 11 was also productive of fawns in 1974. The return of tags has been mostly through hunter-killed animals during the autumn hunting season off the Hanford Reservation. Hunting is not permitted on the Reservation, but a few deer are killed each year by automobile traffic. Since the inception of fawn tagging studies, 18 tags have been returned from hunter-killed animals and 3 from road kills. One animal was killed during the 1974 hunting season 70 miles north of its place of tagging.

Columbia River Bird Census

The Hanford Reach of the Columbia River serves as an important resting stop for migratory waterfowl in winter. During the 1973 migratory waterfowl hunting season, an aerial census

* Service Assessment Funds

TABLE 55. Summary of Canada Goose Nesting on Columbia River Islands, Hanford Reservation, Spring 1974.

ISLAND NUMBER	NUMBER OF NESTS LOCATED	NUMBER OF SUCCESSFUL NESTS	NUMBER OF EGGS LAID	NUMBER OF EGGS HATCHED	PREDATOR DESTROYED NESTS	DESERTED NESTS	FLOODED NESTS
1	27	0	137	0	27	0	0
2	21	16	89	78	0	4	0
3	4	0	21	0	4	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0
8	0	0	0	0	0	0	1
9	2	1	10	6	0	0	0
10	0	0	0	0	0	0	0
11	3	0	UNKNOWN	0	3	0	0
12	29 ⁽¹⁾	15	168	87	1	13	0
13	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0
15	5	3	26	19	0	2	0
16	0	0	0	0	0	0	0
17	24 ⁽²⁾	22	152	123	1	1	0
18	8	6	49	37	0	0	2
19	11	9	64	49	0	0	2
20	3 ⁽³⁾	3	16	13	0	0	0
TOTAL	137	75	732	406	36	20	5

(1) 2 NESTS HATCHED BEFORE APRIL 9

(2) 1 NEST HATCHED BEFORE APRIL 9

(3) 2 NESTS HATCHED BEFORE APRIL 9

TABLE 56. Weights (g) of Individual Canada Goose Eggs from Deserted Nests on Columbia River Islands, Spring 1974.

EGG NUMBER	WHOLE EGG	EGG SHELL	INNER CONTENTS	PERCENT SHELL
1	160.3	22.4	135.3	14.0
2	149.1	19.5	128.7	13.0
3	154.6	23.9	129.1	15.5
4	124.5	21.9	119.7	15.4
5	152.2	23.2	128.4	15.3
6	161.0	22.0	138.1	13.7
$\bar{X}$	153.3	22.2	129.9	14.5
$\pm S.E.$	2.9	0.6	2.6	0.4

FIGURE 46. Map of the Hanford Reservation and Columbia River Showing the Location and Numbering System of Islands Surveyed for Canada Goose Nests.

TABLE 57. Gamma-emitting Radionuclides¹ (dpm/kg/ash) in Unhatched Canada Goose Eggs and in the Muscle and Bone of a Juvenile Goose, 1974.

Radionuclide	Eggs collected April 25, 1974				Collected June 15, 1974	
	² /contents	³ /contents	² /shell	³ /shell	Muscle	Bone
⁷ Be	---	---	720 ± 260	---	---	---
²² Na	42 ± 2	53 ± 3	---	---	---	---
⁴⁰ K	44,000 ± 100	49,000 ± 130	8,000 ± 220	9,400 ± 250	89,000 ± 3,800	15,000 ± 400
⁵⁴ Mn	230 ± 10	91 ± 13	---	---	---	13 ± 6
⁶⁰ Co	87 ± 3	63 ± 3	10 ± 4	17 ± 4	---	16 ± 7
⁶⁵ Zn	950 ± 18	510 ± 22	400 ± 110	---	---	---
⁹⁵ Zr ⁴	150 ± 14	---	180 ± 33	61 ± 42	---	---
¹⁰⁶ Ru	140 ± 27	100 ± 30	130 ± 60	160 ± 68	---	160 ± 81
¹³⁷ Cs	380 ± 8	14,000 ± 25	51 ± 16	71 ± 18	830 ± 130	---
¹⁴⁴ Ce	---	---	210 ± 91	---	---	---
²¹⁴ Bi	400 ± 16	210 ± 16	180 ± 26	470 ± 44	---	---

¹Radiochemistry performed by Radiological Chemistry Department, PNL
²/and ³/ = separate composite samples

⁴Double number listed for ⁹⁵Zr-Nb parent-daughter pair

TABLE 58. Mule Deer Fawns Tagged on the Hanford Reservation, 1974.

Tag #	Sex	Rng.	Tsp.	Sec.	Tag #	Sex	Rng.	Tsp.	Sec.
8788	M	26E	14N	15	A0613	M	27E	13N	3
8789	M	26E	14N	15	A0616	F	27E	13N	3
8790	F	26E	14N	15	A0617	F	27E	13N	3
8791	F	26E	14N	15	8786	M	27E	13N	7
A0605	F	26E	14N	12	8780	M	27E	13N	25
8787	M	26E	14N	12	A0614	F	27E	13N	25
A0604	M	26E	14N	12	A0615	M	27E	13N	25
8781	F	27E	13N	3	A0602	M	27E	14N	20
8782	M	27E	13N	3	A0603	M	27E	14N	20
8784	Unk	27E	13N	3	8795	F	28E	11N	23
8785	M	27E	13N	3	8796	M	28E	11N	23
8792	M	27E	13N	3	8797	M	28E	11N	23
8793	F	27E	13N	3	A0608	F	28E	12N	22
8794	F	27E	13N	3	A0609	F	28E	12N	22
A0601	F	27E	13N	3	A0610	F	28E	12N	22
A0606	F	27E	13N	3	A0611	F	28E	12N	22
A0607	F	27E	13N	3	A0612	M	27E	12N	22

Total = 34: Males = 16; Females = 17; Sex Unknown

FIGURE 47. Map of the Hanford Reservation Showing the Location and Numbers of Mule Deer Fawns Tagged During the Spring of 1974.

was made to estimate the number of ducks, Canadian geese, Great Blue Herons and eagles nesting on the Columbia River. In mid-November, more than 20,000 ducks and 1200 geese were observed resting on the river (Table 59). Most of the ducks, preponderantly Mallards were concentrated between the abandoned townsite of Hanford and 100-F Area. Very few ducks rested on the river upstream from the D and DR Area. Although hunting was permitted downstream from Hanford, ducks and geese used this section of the river for resting purposes, but in smaller numbers. In mid-November, 7 bald eagles and 21 Great Blue Herons were counted along the Columbia River (Table 59).

Ecological Characterization of the 200 Area Plateau* W. H. Rickard

Ecological characterization studies of terrestrial ecosystems on the

200 Area plateau continued. Plant and animal populations with pertinent relationships to the storage and handling of radioactive waste were identified. Studies include vegetational analysis of selected plant communities, small mammal populations dynamics, dietary components of black-tailed hares, uptake of radionuclides by wild plants from 200 Area soil, dietary components of important insects, and their abundance and seasonality.

A document was prepared identifying the wild mammals important to waste management zones (Rickard et al., 1974). Twenty-four mammals exclusive of bats occur on or near the 200 Area plateau. The mule deer, black-tailed hare, coyote, raccoon, badger, ground squirrel, and great basin pocket mouse were identified as important on waste management zones, either as potential contributors of radionuclide transfers in the food chain that can lead to man; predators that can travel beyond the 200 Area and off the Hanford Reservation; mammals that provide an important food base for predators; or have the potential for burrowing deeply and exposing buried radioactive debris.

A document was prepared summarizing a two and a half year survey of seasonal abundance of avifauna associated with low-level waste ponds on the 200 Area plateau (Fitzner and Rickard, 1974). One hundred and twenty-seven species of birds were observed using these ponds and associated vegetation. The abundant waterfowl were the Canada goose, mallard, ringneck duck, American wigeon, bufflehead, scaup, and American coot. Maximal populations by species ranged between 100 and 1000 individuals and are usually attained during the autumn migration. Perching birds not strongly attracted to aquatic habitats per se were most abundant at U-pond, the pond with the oldest and best developed tree-shrub shoreline plant community. Riparian tree communities were very attractive to birds on an otherwise treeless landscape which was low in bird species and abundance of individuals.

Abundance, long residence time, food habits, and nesting populations identify the American coot as the

* Work Supported by ARCHO

TABLE 59. Aerial Counts of Birds Using the Columbia River in November, 1973.

¹ River Section	Ducks	Geese	Great Blue Heron	Golden Eagle	Bald Eagle
-----November 6, 1973-----					
I	1,987	277	4	0	0
II	587	13	0	2	0
III	11,633	86	0	0	1
IV	1,331	126	5	1	0
V	0	25	0	0	0
VI	0	0	0	0	0
Total	15,538	527	9	3	1
-----November 13, 1973-----					
I	5,700	78	5	0	0
II	5,515	448	0	0	0
III	10,212	0	12	1	0
IV	500	495	4	0	7
V	18	56	0	0	0
VI	0	210	0	0	0
Total	21,945	1,287	21	1	7

¹Section I = 300 Area to Ringold; II = Ringold to Hanford; III = Hanford townsite to 100-F Area; IV = 100-F to 100-D and -DR; V = from D and DR Area to N Area; VI = from D and DR to B and C Area.

most important bird of concern for the long distance dispersal of radionuclides from waste ponds.

Important considerations for future studies are dose to biota other than man, trophic level transfers of long-lived radionuclides in ecosystems and the behavior (uptake and retention) of radionuclides in individual animals living on or adjacent to waste ponds and soil-plant interactions over long time periods.

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- **TERRESTRIAL ECOLOGY**

- **GRASSLAND BIOME STUDY**

The Terrestrial Ecology program has undertaken, over a period of 10 years, to quantitatively characterize major ecosystems of the Arid Lands Ecology (ALE) Reserve. Two of the major landscape units are steppe-shrub and the grasslands systems. The Grasslands Biome Study, cosponsored with the National Science Foundation, looks primarily at the effect of grazing (cattle) stress on the vegetation dynamics of grasslands. The separate Terrestrial Ecology project looks at the effects of other stressors, and it provides the continuing basis for developing quantitative methodology suitable for studying terrestrial ecosystems. At this point, major efforts at characterization have been completed, extensive quantitative data have been stored in computer retrievable form, and statistical evaluation is in process. Also in progress are beginning synthesis and modeling efforts (see subsequent 189, "Animal Ecology").

Important new information was obtained during the 1973-74 growing season concerning the response of steppe region biota to environmental stresses: wildfire, cattle grazing, and an above-average year of precipitation. The most important forage grass, bluebunch wheatgrass, failed to respond to the improved moisture regime; however, cheatgrass responded by increasing yields. Cheatgrass communities continue to produce more dry matter per unit area than native perennial grass communities. Bluebunch wheatgrass showed substantial yield increases in dry matter production the year after wildfire. Apparently, burning removes the constraints present in unburned communities. The response of small mammal and bird populations to environmental stresses are reported, as well as information on weight gains and forage consumption by steers.

Environmental and Biological Stresses in Steppe Communities

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Environmental Stresses

During the annual cycle of growth and reproduction, terrestrial plants and animals experience periods of environmental stress. These stresses are droughts and periods with above-average warm or cold temperatures. Some stresses, such as wildfire, occur with erratic frequency.

Precipitation

The scarcity of water is an annual event in the steppe region, but some years have greater contributions of precipitation than others. Almost three times more precipitation was recorded in the 1973-74 growing seasons as in the preceding season (Table 60). The October to May precipitation was 200% greater during the 1973-74 growing season than in the 1972-73 season.

Primary Production in a Perennial Grass Community

The increased precipitation in 1974 as compared to 1973 resulted in a total herbaceous yield 29% greater (Table 61). The important feature, however, is that bluebunch wheatgrass, *Agropyron spicatum*, the dominant member of the community, did not grow in proportion to the increased moisture supply.

Primary Productivity in an Annual Grass Community

The annual grass community studied produced more dry matter than the perennial grass community in both 1973 and 1974, even though the total amount of precipitation received was

slightly less in this community (Table 60). The important observation here is that cheatgrass, *Bromus tectorum*, the dominant grass, responded to the increased precipitation in 1974 by a 100% increase in dry weight (Table 62).

Primary Productivity in a Perennial Grass Community After Wildfire

Wildfire is an important stress in steppe communities. A fire swept through the perennial grass community August 13, 1973, and effectively combusted aboveground plant material. However, the perennial grasses responded to the burning by regenerating shoots from ground-level crowns. The yield of perennial grasses, primarily bluebunch wheatgrass, was dramatically increased the year following burning (Table 63). However, yields of associated species were reduced. These data indicate that burning is a stimulus to growth of bluebunch wheatgrass, at least during the first season following summer burning. The important observation here is that burning removed the stress that was constraining herbage production in bluebunch wheatgrass.

Phenological Observations in a Perennial Grass Community

Phenology is the study of the cyclical occurrence of developmental

TABLE 60. Comparison of October-May Precipitation (cm for the 1972-73 and 1973-74 Growing Seasons in Perennial Grass and Annual Grass Communities).

	1972-1973		1973-1974	
	Perennial Grass Community	Annual Grass Community	Perennial Grass Community	Annual Grass Community
Oct.	0.3	0.1	4.6	5.0
Nov.	2.7	1.9	8.5	8.4
Dec.	3.8	4.1	8.9	7.7
Jan.	2.3	2.7	4.6	4.1
Feb.	1.7	1.3	4.4	2.4
Mar.	0.4	0.2	3.0	1.8
Apr.	0.8	0.9	1.5	1.3
May	1.0	0.5	0.6	0.8
Total	13.0	11.7	36.1	31.5

* NORCUS Appointee

TABLE 61. Comparison of Live Aboveground Biomass ($\text{g/m}^2 \pm \text{SE}$) at Peak Harvest Yield in a Perennial Grass Community in 1973 and 1974.

Taxa	1973	1974
Perennial grasses		
<u>Agropyron spicatum</u>	35.2 $\pm$ 4.5	38.6 $\pm$ 7.2
<u>Poa cusickii</u>	0.4 $\pm$ 0.4	2.0 $\pm$ 2.0
<u>Poa secunda</u>	0.4 $\pm$ 0.4	8.3 $\pm$ 1.4
<u>Stipa thurberiana</u>	0.3 $\pm$ 0.3	1.7 $\pm$ 1.2
Total	36.3	50.6
Perennial forbs		
<u>Crepis atrabarba</u>	0.1 $\pm$ 0.1	0.7 $\pm$ 0.5
<u>Calochortus macrocarpus</u>	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1
<u>Lomatium macrocarpum</u>	0.6 $\pm$ 0.3	0
<u>Lupinus sp.</u>	0.2 $\pm$ 0.2	0
<u>Astragalus sp.</u>	2.9 $\pm$ 1.8	3.6 $\pm$ 3.6
<u>Brodiaea douglasii</u>	0.01 $\pm$ 0.01	0
Total	4.0	4.5
Semi-woody perennials		
<u>Antennaria dimorpha</u>	3.1 $\pm$ 1.8	1.2 $\pm$ 1.1
<u>Phlox longifolia</u>	0	2.0 $\pm$ 1.5
<u>Erigeron filifolius</u>	7.6 $\pm$ 4.6	1.0 $\pm$ 0.8
Total	10.7	4.2
Annual herbs		
<u>Bromus tectorum</u>	0.2 $\pm$ 0.2	0.4 $\pm$ 0.2
<u>Descurainaea pinnata</u>	0.1 $\pm$ 0.1	1.3 $\pm$ 0.3
<u>Plantago patagonica</u>	0.01 $\pm$ 0.01	0.09 $\pm$ 0.06
<u>Festuca octoflora</u>	0.30 $\pm$ 0.10	4.3 $\pm$ 0.7
Total	0.61	6.0
Grand Total	51.6	65.3

TABLE 62. Comparison of Live Aboveground Biomass (g/m^2) at Peak Harvest Yields in an Annual Grass Community in 1973 and 1974.

Taxa	1973	1974
Annual herbs		
<u>Bromus tectorum</u>	166 $\pm$ 12	328 $\pm$ 16
<u>Sisymbrium altissimum</u>	34 $\pm$ 10	30 $\pm$ 7
<u>Holosteum umbellatum</u>	0.2 $\pm$ 0.2	Tr
<u>Amsinckia lycopsoides</u>	5.3 $\pm$ 3.6	Tr
Perennial grass		
<u>Poa secunda</u>	0.07 $\pm$ 0.07	Tr
Grand Total	205 $\pm$ 14	359 $\pm$ 25

Tr = Trace

TABLE 63. Comparison of Live Above-ground Biomass of Large Perennial Bunch Grasses in Adjacent Burned and Control Pastures, June 1974.

Taxa	Control	Burned
<i>Agropyron spicatum</i>	38.6 ± 7.2	61.4 ± 11.3
<i>Poa cusickii</i>	2.0 ± 2.0	0.3 ± 0.3
<i>Stipa thurberiana</i>	1.7 ± 1.2	0.8 ± 0.5
Total	42.3	62.5

phases, such as spring initiation of growth and flowering in plants. A comparison of phenological development in 1973 and a wet growing season in 1974 for the major species in a perennial grass community shows the different adaptation characteristics of these species. Field observations on the six principal species is shown in Figure 48. *Agropyron spicatum* (bluebunch wheatgrass) started growth and flowering later in 1974, and essentially grew or maintained some green leaves throughout most of the summer of 1974, in contrast to the early summer shoot senescence in 1973. *Poa secunda* (Sandberg bluegrass) initiated floral buds at approximately the same time in 1973 and 1974, but flowered a little later in 1974 and grew later into the summer. *Poa cusickii* (Cusick's bluegrass) started flowering about the same time in both years, but continued to flower and grow for a longer time in 1974. *Artemisia tridentata* (Big sagebrush) initiated growth and flower bud appearance a little earlier in 1974 than 1973 and also flowered earlier in 1974. Observations on this autumn flowering species are still being taken so the data are at this time incomplete. *Erigeron filifolius* (flea-bane) initiated floral buds slightly later in the wet year, but flowering and senescence were markedly delayed, with growth lasting essentially all summer. *Crepis atrabarba* (slender hawk's beard) appeared to open flowers about the same time in the wet and dry years, but maturing fruit and growth lasted longer in the wet year.

These data show the phenological response of these important species is not uniform. Perhaps water availability, as determined by different

FIGURE 48. Phenological Calendar of Events for Important Steppe Plants in 1973 and 1974.

rooting depths, dictates when growth stops. An alternate interpretation is that some species continue to grow as long as soil water is available, while others key on some other environmental characteristic, such as temperature or photoperiod.

Those species that can use water as long as available could be expected to grow more during wet years than those that go dormant while water is still available.

Avian Census in a Perennial Grass Community After Wildfire

The lightning-caused range fire which burned several thousand acres of the ALE Reserve in August 1973, also consumed most of a 9-hectare bird study plot. This provided a unique opportunity to observe the detailed effects of a natural alteration of habitat upon the population

structure of the avian community (horned larks, meadowlarks and sage sparrows) because this plot has been censused in the spring of each year since 1971. Two additional plots sampled currently in the spring of 1974 provided further comparisons. One plot is dominated by the Artemisia/Agropyron habitat type and essentially replicates the pre-burn vegetation of the original plot. The other consists largely of Agropyron only, having been burned in 1957, and is very similar to the recently burned area. The results of these censuses are presented in Table 64.

It is interesting to note that avian density and biomass show relatively little difference between burned and unburned areas, especially when considered against yearly variation. However, the apportionment of those numbers and weight among the species changes considerably. Species diversity decreases by an average 12% and species dominance increases over 20%. This is largely a result of the decreased structural heterogeneity of the vegetation brought about by the removal of almost all the sagebrush by fire. The nearly 20% change in biomass parti-

tioning, reflected by reduced biomass diversity, results from a reduction of mainly sagebrush-associated sage sparrows (small, 20 g) with a relative increase in horned larks (medium, 31 g). The average weight of the hypothetical "average bird" increased 18% post-burn.

This increase in size has important implications for energy flux through the bird community. A large organism requires less energy to support a gram of biomass than a smaller one. Consequently, by increasing the relative size of the individuals which make up a trophic level or occupy similar niches, a larger standing crop may be maintained on the same amount of available energy. In this case, we might suggest that an increase in bird size allows maintenance of pre-burn standing crop despite the post-burn reduction in available food in the form of insects.

Biological Stresses

The most wide-spread biological stress imposed upon steppe communities in western North America is that of livestock grazing, especially cattle and sheep.

TABLE 64. Results of Avian Censuses Taken Pre- and Post-Burn, 1971-1974.

Avian Community Attribute	Pre-burn			Post-burn, 1974		
	1971	1972	1973	Unburned "replicate"	Burned 1973	Burned 1957
Total density (birds/km ²)	291	206	230	240	223	204
Species diversity ¹	1.11	1.12	1.03	1.07	0.99	0.94
Dominance ²	0.49	0.46	0.49	0.44	0.56	0.60
Total biomass (kg live wgt/km ²)	11.51	7.71	9.34	10.31	9.94	10.31
Biomass diversity ¹	1.28	1.09	1.01	0.98	0.93	0.91
Avg. wgt/bird ³ (gm/individual)	39.6	37.4	40.6	43.0	44.6	50.5
Number of species	4	4	3	3	3	3

¹Diversity = $-\sum_{i=1}^n P_i \log_e P_i$ where P_i is the proportion of individuals (or biomass) belonging to the i th species

²Dominance - percent composition of the most abundant species

³Total Biomass ÷ Total Density

One of the aims of the International Biological Program's Grassland Biome studies is the investigation of the role of cattle grazing in native perennial grass communities representative of steppe vegetation in the Pacific Northwest.

Controlled grazing by yearling steers has been conducted for 4 consecutive years in previously ungrazed pastures on the Arid Lands Ecology Reserve. This report summarizes some of the salient features of cattle grazing on these pastures.

Growth Performance of Steers

The weight gains of steers over four spring grazing seasons are summarized in Table 65. The average gain in weight was 1.5 lbs per day and ranged between 1.40 and 1.56. The lowest gains were made in the spring of 1973 which was the driest of the 4 years. The weight gain per acre averaged 21.6 lbs.

Estimates of Forage Intake by Steers

The water intake method of determining forage intake was used in the spring of 1974. The results are summarized in Table 66. As expected, the moisture content of forage decreased as the growing season progressed. Water consumption by steers increased as daily air temperatures increased. Water consumption averaged 3.7 gal/head/day early in the spring and increased to 6.3 gal/head/day later in the spring.

The amount of forage consumed was estimated at 35 lbs per head per day at the beginning of the grazing season and only 18 lbs per head per day at the end of the grazing season. This is probably a reflection of the declining availability of palatable herbage at the end of the grazing period.

Cattle Consumption of Bluebunch Wheatgrass

Bluebunch wheatgrass, *Agropyron spicatum*, is the most important cattle forage in native grasslands in the steppe region of the Pacific Northwest. Bluebunch wheatgrass produced about 46 g of aboveground herbage per square meter during the spring of 1974 on ungrazed pastures (Figure 49). At the end of the cattle grazing season in 1974 the grazed pasture yielded only 18 g of bluebunch wheatgrass. The difference of 28 g/m² in a rough way represents the amount of grass consumed by cattle.

Potential Selectivity of Cattle

Cattle, while grazing in perennial grass communities, have the opportunity to select those species that are most palatable. As a general rule, cattle select herbage of higher than average nutrient quality. During 1973 and 1974, cattle fecal droppings were periodically collected and examined for botanical composition using a microscopic examination technique. Although more than 20 plant species grew in the pasture, four species

TABLE 65. Summary of Performance of Steers on a Spring Grazing Regime.

Year	Grazing Period	No. Steers	Acres	Days on Pasture	Initial Wt. of Steers (lb)	Final Wt. of Steers (lb)	Total Gain (lb)	Daily Gain/hd (lb)	Total Gain/Acre (lb)
71	4/14-6/01	15	44.5	58	6615	7970	1355	1.56	30.5
72	4/05-5/15	15	44.5	41	7742	8693	951	1.55	21.4
73	4/11-5/15	7	22.2	35	3528	3871	343	1.40	15.4
74	4/03-5/29	5	22.2	57	2545	2974	429	1.51	19.3

TABLE 66. Amount of Dry Forage Consumed by Yearling Steers During the Spring Grazing Period of 1974 as Estimated by the Water-Intake Method.

Grazing Period	No. Days	No. Steers	Moisture ¹ Content of Forage (adj.) %	Average Air Temp. (°F)	Daily Water Intake (gal)	Dry Matter Intake/Gal of Water Drunk (lb)	Dry Matter Intake (lb/hd/day)	Dry Matter ² Intake (adj.) (lb/hd/day)
4/02-4/19	17	5	72.5	51	3.7	10.4	38.7	34.8
4/20-5/01	12	5	72.6	56	4.1	8.3	34.1	29.9
5/02-5/17	16	5	65.3	56	5.2	4.7	24.5	20.8
5/18-5/29	12	5	59.3	60	6.3	3.5	22.0	18.3

¹Moisture content of the forage was adjusted for dietary composition of the steers.

²Forage intake adjusted to a constant metabolic size ($W^{0.75}$) to 100.

FIGURE 49. Comparison of Live Biomass of *Agropyron spicatum* in Grazed and Ungrazed Pastures During the Spring of 1974.

comprised more than 90% of the dietary intake (Table 67). *Agropyron spicatum* was by far the most abundant forage species in the field, and it also dominated the fecal material. *Poa cusickii* and *Crepis atrabarba*, although sparingly represented in the field, were well represented in fecal samples. These data support field observations that cattle preferentially select *P. cusickii* and *C. atrabarba* over *A. spicatum*, and indicate that stresses imposed by successive years of spring grazing will be most severe upon *P. cusickii* and *C. atrabarba*.

Belowground Biomass

Roots play an important role within the steppe ecosystem. They absorb water and essential mineral nutrients. Roots play important roles in soil development and stability. The effect of cattle grazing upon root systems in steppe communities has received little study.

The results show that root biomass in the upper 80 cm of soil profile varied significantly between sampling dates and among years (Table 68). There appears to be no consistent difference resulting from 4 consecutive years of moderate spring grazing by steers.

Moisture Stresses in Sagebrush Leaves

Leaf water potentials in sagebrush were measured during the 1974 growing season. The winter rainfall during this year was a record-breaking high, in contrast to the relatively dry 1973 season. Dawn and noontime leaf water potentials are illustrated in Table 69 for both years, for both grazed and ungrazed pastures. The difference between grazing treatments was not noticeable in 1973 but seems consistently observable in 1974, with the ungrazed pastures possessing lower potentials, indicating greater moisture stress.

TABLE 67. Average Botanical Composition of Cattle Fecal Material, 1973 and 1974.

Species	1973 (%)	1974 (%)
Perennial Grasses		
<u>Agropyron spicatum</u>	39.2	32.0
<u>Poa cusickii</u>	35.7	24.9
<u>Poa secunda</u>	1.2	0.3
<u>Stipa thurberiana</u>	5.4	11.9
Annual Grasses		
<u>Festuca octoflora</u>	N.O.	4.1
Perennial Forbs		
<u>Crepis atrabarba</u>	14.0	22.3
Annual Forbs		
<u>Descuriana pinnata</u>	0.1	1.4
Half-shrubs		
<u>Phlox longifolia</u>	0.3	1.2
Lichen	0.6	0.5
Miscellaneous	3.5	1.4
N.O. = Not Observed		

TABLE 68. Root Biomass g/m²/Ash-Free Weight in the Upper 8 dm of Soil Profile.

	Grazed Pasture		Ungrazed Pasture	
1972				
Mar	776 ± 125		693 ± 66	
Apr	1033 ± 150	Avg. 1300	853 ± 71	Avg. 1050
May	1601 ± 241		1325 ± 92	
Oct	1799 ± 180		1338 ± 226	
1973				
Mar	1022 ± 107		946 ± 85	
Jun	991 ± 92	Avg. 1025	1226 ± 126	Avg. 1010
Sep	805 ± 79		855 ± 76	
1974				
Mar	1025 ± 91	Avg. 1100	924 ± 83	Avg. 1010
May	919 ± 128		795 ± 75	

TABLE 69. Significance Attached to Combined Observations of Difference Between Grazed and Ungrazed Pastures Upon Leaf Water Potential in Leaves of Big Sagebrush.

Time Of Day	Year	Difference Between Grazed and Ungrazed (bars)	No. of Tests	(Chi) ²	df	Probability of Occurrence
Dawn	1973	+0.04	10	39.0	20	6 x 10 ⁻³
	1974	0.6	13	87.9	26	1 x 10 ⁻⁸
Noon	1973	-0.6	11	62.8	22	9 x 10 ⁻⁶
	1974	-1.0	4	43.8	8	6 x 10 ⁻⁷
All	--	-0.5	38	233.5	76	<10 ⁻¹²

A test of this expectation was performed using nearly 1000 individual readings from pressure chamber estimates of water potential, using an unusual statistic to combine several observations of the differences between grazed and ungrazed treatments. The statistic is

$$-2 \sum_{i=1}^k (\log_e P_i),$$

where P_i is the probability of a t-statistic smaller than that observed each observation period (Winer, 1962.) This statistic combines several observations of differences resulting from several independent tests of the same hypothesis, allowing an overall estimate of the probability of whether a difference truly exists. This statistic is distributed as chi-square, and the results are summarized in Table 69. The differences between grazed and ungrazed values are not due to chance; it is concluded that grazing alters sagebrush water relations favorably, probably through reduction of foliage of associated plant species by cattle.

Anomalous behavior was observed both years in mid- to late June; in 1973, the morning potentials were transiently higher than the afternoon readings, contrary to all other reading periods, and in 1974 there was an abrupt increase in dryness of the shrubs in mid-June. Both of these

phenomena may be related to changes in phenophase at this time because sagebrush seems to change from vegetative to reproductive growth. Sagebrush flowers are initiated at about this time, but floral bud expansion is very slow; full flowering activity is not reached until October (Figure 48).

Small Mammal Populations 1973 and 1974

Small mammal populations were studied using a live-trap, mark and recapture technique. Five species were consistently captured in live traps. These were the deer mouse, Peromyscus maniculatus; pocket mouse, Perognathus parvus; grasshopper mouse, Onychomys leucogaster; ground squirrel, Citellus townsendii; and the sagebrush vole, Lagurus curtatus. The pocket gopher, Thomomys talpoides, was caught only once.

The pocket mouse was the most often trapped animal. In 1973 the peak catch in the grazed pasture was 35 animals in April. The peak catch in the ungrazed pasture was 31 animals in March (Table 70). In 1974 the peak catch was 150 animals in October in the grazed pasture and 95 animals in the ungrazed pasture, also in October.

The autumn influx of animals in 1974 is attributed to juvenile animals entering the population. This

TABLE 70. Small Mammal Trap Catches in Grazed and Un-grazed Pastures During 1973 and 1974.

Species	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Total Captures
-----Grazed Pasture-----										
<u>1973</u>										
Deer mouse	4	8	8	3	1	0	-	1	1	26
Pocket mouse	2	24	35	28	23	20	-	8	5	145
Grasshopper mouse	3	3	3	6	2	2	-	2	1	22
Sagebrush vole	0	2	0	0	0	0	-	0	0	2
Ground squirrel	10	14	21	17	1	0	-	0	0	63
<u>1974</u>										
Deer mouse	-	0	7	1	1	0	0	-	0	9
Pocket mouse	-	5	26	25	17	6	41	-	150	280
Grasshopper mouse	-	1	2	2	4	2	1	-	3	15
Sagebrush vole	-	0	0	0	0	0	0	-	0	0
Ground Squirrel	-	9	35	27	0	0	0	-	0	71
-----Ungrazed Pasture-----										
<u>1973</u>										
Deer mouse	7	10	4	2	1	1	¹	1	0	25
Pocket mouse	0	31	28	20	15	16	-	7	0	117
Grasshopper mouse	3	3	2	2	3	1	-	3	3	20
Sagebrush vole	1	2	1	0	0	0	-	0	0	4
Ground squirrel	7	16	27	25	2	0	-	0	0	77
Pocket gopher	0	0	1	0	0	0	-	0	0	1
<u>1974</u>										
Deer mouse	-	3	9	1	2	0	0	-	0	15
Pocket mouse	-	6	26	16	5	15	41	-	95	204
Grasshopper mouse	-	1	0	1	1	3	2	-	0	8
Sagebrush vole	-	1	0	0	0	0	0	-	0	1
Ground squirrel	-	9	40	25	2	0	0	-	0	76

- = No trap session
¹Wildfire occurrence

influx is coincident with a better than average moisture year and presumably an increase in mouse food. It is interesting to note that the dominant grass in the grazed pasture had very few seeds available for pocket mice since grazing cattle removed the seedbearing heads as they were being formed.

The ground squirrel was the most important small mammal in terms of live weight. They were present aboveground from February to June and disappeared with the onset of summer aestivation. A peak catch of 21 animals was recorded in the grazed pasture and 27 in the ungrazed pasture in April 1973 (Table 70). In 1974

the peak catch in the grazed pasture amounted to 35 animals, an increase of 14 animals over 1973. The peak catch in the ungrazed pasture was 40 animals, an increase of 13 animals over 1973.

These data suggest that pocket mice and ground squirrels, both vegetation eaters, responded to a salubrious precipitational regime by increased populations during the 1974 growing season.

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Manipulation of Herbaceous Ground Cover by Irrigation and Selective Herbicide Applications

J. F. Cline and D. W. Uresk

Three 5.75 hectare study areas were established in an Artemisia tridentata (sagebrush)-Bromus tectorum (cheatgrass) community located near Rattlesnake Springs on the ALE Reserve. One site was irrigated with an automated overhead sprinkling system to simulate a year of high precipitation. A selective herbicide was applied to the second to kill Bromus tectorum. The third site remained as control with no manipulation.

In early June of 1974, percent herbaceous cover was estimated using 0.1 m² quadrats placed at 1 m intervals along 50 m long line transects. Two hundred quadrat measurements were made along four 50 m transects in each

treatment. Each species was estimated separately; however, the data are summarized in three groups--cheatgrass, all-species-except-cheatgrass, and sum of all species.

Cheatgrass was the dominant species on the control and irrigated sites (Table 71). Irrigation increased ground cover by cheatgrass from 38% in control plots to 71% in the irrigated plots. The herbicide was very effective in controlling cheatgrass germination and growth. The herbaceous ground cover among control, herbicide, and irrigated plots was significantly different ($P < 0.001$). The all-species-except-cheatgrass category consisted mostly of annual forbs that were not as responsive to irrigation or herbicide treatments as cheatgrass. Using the Kuels multiple range test, the following statistical relationships of the forbs were determined. The percent cover between the irrigation and control plots was significantly different ($P < 0.013$). However, the difference between percent cover of the irrigation and herbicide plots, and between the herbicide and control plots were not significantly different. Therefore, the herbicide treatment did not reduce forbs. The reduced forb cover in the irrigated plots may be due to the intensive competition from the cheatgrass. This crowding out of forbs as Russian thistle by cheatgrass is an important consideration for management of waste burial grounds because forbs generally are better accumulators of radioactive nuclides from soils than grasses and also generally have a taproot that

TABLE 71. Percent of Herbaceous Ground Cover on Irrigated, Herbicide and Control Plots, June 1974.

Treatments	Cheatgrass Only	All Species Except Cheatgrass	Total
Control	38.2 ± 2.0	7.74 ± 0.89	46.0 ± 1.4
Herbicide	0.64 ± 0.29	5.11 ± 1.0	5.75 ± 1.0
Irrigation	70.9 ± 1.7	3.14 ± 0.5	74.0 ± 1.4

$\bar{X} \pm S.E.$
 $n = 200$

reaches deeper into the soil profile and therefore a greater potential for growing into buried waste zones.

Plant Ecology Studies with Practical Applications to Management of Waste Burial Grounds

W. H. Rickard, J. F. Cline,

D. W. Uresk and R. H. Sauer

Technical Assistance: V. D. Charles

Plants play fundamental roles in food chains, especially the uptake of radionuclides from soil by roots and transfers to herbivorous animals. Plants also play an important role in returning soil moisture derived from precipitation back into the atmosphere through the evapotranspiration process. Water not returned to the atmosphere does not accumulate in the soil but

eventually percolates downward through buried waste and on to ground water with a potential for uncontrolled long distance dispersal.

Historically, radioactive debris has been deposited in open trenches and backfilled with soil to conform to existing land forms. Trench excavation destroys the existing vegetation and displaces or destroys animal populations at least temporarily. Revegetation of backfilled trenches at Hanford has mostly been through the natural mechanisms of seed dispersal. Because backfill is often a heterogeneous mixture of soil and rocks, vegetation cover remains patchy even after a decade or more of time after backfilling (Figure 50) and favors the establishment and

FIGURE 50. Backfill Surface with Abundant Rock Content and Sparse Vegetative Cover.

growth of Russian thistle rather than cheatgrass. Russian thistle is undesirable because of a high affinity for ^{90}Sr .

The elements of biological restoration of waste burial grounds are not necessarily the same as those deemed desirable by livestock grazers, wildlife managers, or agronomists. Some of the desirable features of vegetation as cover for waste burial sites are listed below.

- A self-sustaining plant community capable of resisting soil erosion over long periods of time (decades, centuries) without added water or fertilizer.
- A community comprised of only a few species, all shallow rooted with low mineral nutrient requirements and low affinity for radionuclide uptake.
- A plant community that is effective in using soil water derived from precipitation each year.
- A plant community that is not attractive to wildlife as food or shelter, especially those animals that have potential to burrow deeply and/or to travel long distances (tens of miles) and can be killed and eaten by people.

Prior to the establishment of the Hanford Reservation in the early 1940's, native plant communities were

destroyed and replaced by agricultural crops. After the establishment of the reservation, these fields were abandoned and re-invasion by plants and animals has been in progress for 30 years. Observations show that the most important plant colonizer is the alien grass, cheatgrass. After 30 years, cheatgrass maintains a dominant role in the botanical composition of these fields (Table 72) and can effectively compete with Russian thistle. Field observations and samples taken from abandoned fields show that it is reasonable to expect cheatgrass to provide many of the desirable plant community features needed for long-term maintenance of burial grounds.

The shallowness of roots in a cheatgrass community is illustrated in Figure 51. Ninety percent of the root biomass is confined to the upper 3 dm of soil profile. Few roots are present at a depth of 8 dm. The Hanford Reservation precipitational regime is one of fall and winter precipitation followed by a long period of summer drought. Cheatgrass normally begins growth in the fall with the onset of the rainy season. Soil water accumulates during winter and is depleted in the spring when warming temperatures accelerate plant growth which results in depleted soil water before the onset of the summer drought (Table 73).

Cheatgrass is not known as an avid mineral nutrient accumulator. Potassium, nitrogen, and calcium are the

TABLE 72. Botanical Composition and Aboveground Yield of Dry Matter in a 30-year-old Cheatgrass-dominated Community, May 7, 1973.

Taxa	Yield g/m ² dry wt.	Percent of total
Cheatgrass	166	81
Tumble Mustard	34	17
Filaree	5	2
Fiddleneck Tarweed		
Tansy Mustard		
Sandberg Bluegrass		
Total	205 ± 14	

abundant mineral constituents of cheatgrass tissues (Table 74). Less than 1% of the dry weight of tissues is comprised of any one of these important nutrients.

Cheatgrass communities retard soil erosion, are self-sustaining, resist invasion of Russian thistle, have relatively shallow root systems, and have a low requirement for mineral nutrients.

FIGURE 51. Distribution of Root Bio-mass in the Upper 8 dm of Soil Profile in a Cheatgrass Community.

TABLE 74. Mineral Content of Live Cheatgrass Shoots Harvested from a 30-year-old Cheatgrass Community May 7, 1973.

	Average Percent Dry Wt. ± S.E.
Potassium	.84 ± .006
Nitrogen	.80 ± .012
Calcium	.48 ± .020
Magnesium	.23 ± .009
Phosphorus	.12 ± .006
Sulfur	.08 ± .029
Total	2.55

TABLE 73. The Distribution of Soil Water (Percent Dry Weight) in the Upper Meter of Soil Profile in a 30-year-old Cheatgrass Community, 1973.

Soil Depth (dm)	Growth Water Available					Growth Water Not Available						12/4
	1/17	2/16	3/6	3/21	4/11	5/3	5/30	7/20	8/2	9/10	10/10	
0-1	19.6	15.6	12.0	9.2	6.1	4.4	3.5	1.2	1.8	3.1	5.8	16.2
1-2	16.5	14.0	11.3	10.2	6.8	5.4	4.7	2.6	3.5	4.1	3.6	14.5
2-3	15.1	14.2	11.2	11.0	7.3	6.5	5.5	3.9	5.1	5.4	4.4	13.9
3-4	13.6	13.5	10.9	11.4	7.8	6.5	5.6	4.3	5.9	6.6	5.7	13.6
4-5	10.6	13.4	10.8	11.6	8.7	6.7	5.9	4.6	6.0	6.6	6.3	13.2
5-6	9.1	13.4	10.4	11.7	9.5	6.6	6.1	4.6	5.5	6.7	6.6	12.5
6-7	7.9	12.1	9.8	11.6	10.1	7.3	6.2	4.8	6.0	7.1	6.9	10.6
7-8	7.0	10.9	9.1	10.6	9.6	7.7	6.6	4.8	5.8	7.3	7.6	10.9
8-9	7.2	12.1	9.2	10.7	9.6	8.1	6.8	5.1	5.5	6.9	7.9	9.7
9-10	7.1	10.9	6.2	9.0	10.2	9.5	6.9	4.5	6.9	7.9	7.5	7.3

• ANIMAL ECOLOGY

The Animal Ecology Project is an integral part of the terrestrial ecology program as described in the preceding section. For convenience, it is reported in a separate 189-schedule because of the specialized nature of its technics. It includes studies to characterize faunal populations taxonomically and to estimate density and biomass of important mammal, bird, herpetofauna, and invertebrate populations. Important small mammals of the ALE Reserve have been fairly well characterized during the past 5 years, and effort has shifted during the past year to other major fauna of the Reserve. Projected future studies include animal populations in relation to the biologic dispersal radionuclides from waste burial grounds and ponds. Dietary analyses are performed to construct factual food web diagrams. Radiochemical analyses of animal foods and animal tissues provide information useful for estimating dose received by individual animals living in waste management zones.

An intensive 4-year study of the behavior of small mammals in response to irrigation and selective herbicides reached a technical termination point in December 1974 and the results will be published in the open literature.

Nematode Biomass and Density in the Shrub-Steppe Ecosystem L. E. Rogers and J. Smolik*

The role of soil nematodes in grassland ecosystems appears to closely parallel that of other invertebrate organisms. They occupy phytophagous, carnivorous and saprophagous trophic levels. A listing of major nematode taxa and associated trophic levels is shown in Table 75. A discussion of nematode abundance was included in last year's (1973) report. Further analysis of these data now permits expres-

sion of density and biomass values on a unit area basis.

Highest densities occurred in the top 20 cm of the soil (Figure 52). This is the area of greatest root concentration--indicating a close association between rooting depth and nematode abundance. An estimate of total density and biomass values is shown in Table 76. The highest density and biomass values occur in an ungrazed area although it is not yet known if the impact of cattle grazing results in significant reductions. Nematode biomass estimates for grazed and ungrazed study areas (0.3 and 0.4 g/m² respectively) are higher than current estimates of either insect or small mammal biomass.

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TABLE 75. Major Nematode Taxa and Associated Trophic Levels.

Phytophagous	Predaceous	Saprophagous
<u>Aphelenchoides</u> spp.	<u>Aporcelaimellus</u> sp.	<u>Acrobeles</u> sp.
<u>Axonchium</u> sp.	<u>Discolaimus</u> sp.	<u>Acrobeloides</u> sp.
<u>Ditylenchus</u> sp.	<u>Eudorylaimus</u> sp.	<u>Aphelenchus</u> sp.
<u>Heterodera</u> larvae		<u>Cephalobus</u> sp.
<u>Nothotylenchus</u> sp.		<u>Cervideilus</u> sp.
<u>Paratylenchus</u> sp.		<u>Chiloplacus</u> sp.
<u>Pungentus</u> sp.		<u>Eucephalobus</u> sp.
<u>Scutellonema</u> sp.		
<u>Steineria</u> sp.		
<u>Tylenchorhynchus acutus</u>		
<u>Tylenchorhynchus</u> sp.		
<u>Tylenchus</u> spp.

FIGURE 52. Relationship Between Soil Depth and Nematode Abundance.

A more intensive sampling plan was conducted during 1974 with collection of three data sets at initiation of growth, during peak standing crop and at the end of the growing season. Samples were also collected from a severely burned area to assess the effects of fire on the nematode fauna. These results will be included next year.

Dietary Analysis of the Side-Blotched Lizard, *Uta stansburiana stansburiana*, from a Big Sage Community in South-eastern Washington
J. K. Sheldon* and L. E. Rogers

The feeding habits of the Side-blotched lizard, *Uta stansburiana stansburiana*, were analyzed during the summer of 1974 to determine the position of these species in the food web in a big sagebrush (*Artemisia tridentata*) community. As a known insectivore and conspicuous component of the desert biome, *U. s. stansburiana* may account for a significant portion of the energy flow associated with the insect population. To the extent that waste nucleides are incorporated in insect tissue, this species may also play a role in the dispersal of these waste products. The purpose of this report is to present preliminary results of the diet analysis.

Thirteen adult lizards were collected during July 1974. Upon capture, the adults were immediately killed and

* NORCUS appointee

TABLE 76. Nematode Density and Biomass in the Shrub-Steppe Ecosystem.¹

Soil Depth ²	Phytophagous		Predaceous		Saprophagous		Total	
	Density	Biomass	Density	Biomass	Density	Biomass	Density	Biomass
-----Grazed-----								
0-5	839.1	36.7	0.4	0.1	896.5	80.7	1,736.0	117.5
5-10	732.3	35.7	19.2	6.0	738.5	66.5	1,490.0	108.2
10-20	371.4	22.3	33.6	10.5	243.0	21.9	648.0	54.7
20-30	217.0	12.3	0.0	0.0	94.0	8.5	311.0	20.8
30-40	123.0	6.0	8.0	2.5	29.0	2.6	160.0	11.1
40-50	102.0	4.2	0.0	0.0	69.0	6.2	171.0	10.4
50-60	<u>62.0</u>	<u>2.8</u>	<u>4.0</u>	<u>1.2</u>	<u>28.0</u>	<u>2.5</u>	<u>94.0</u>	<u>6.5</u>
Total	2,446.8	120.0	65.2	20.3	2,098.0	188.9	4,610.0	329.2
-----Ungrazed-----								
0-5	1,037.5	43.8	276.0	86.2	1,190.5	107.1	2,504.0	237.1
5-10	560.5	27.3	24.0	7.5	606.0	54.5	1,190.5	89.3
10-20	480.0	28.0	12.0	3.7	281.0	25.3	773.0	57.0
20-30	115.0	5.5	0.0	0.0	92.0	8.3	207.0	13.8
30-40	130.0	6.4	12.0	3.7	100.0	9.0	242.0	19.1
40-50	72.0	2.8	0.0	0.0	70.0	6.3	142.0	9.1
50-60	<u>99.0</u>	<u>4.6</u>	<u>0.0</u>	<u>0.0</u>	<u>38.0</u>	<u>3.4</u>	<u>137.0</u>	<u>8.0</u>
Total	2,494.0	118.4	324.0	101.1	2,377.5	213.9	5,195.5	433.4

¹Density expressed as thousands of nematodes/m². Biomass expressed in mg/m² dry weight.

injected interperitoneally with 95% ETOH to stop all digestive processes. The stomachs were later removed and the contents examined.

Table 77 shows a broad selectivity of food types, indicating an opportunistic type of feeding behavior. Three groups of non-insect arthropods were encountered. Of these, red mites and spiders appear to occupy an important position in the overall diet. In-

sects representing 8 orders in 17 identified families comprised the remainder of the diet. Nematode parasites were found in two individuals and a hynenopterian parasitoid which was contained in an ingested leafhopper was found in a third. In addition, leaf fragments, a seed, and numerous grains of sand were encountered. These latter components were undoubtedly present due to accidental ingestion.

TABLE 77. Dietary Composition of the Side-Blotched Lizard, Uta stansburiana stansburiana, from a Big Sage Community in Southeastern Washington.

Taxa	Total Number In Stomach Contents	Number of Lizards Containing Food Item	Mean Number Per Lizard
Non-Insect Arthropoda			
Acarina (Erythraeidae)	262	8	20.15
Araneida	12	8	0.92
Solpugida	3	3	0.23
Insects			
Machilidae	2	1	0.15
Acrididae	10	6	0.77
Mantidae	1	1	0.08
Tettigoniidae	1	1	0.08
Dictyopharidae	1	1	0.08
Cicadellidae	3	1	0.23
Cydnidae	1	1	0.08
Lygaeidae	10	5	0.77
Nabidae	1	1	0.08
Bruchidae	1	1	0.08
Chrysomelidae	3	1	0.23
Curculionidae	1	1	0.08
Bombyliidae	1	1	0.08
Chalcidoidea	1	1	0.08
Formicidae	2	2	0.15
Halictidae	1	1	0.08
Tiphiidae	2	1	0.15
Unidentified Hemiptera	1	1	0.08
Unidentified Coleoptera larvae	12	1	0.92
Unidentified Lepidoptera larvae	4	2	0.31
Unidentified Diptera	1	1	0.08
Unidentified Hymenoptera	1	1	0.08
Other			
Nematode parasite	2	2	0.15
Hymenoptera parasitoid from Cicadellidae	1	1	0.08
Leaf fragments	2	1	0.15
Seeds (<u>Bromus tectorum</u>)	1	1	0.08

Pitfall Trap Survey of Darkling
Beetles in Greasewood and Sagebrush
Communities
L. E. Rogers

Ground dwelling darkling beetles (Tenebrionidae) are an abundant animal component of semi-arid regions of the western United States and occasionally become so numerous as to constitute a serious pest to agriculture. Little is known concerning beetle pop-

ulations in natural areas or what might have constituted a "normal" range of beetle density in greasewood (Sarcobatus vermiculatis) and sagebrush (Artemisia tridentata) communities during the fall of 1973.

Adjacent stands of greasewood and big sagebrush were selected as study sites. Pitfall collections were initiated in September and continued through the first part of December.

Forty-nine pitfall traps were used in each study area. Seven beetle species were caught from the sagebrush community and only five species from the greasewood community (Table 78). More beetles were collected from the sagebrush plot. The major difference between these communities was the abundance of Philolithus densicollis and Stenomorpha puncticollis. Stenomorpha was abundant on the greasewood plot but occurred infrequently on the sagebrush plot. Philolithus, on the other hand, was abundant in the sagebrush area but occurred infrequently in the greasewood community. Coniontis setosa also occurred much more frequently in greasewood pitfall traps. These differences are attributed to vegetative differences of the respective areas. A dense stand of cheatgrass, Bromus tectorum, occurred within the greasewood community while the sagebrush community supported a more open herb understory with a prevalence of bare ground. We postulate that the more open sagebrush community favors Philolithus populations while the dense understory associated with the greasewood community favors Coniontis and Stenomorpha populations. The mechanisms involved that favor the propagation of one beetle species over another in plant communities that differ in density are unknown at this time.

Ecological Data Synthesis--Trophic Analysis

L. E. Rogers

A data synthesis effort involving most members of the terrestrial ecology staff is currently underway in an attempt to integrate study results covering all phases of ecosystem analysis. The goal of this effort is to describe both the structure and functioning of the shrub-steppe ecosystem. A major effort during the past 4 years consists of the grassland biome--Arid Lands Ecology studies designed to obtain quantitative data on plant productivity using harvest methods and animal productivity using a variety of collecting techniques including mark-release-recapture as well as direct counting. Data are available in computer retrievable form and are currently undergoing preliminary synthesis scanning.

As a first step, biomass values are being extracted from various data sets established for the ALE Reserve (i.e., producer, mammal, invertebrate, etc.) and assigned to appropriate trophic levels. Comparisons may then be made of the size and shape, and seasonal differences between pyramids constructed for the shrub-steppe and other ecosystems. A representative pyramid is shown in Figure 53 constructed for May 1973. As with all

TABLE 78. Pitfall Catches of Darkling Beetles in Greasewood and Sagebrush Communities, Autumn 1973.

Species	Communities	
	Greasewood	Sagebrush
<u>Eleodes humeralis</u>	0	4
<u>Eleodes novoverrucula</u>	0	3
<u>Eleodes hispilabris</u>	5	9
<u>Eleodes nigrina</u>	12	7
<u>Coniontis setosa</u>	56	23
<u>Philolithus densicollis</u>	49	395
<u>Stenomorpha puncticollis</u>	370	99
Total	492	540

FIGURE 53. Trophic Pyramid of Biomass, Showing Relationship of Producers and Consumers in Terms of Dry Weight for May 1973, Based on ALE Data Sets.

trophic pyramids, plants form the first trophic level and support the higher level consumer groups. The producer level is comprised of live vegetation, litter, standing dead vegetation, and grass crowns. The percentage composition of each component is shown in Figure 54. Insects dominate the total biomass of both first and second consumer levels, followed by small mammals which account for an appreciable portion of the primary consumer group (22%) but only about 1% of the secondary consumer total. Passerine birds are nesting and primarily insectivorous during May. The greatest portion of their biomass is therefore reflected in the secondary consumer level. Coyotes, bobcats, hawks, and owls are all top carnivores of the area. Biomass estimates for hawks and owls were assumed to be identical with the shortgrass prairie of northeastern Colorado since the raptor density between the two areas was found to be about the same (Olen-dorff, 1973). Coyote and bobcat biomass estimates were made, assuming densities of one per 5 square miles and one per 20 square miles, respectively. Badgers also occur in the area, but the paucity of data concerning their density precludes a biomass estimate at this time.

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Raptor Nesting on the Hanford Reservation

R. E. Fitzner and L. E. Boyd*

The relatively undeveloped land of the Hanford Reservation and its isolation from resident human populations make it attractive to nesting raptors. One of the most abundant raptors is the Swainson's Hawk (*Buteo swainsoni*). There has been a general decline in abundance of the larger birds of prey in North America, mostly attributed to changes in land use patterns imposed by expanding human populations. On the Hanford Reservation, Swainson's Hawks prefer to nest in trees 8 to 30 ft aboveground (Table 79). Historically, trees are scarce on the Hanford Reservation and are confined to a few scattered locations along water courses in the Rattlesnake Hills and along the Columbia River. However, with the advent of agriculture and industry, trees have been planted as ornamentals. Once root systems have developed, trees can survive for many years without further need of irrigation. Trees around abandoned farmsteads and military installations now provide opportunities for hawk nesting and permit exploitation of territory not available prior to tree plantings. Even so, only 10 active nests were located on the Hanford Reservation west of the Columbia River. This amounts to only one nest per 40 square miles (26,000 acres).

Bird Use of Riparian Vegetation

R. E. Fitzner and J. T. Rotenberry**

Although steppe vegetation does not support diverse nor abundant bird populations, riparian tree and shrub communities are especially attractive to song birds. Riparian vegetation provides nest and perching sites as well as food and shelter during periods of inclement weather.

One of the few scattered riparian plant communities not associated with the Columbia River is the community in Snively Gulch on the Arid Lands Ecology Reserve. Here the vegetation

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FIGURE 54. Percentage Composition of Producer and Consumer Trophic Levels in Terms of Dry Weight, Based on ALE Data Sets.

TABLE 79. Location of Active Swainson's Hawk Nests and Number of Young per Nest, July 11 and 12, 1974.

Location	Nest Support	Number of Young
Gate 119	Tree	3
Gate 120	Tree	1
300 Area	Tree	Young known to be in nest before 7/12/74
200 East	Tree	2
Hanford	Tree	2
100 F	Tree	Dead chick in nest
100 H	Tree	Nest inaccessible, live young observed in nest
Exxon	Tree	3
White Bluffs	Tree	2
100 D	Tree	2
Total Banded Young		15

consists of a narrow band of deciduous trees and shrubs with cottonwood, willow, blue elderberry, chokecherry, golden current, mock orange, smooth sumac, service berry, wild rose, and Virgin's bower forming a dense tangle. An abandoned farmstead is located at the head of Snively Gulch and supports a few black locust and white poplar trees which add to the arboreal aspects of the vegetation.

Thirty-five species were recorded from Snively Gulch in the summer of 1974 (Table 80). The Western Meadow

Lark, Chukar, and Vesper Sparrow were abundant but were associated with the grassland vegetation adjacent to the tree-shrub community. Mourning Doves used the trees and shrubs as perches and probably nested in trees and shrubs as well as on the ground. California Quail were scarce although superficially the habitat seemed favorable. Fourteen Sage grouse were observed on July 2. These data are of interest because they represent bird populations remote from agricultural disturbances, urban residential activity, and industrial operations.

TABLE 80. Abundance of Birds in Snively Gulch, Summer 1974.

Species	6/19	7/2	7/17	8/6	8/21
Brewer's Blackbird	1	21	2	-	-
Eastern Kingbird	1	1	4	5	2
Western Meadowlark	6	17	32	9	35
Mourning Dove	30	39	42	24	38
Black-billed Magpie	7	5	1	4	17
Bullock's Oriole	10	14	9	3	1
California Quail	1	1	-	-	1
Barn Swallow	3	15	5	13	9
Killdeer	1	-	-	-	-
Yellow-breasted Chat	3	2	1	1	-
Lazuli Bunting	2	5	5	10	8
Rock Wren	1	1	4	-	-
Red-tailed Hawk	1	-	1	-	-
Sparrow Hawk	1	-	-	-	3
Song Sparrow	1	2	1	-	-
Loggerhead Shrike	1	-	2	-	-
Canyon Wren	-	1	-	-	-
Sagegrouse	-	14	-	1	-
Audubon's Warbler	-	1	-	-	-
Vesper Sparrow	-	10	2	39	27
Lark Sparrow	-	-	3	-	-
Western Kingbird	-	1	1	-	-
Western Tanager	-	1	1	10	-
Chukar	-	4	-	21	72
Sage Grouse	-	-	-	1	-
Golden Eagle	-	-	-	2	-
Rufous Hummingbird	-	-	-	1	1
Traill's Flycatcher	-	-	-	-	3
Wilson's Warbler	-	-	-	-	23
House Wren	-	-	-	-	2
Red-shafted Flicker	-	-	-	-	1
Nashville Warbler	-	-	-	-	1
Long-billed Marsh Wren	-	-	-	-	2
Savannah Sparrow	-	-	-	-	3
Western Wood Pewee	-	-	-	-	3
Total Species (35)	16	19	17	15	20

Heron Nesting on the Hanford
Reservation
W. H. Rickard

One of the largest and most conspicuous birds associated with the avifauna of the Hanford Reservation is the Great Blue Heron (*Ardea herodias*). Although the Great Blue Heron has historically been associated with the Columbia River, nesting has been restricted by the scarcity of large trees. The present nesting population on the Hanford Reservation is located near the White Bluffs ferry landing in a large deciduous tree (*Populus alba*) rooted on the west bank of the Colum-

bia River. The nearest known other colony is located immediately below McNary Dam, Umatilla County, Oregon. The Great Blue Heron (Figure 55) and Black-crowned Night Heron, the smaller, are of interest to radiological and ecological monitoring because of their ability to fly great distances (tens of miles) in search of food and because the diet consists of fish, frogs, garter snakes, and other prey associated with waste ponds. As a resident bird, the Great Blue Heron derives its food supply from a relatively small geographic region as compared to the long distance travels of migratory waterfowl (hundreds of miles).

FIGURE 55. Great Blue Heron Nesting on the Hanford Reservation.

• ECOLOGICAL MICROMETEOROLOGY AND CLIMATOLOGY

Precipitation data obtained from the network of climatological stations on the Arid Lands Ecology (ALE) Reserve since 1969 were analyzed for similarities of precipitation regimes and were related to precipitation events at the meteorological station located in the 200 Areas. A cluster analysis of regression coefficients is displayed as a dendrogram, revealing only two distinctive groups as stations on the ALE Reserve.

Wind is an important factor in waste management zones, particularly in the movement of surface soils that may be denuded of vegetation by fire. The ability of plants to re-colonize burned areas is an important consideration in maintaining surface soil stability. Wind erosion in relation to soil texture is presented in this section of the annual report.

Similarities Between Precipitation Regimes of the ALE Reserve

W. T. Hinds, J. M. Thorp* and
J. T. Rotenberry**

Patterns of precipitation on the Rattlesnake Hills and nearby lowlands have been presented annually since 1969. Generally, these patterns have indicated that precipitation increases more or less uniformly with elevation, with an indication that the eastern end of the hills may receive less than the western end. The relatively short record from the ALE network may be extended by relating the individual stations to the 29-year record from the Hanford Meteorological Tower and most readily by regression analyses of the ALE precipitation on the Tower data. The regressions showed only that the average intercept for the 26 stations was zero (indicating that rain on the Reserve coincided with rain at the Tower),

and yielded a uniform distribution between 1.0 and 1.7 of regression coefficients (which measures rain at the ALE sites per unit received at the Tower).

Precipitation regimes on the ALE Reserve were examined for similarities between stations by subjecting the regression coefficients to a cluster analysis (weighted pair group clustering). This type of analysis can be displayed as a dendrogram that exhibits the degree of differentiation between dissimilar sites, and the degree of similarity between closely related sites. The dendrogram for the precipitation analysis is shown in Figure 56, where the array of sites can be seen to be differentiated into two quite distinct groups, each of which contains two distinct sub-groups, within which less distinct groups may be discerned. To illustrate the geographical characteristics of the various groupings, the stations which are similar to a given

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FIGURE 56. Dendrogram Illustrating the Degree of Similarity Between Stations on the ALE Reserve, and the Interpretation of the Similarity in Terms of Precipitation.

degree are indicated on a topographical map of the ALE Reserve (Figure 57). The solid, dashed, and dotted lines delineate areas differing by uniform amounts, and regions surrounded by similar lines contain clusters of stations similar to each other.

This analysis used only a single dimension to differentiate between stations; consequently, the patterns strongly resemble lines of constant precipitation (isohyets) in that the clusters can be characterized as I: Dry vs. II: Wet; and A: Driest vs. B: Moistest vs. C: Moistest yet vs. D: Wettest. A three-dimensional clustering adding maximum and minimum temperatures (currently in progress) will probably prove much more illuminating in pointing out closely related microclimates.

Clustering of climatic types has in the past frequently depended upon subjective reasoning. We are not aware of any earlier application of this objective approach to climatic mapping.

Soil Texture and Wind Erosion W. T. Hinds

Erosion by wind is a variable problem in semi-arid regions, being very prominent in regions with relatively sandy soils but unimportant in regions with silty soils. The crucial importance of the texture of the surface layer of soils was examined in the aftermath of the fire on ALE in August 1973. This fire removed entirely the vegetation overlying a junction of two contrasting soil textures--one sandy, the other silty--over a distance of less than 100 m. The first windy season removed great amounts of soil from the sandy region, but left the silty region untouched. To isolate the physical and ecological properties producing this difference, transects in both burned and unburned areas were laid out for soil and erosion measurements.

Erosion was measured by the projection of burned sagebrush stems, grass

FIGURE 57. Map of Clusters of Similar Precipitation Regimes on the ALE Reserve. The Regions Identified Here (I, II; A, B, C, D) Correspond to Similar Collections in Figure 56.

bunches, etc., above the eroded surface in 50 plots along several transects (Figure 58). Usually, each plot contained several such stems or stumps blackened by the fire and burned to the ground the previous summer; no plot contained fewer than 2 such stems, many had 10 or more. The characteristics of the unburned soil surface were measured in an unburned segment (Site II, Figure 58); the surface 3 cm (1 in.) was removed and sieved through standard sieves.

The relation between wind erosion during the first windy season and fraction of the surface centimeters in erodible-size fractions (0.06 to 0.5 mm) is shown in Figure 59. The data indicate that soils become easily erodible only when the surface layer contains more than about 60% of easily erodible sizes; soils with lesser proportions of erodible size fractions apparently keep the erodible fractions protected by non-erodible sizes. However, the integrity of the surface against wind abrasion is assisted by an algal-lichen-moss carpet

that is physiologically active only between February and April, but remains physically intact other seasons. The proportion of the surface layers protected by this carpet might be estimated by the amount of organic matter in the surface layer, as shown in Figure 60. Apparently, the erodible soils not only have a larger fraction in erodible sizes, they also have less protection from wind abrasion provided by the algal-moss mat. Consequently, it now appears that vegetation on sandy soils is doubly important; without it, the soil is both more mobile and more exposed to wind abrasion.

Secondary succession on the burned areas during the first spring was mostly due to an increase in the relative importance of winter annuals in relation to their importance in the unburned community (Figure 61), so long as erosion was moderate. In badly eroded areas, the relative importance of these winter annuals fell off sharply, probably due to the loss of the seed source along with the

FIGURE 58. Sketch Map (to scale) Showing Location of Soil Sample Sites and Transect Locations in Burned and Unburned Regions.

FIGURE 59. Relation Between Soil Depth Lost Due to Wind Erosion, During the First Windy Season Following Fire, and Fraction of the Surface Centimeter in Erodible Particle Sizes (Between 0.06 mm and 0.5 mm).

FIGURE 60. Organic Matter Due to Presence of an Algal-Moss Carpet Related to Erosion Loss in First Windy Season.

FIGURE 61. Relative Cover of Annuals as a Function of Erosion Loss During the First Windy Season.

soil (the important vegetation in the eroded areas were bunchgrasses that survived the fire to sprout again).

The badly eroded area was not colonized to any great degree during the spring season, leaving a relatively large amount of soil water still available in the profile. This water

provided a resource for vigorous colonization of the eroded areas by tap-rooted summer annuals, chiefly slim-leaf goosefoot (*Chenopodium leptophyllum*) and tumbleweed (*Salsola* spp.). These species started late but grew vigorously during the summer (Figure 62) when other species were dormant for lack of water in their communities.

FIGURE 62. Increase of Cover of Summer Annuals (Tumbleweed and Slim Leaf Goosefoot) During the First Summer Following Wildfire, in Relation to Erosion Loss of Soil.

RADIONUCLIDES IN THE ENVIRONMENT

- **WEATHERING AND AGING OF TRANSURANICS IN SOIL**
- **RADIOECOLOGY OF IODINE-129 AND TECHNETIUM**
- **RADIONUCLIDES IN ALASKA**

Two new 189-schedules are included under this topic, "Weathering and Aging of Transuranics," funded in July 1974, and "Radioecology of Iodine-129" funded 1 year earlier. The third 189-schedule, "Radionuclides in Alaska," is nearing completion at this time and will be supplemented by radioecology studies focused on the problem of biological availability.

One important consideration about the long-term availability of radioelements concerns chemical and biological transformation. These mechanisms are now being defined for certain radioelements, and they are reported in the Environmental Chemistry section of this report. A second important consideration is the quantitative behavior of radioelements when exposed in the natural environment to "weathering", i.e., varying degrees of climatic, biological, and edaphic activities. Such effort is needed as an intermediate step between the retrospective observational studies of contaminated landscapes and the designed experiments carried out under systematically controlled test conditions in the laboratory.

Long-lived radionuclides of plutonium are of particular concern because of long residence times and potentials for participation in mineral cycling and food chain transfers. Because of the long residence time, aging and weathering conditions are likely to be critically important in understanding the ecological ramifications of the fate and behavior of the transuranium elements. In some cases not all factors operative in the natural environment are sufficiently delineated to bring under laboratory control.

The radioecological studies reported here have involved an unusually high degree of interaction among different specialists, and in all cases represent cooperative projects.

Radionuclides in the Environment

W. H. Rickard, E. L. Klepper,
W. T. Hinds, J. F. Cline,
J. D. Hedlund, R. G. Schreckhise,
D. G. Watson, F. B. Brauer,
J. K. Soldat and J. E. Fager.
Technical Assistance: V. D. Charles
and M. J. Harris

In recent years the behavior and fate of radionuclides with very long physical half-times has attracted considerable scientific attention, especially in regard to a potential for increased biological availability as affected by aging, weathering and biological processes operating in surface soils and attendant vegetative cover. Two radioelements important to the nuclear industry are plutonium and ^{129}I . Other shorter-lived isotopes, such as ^{137}Cs , which are widely dispersed in the global environment, also occur as important constituents of waste ponds and burial trenches on the Hanford Reservation.

Plutonium-238 Uptake by Range and Crop Plants in Field Environments

A special lysimeter array has been developed for growing plants in plutonium contaminated soil under outdoor conditions with little chance for inadvertent dispersal from the lysimeters. The lysimeter development and validation of technic was initiated under other 189 funding. Its application to plutonium uptake was commenced under the 189, "Weathering and Aging of Transuranics," in July 1974. The lysimeters are durable enough to persist for 20 years or more so that chemical and bio-chemical transformations in the soil that occur with exposure to climate, microbial activity, and time can be related to increases or decreases in availability to plants growing in the soil. Other transuranics will also be used in the lysimeters.

First year uptake of ^{238}Pu by cheatgrass, mustard, barley, and peas were completed in early summer, 1974, using a lysimetric technique in the field. The results are summarized in Table 81, in terms of both concentration in the plant tissue and the fraction translocated to various above-ground plant organs.

Rangeland species, cheatgrass and tansy mustard, exhibited relatively

low concentration of plutonium in shoot tissues, averaging about 20 pCi g^{-1} dry weight in stems and leaves and about 2 pCi g^{-1} in cheatgrass seeds. The uptake into plant tissues was 1 to 3 ppm in relation to the soil activity. This is a factor of five times less than that reported for cheatgrass grown in growth chamber studies using ^{239}Pu with the same source geometry.

Crop species, barley and peas, exhibited a wide difference in uptake. Barley had uptake values comparable to cheatgrass, about 20 pCi g^{-1} in the stems and leaves and about 2 pCi g^{-1} in the grain. The peas, however, were nearly an order of magnitude greater in their uptake, about 200 pCi g^{-1} in the vegetative organs, but only about 3 pCi g^{-1} in the seed. This was quite comparable to the seed concentrations of the other species. The uptake from the soil inventory by crop plants was about twice that of the wild plants (due to barley's productivity only) and about 10 times as high in peas, due to greater uptake, not productivity. These same lysimeters will be replanted in 1974-75 and in future years to assess changes in uptake as affected by aging, weathering and biological activity. The lysimeters also provide an opportunity to assess downward movement of plutonium by gravity, leaching and by root growth extension, death, and subsequent decay processes.

Concentration Factors for Radioiodine in Belowground Plant Parts

Growth-chamber experiments, begun last year to determine concentration factors for radioiodine (^{125}I) in crop plants, are being extended to allow studies on larger plants that take long times to mature and that require relatively large volumes of soil to grow satisfactorily. Also, an effort has been made to include crops which grow belowground and which could receive significant contamination directly from the soil.

Methods of soil spiking and mixing were similar to those reported last year except that 360 g (dry weight basis) of soil were used in each plot. Sodium-Iodine-125 was added to the soil in a NaI solution so that at planting time soil contained 0.1 $\mu\text{Ci/g}$ dry weight and 8 ppb iodide. There were four replicates of each species treatment.

TABLE 81. Uptake of ^{238}Pu from a Silt Loam Soil with 0.084 Ci m^{-2} .

Species	Biomass g m^{-2}	^{238}Pu pCi g^{-1}	Pu Uptake nCi m^{-2}	Uptake ppm $\mu\text{Ci/Ci}$	Sample Size
<u>B. tectorum</u> = Cheatgrass					
Stems	146 ± 17	18.9 ± 1.4	2.76	3.3	50
Seeds	<u>150 ± 10</u>	2.5 ± 0.36	<u>0.38</u>	<u>0.4</u>	<u>50</u>
Total	296 ± 20		3.14	3.7	50
<u>D. pinnata</u> = Mustard					
Total	77 ± 12	15.7 ± 25	1.21	1.4	37
<u>H. vulgare</u> = Barley					
Stems	724 ± 30	20 ± 1.9	5.45	6.5	10
Seeds	<u>354 ± 36</u>	2.7 ± 0.3	<u>0.96</u>	<u>1.1</u>	<u>10</u>
Total	1078 ± 47		6.41	7.6	10
<u>P. sativa</u> = Peas					
Stems	60 ± 4.3	155 ± 37	9.30	11.1	10
Leaves	112 ± 13	250 ± 48	28.00	33.3	10
Pods	92 ± 6.5	43 ± 12	3.96	4.7	10
Seeds	<u>333 ± 37</u>	3.2 ± 0.3	<u>1.07</u>	<u>1.3</u>	<u>10</u>
Total	597 ± 40		42.33	50.4	10

Table 82 shows radioiodine concentrations for three belowground crops. At harvest time, soil had $0.04 \mu\text{Ci/g}$ dry weight for carrot and potato and $0.07 \mu\text{Ci/g}$ dry weight for radish. In every case, edible portions of these plants had lower concentrations of iodine than aboveground parts. Generally, these concentrations were lower by about an order of magnitude. The edible portions of these three crops are anatomically different. Potato is stem or tuber tissue; carrot is root; and radish is mostly swollen hypocotyl, although the lower portion of the edible part is enlarged taproot. Data in Table 82 show that the lower end of the radish and the taproot have higher concentrations of iodine than the upper two-thirds of the tissue which comes from the hypocotyl.

Concentration factors for the edible parts of these plants range from 5×10^{-3} for the center parts of the potato to 10^{-1} for the lower third of the radish. The fact that starchy food products have lower concentration factors for iodine than do leafy shoots is in agreement with previous studies and may indicate that the mobility of iodine in the phloem is somewhat restricted.

Iodine-129 in Litter and Soil from Grass and Forest Communities

During the past 30 years, ^{129}I has become widely distributed in terrestrial plant communities derived from worldwide fallout and from stack release at sites that reprocess nuclear fuels. Because ^{129}I has a very long

TABLE 82. Internal Distribution of Radioiodine in Above- and Belowground Plant Parts (μCi per g dry weight $\pm$ S.E.).

		Potatoes	Carrots	Radishes
Leaves	Bottom leaves	$0.0166 \pm .0017$	$0.0102 \pm .0011$	$0.0153 \pm .0019$
	Middle leaves	$0.0211 \pm .0032$	$0.0064 \pm .0013$	$0.0238 \pm .0026$
	Upper leaves	$0.0224 \pm .0021$	$0.0068 \pm .0011$	$0.0220 \pm .0025$
Potato	Peeling	$0.0030 \pm .0004$	---	---
	Center	0.0002 ± 0	---	---
Carrot		---	$0.0012 \pm .0001$	---
Radish	Upper Third	---	---	$0.0048 \pm .0006$
	Middle Third	---	---	$0.0039 \pm .0003$
	Lower Third	---	---	$0.0071 \pm .0005$
	Taproot	----	---	$0.0555 \pm .0155$

physical half-time, it can be expected to persist on the environment for centuries and become incorporated into the mineral cycling behavior of managed and wild plant communities.

Iodine-129 was measured in soils and plants and animals at nuclear fuel reprocessing facilities at West Valley, New York, and at Hanford, Washington. Iodine-129 was concentrated in litter and surface soil with more ^{129}I present in the forest soil than in adjacent grassy meadow communities. Iodine-129 appears to be retained in the surface soil layers rather than to move rapidly to ground water.

Samples of litter from grass and forest communities and underlying mineral surface soil and subsurface soil (6 in.) were collected at two locations with a history of nuclear fuels reprocessing, i.e., Richland, Washington, and West Valley, New York. A third site, Morris, Illinois, is not near any nuclear fuel facility but is expected to become such a site in the future.

The results of radiochemical analyses are shown in Table 83. These data show the highest concentrations of ^{129}I associated with samples from West Valley and Richland. However, the values associated with West Valley forest litter and surface soils are approximately 50 times higher than Richland samples, but grass community samples were only 2-3 times greater. Iodine-129 values at Morris are very low and represent worldwide fallout. The forest communities at West Valley are apparently much more efficient at accumulating ^{129}I in the litter and surface soil than are grass communities in the same area with average values ranging from 10-20 times greater.

Subsurface soil samples were always lower in ^{129}I than associated surface samples showing that radioiodine is currently associated with the soil surface. However, with time radioiodine can be expected to become more thoroughly mixed in the soil profile through transfers through root systems, weathering, leaching, and the activities of burrowing animals and microorganisms.

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TABLE 83, PAGE 195

The table is presently in pCi/g dry wt. and it should read
fCi/g dry wt.

Please make this change in your copy of the 1974 Annual Report.

TABLE 83. Average Concentrations $\pm$ SE of ^{129}I in Forest and Grass Communities at Richland, Washington; Morris, Illinois; and West Valley, New York.

	West Valley	f Morris	Richland
	-----($\mu\text{Ci/g dry wt.}$)-----		
Forest Communities			
Litter	110 $\pm$ 48	.0045 $\pm$.00092	---
Surface Soil	97 $\pm$ 46	.0044 $\pm$.00080	---
Subsurface Soil	1.1 $\pm$ 0.32	.0011 $\pm$.0036	---
Grass Communities			
Litter	5.7 $\pm$ 4.3	.0024 $\pm$.0005	2.3 $\pm$ 0.38
Surface Soil	9.2 $\pm$ 0.30	.0036 $\pm$.0009	2.2 $\pm$ 0.22
Subsurface Soil	.35 $\pm$ 0.01	<.00052 $\pm$	0.044 $\pm$ 0.004

Iodine-129 in Freshwater Aquatic Organisms

Field sampling was conducted in the streams near the Nuclear Fuels Services (NFS) fuels processing plant near West Valley in western New York State in the spring and fall of 1973. This plant, which began operation in 1966 and temporarily discontinued operation in the fall of 1971, discharges small amounts of ^{129}I in both its gaseous and liquid effluents. Low-level radioactive wastes are discharged to Frank's Creek, a tributary of Buttermilk Creek, which in turn flows into Cattaraugus Creek (Figure 63).

In the area of sample collection the stream bottom is composed of sediments and fragmented shale and siltstone. The streams are subject to rapid fluctuations in flow and scouring of the stream bed. Average flows of Buttermilk and Cattaraugus Creeks are about 46 and 354 cfs, respectively, and the annual precipitation in the region is approximately 40 in.

The concentrations of ^{129}I in aquatic organisms collected from Buttermilk and Cattaraugus Creeks are shown in Figure 64. In the water, sediments, and sessile aquatic plants the concentrations of ^{129}I taken at sampling Location B are two orders of

magnitude or more greater than those from Location A. This reflects the difference between the contribution of ^{129}I from direct liquid effluent

FIGURE 63. Map of the Environs of the Nuclear Fuels Services (NFS) Plant Showing Sampling Locations (A, B, C).

FIGURE 64. ^{129}I in Aquatic Organisms Near Nuclear Fuels Services Plant.

discharge to Buttermilk Creek and that coming indirectly from gaseous effluents that are deposited on land and subsequently flushed into the stream by precipitation runoff. This conclusion that liquid effluents are the major source of ^{129}I to the stream environment is also supported by lower levels of radioiodine in the terrestrial environment. Iodine-129 levels in vegetation and soil from the environs of the NFS plant were an order of magnitude or more lower than in the aquatic samples.

The differences in ^{129}I burdens in the mobile organisms, i.e., crayfish and fish, collected in Buttermilk Creek above and below the mouth of Frank's Creek is not as great as the sessile forms. Most probably this is because the upstream collections were made within the normal range of upstream movement of these animals.

This indicates that the animals collected at the upstream site had spent part of their lives downstream of the plant discharge. The same line of reasoning would also suggest that the ^{129}I levels in the fish and crayfish sampled at the downstream site may have been reduced by recruitment of relatively uncontaminated animals from the upstream location.

With the exception of one filamentous algae sample taken from Buttermilk Creek below the point of effluent discharge, crayfish consistently had the highest concentrations of ^{129}I , and consequently may be a useful indicator organism in future monitoring. Crayfish probably are not a direct source of ^{129}I to man but may be an important food of fish, such as bass and trout, which are consumed by humans.

The relationship of ^{129}I to stable ^{127}I , an important consideration in food chain relationships, is given in Table 84. The specific activity of ^{129}I in the sediment samples from Buttermilk Creek downstream from Frank's Creek, and from Cattaraugus Creek are much closer to that of the organisms than that of the water. This suggests that the sediments may be the source of ^{129}I in the aquatic ecosystem. There is also indication that

the specific activity in the stream biota is inversely related to distance from the source of effluent discharge. The specific activity of the biota from the lower Buttermilk location is about 5.6 times greater than that of Cattaraugus Creek samples. Based on average annual flows, Buttermilk Creek is diluted about 7.7-fold after joining Cattaraugus Creek. Better agreement between the stream flows and specific activity may be

TABLE 84. Relationship of ^{129}I to ^{127}I (Stable Iodine) in Aquatic Organisms from Buttermilk and Cattaraugus Creeks, 1973.

LOCATION/SAMPLE TYPE	NO. OF SAMPLES	SPECIFIC ACTIVITY $\mu\text{Ci } ^{129}\text{I/g } ^{127}\text{I}$	ATOM RATIO $^{129}\text{I}/^{127}\text{I}$	^{127}I ng/g dry wt
BUTTERMILK CREEK (UPSTREAM OF NFS EFFLUENT DISCHARGE)				
WATER	1	2.6×10^{-4}	1.4×10^{-6}	$1.3^{(1)}$
SEDIMENT	1	8.5×10^{-4}	4.7×10^{-6}	5.3×10^2
FILAMENTOUS GREEN ALGAE	1	5.3×10^{-4}	3.0×10^{-6}	8.8×10^3
WATERCRESS	1	1.2×10^{-3}	6.6×10^{-6}	6.7×10
CRAYFISH	2	6.2×10^{-2}	3.2×10^{-4}	4.9×10^3
FISH	5	1.8×10^{-1}	3.5×10^{-4}	2.2×10^2
BUTTERMILK CREEK (DOWNSTREAM OF NFS EFFLUENT DISCHARGE)				
WATER	1	4.0×10^{-2}	2.1×10^{-3}	$1.0^{(1)}$
SEDIMENT	1	2.8×10^{-1}	1.5×10^{-3}	1.2×10^3
FILAMENTOUS GREEN ALGAE	1	2.6×10^{-1}	----	9.9×10^3
DIATOMS	1	1.0×10^{-1}	5.8×10^{-4}	2.7×10^3
WATERCRESS	1	2.5×10^{-2}	1.4×10^{-4}	6.4×10^2
CRAYFISH	2	6.6×10^{-1}	7.6×10^{-4}	9.4×10^3
FISH	6	2.3×10^{-1}	1.3×10^{-3}	3.5×10^3
CATTARAUGUS CREEK				
WATER	1	9.2×10^{-3}	5.0×10^{-5}	$1.3^{(1)}$
SEDIMENT	2	1.3×10^{-2}	7.6×10^{-5}	7.0×10^2
DIATOMS	1	1.7×10^{-2}	9.5×10^{-5}	1.6×10^3
WATERCRESS	1	1.8×10^{-2}	1.0×10^{-4}	6.5×10^2
CRAYFISH	3	8.9×10^{-2}	7.4×10^{-4}	6.6×10^3
FISH	8	5.6×10^{-2}	1.9×10^{-4}	1.0×10^3

(1) ng/ml

the result of low numbers of ^{129}I measurements or a different flow of relationships between Buttermilk and Cattaraugus Creeks at the season of sampling.

An average specific activity of $5.3 \times 10^{-1} \mu\text{Ci/g}$ iodine was reported for aquatic samples collected from Buttermilk Creek in 1971 (Magno et al., 1972). This is comparable to an average of $2.6 \times 10^{-1} \mu\text{Ci } ^{129}\text{I/g}$ iodine observed in 1973. This may indicate the degree of persistence of ^{129}I in the aquatic environment or, more likely, the continued discharge of radioactive liquid effluents after fuels reprocessing was stopped in November of 1971.

There appears to be an increase in the specific activity and atom ratio with progression up the food web. More information on food relationships and additional ^{129}I measurements need to be made in order to confirm this relationship.

Measurements for ^{127}I and ^{129}I in Pig Tissues

Several samples of selected tissues from pigs raised locally were analyzed for both ^{129}I and stable ^{127}I . The following items were also analyzed; feed (Hog Finisher), ground cover (to 3/4 in. deep), and soil beneath the ground cover (12 in.-14 in. deep). The pigs were weaned at 6 weeks of age, slaughtered at 6 months and had been on Hog Finisher feed for the last 4 months of their life. The soil was analyzed because observation of the animals indicated they could have ingested and/or inhaled measurable quantities of dust (between 10 and 10^3 g/day) while rooting in an unplanted pasture.

The results of the tissue analyses are summarized in Table 85. The results of the analysis of feed and soil are reported in Table 86 along with previously reported (Brauer, 1974) radiochemical analyses of local air and water. The data in Table 86 were used to estimate the daily intakes of ^{129}I and ^{127}I by the animals. These estimates are listed in Table 87. Included in this table are a range of possible intakes depending upon the quantity of upper level soil assumed to be ingested.

Assuming that the ^{129}I and the ^{127}I are equally soluble in the digestive tract of the animals and that there was no discrimination between the two nuclides then the $^{129}\text{I} : ^{127}\text{I}$ atom ratio in the various organs should be identical to the ratio in the animals diet. The principal source of ^{127}I is extrinsic and should be readily available. It is deliberately added by the feed producer for health reasons. The $^{129}\text{I} : ^{127}\text{I}$ ratio in the various tissues ranged from a low of 7×10^{-8} in the thyroid samples to a high of 5×10^{-7} in the mammary gland. Even the lowest atom ratio found in the animal tissues was higher than the highest ratio found in feed, 1.7×10^{-8} . Air, water and the upper soil layer all had significantly higher atom ratios than feed but the intake rates of air and water are not high enough to account for the increased atom ratios measured in tissue samples (even if one were to assume the ^{127}I in these media were in a form which was unavailable for uptake from the gastrointestinal (GI) tract).

It is conceivable that ^{127}I in soil was in an "unsoluble" form while the more recently deposited ^{129}I was in a "soluble" form. However, there is so little stable ^{127}I in local soil that the question of its availability is academic. Even if completely available, it could not have added 10% to the ^{127}I present in feed.

Estimated factors for transfer of iodine from diet to pig tissues are listed in Table 88. The transfer factor for ^{129}I to meat ranged from $0.027 \text{ pCi/kg meat per pCi/day intake}$ (i.e., day/kg) for no soil ingestion to 0.001 day/kg for a hypothetical soil ingestion rate of 1.4 kg/day (the soil intake required to yield the observed transfer factor to meat for ^{127}I). By comparison the value previously estimated for use in the Year 2000 dose studies (Fletcher et al., 1971) was $0.09 \text{ pCi/kg per pCi/day}$. A value of about 0.03 was found for sheep in a study performed at Hanford (Comar, 1966).

Transfer factors from diet to thyroid averaged $1.9 \times 10^5 \text{ day/kg}$ for no soil ingestion and $2.9 \times 10^4 \text{ day/kg}$ for a soil ingestion rate of 300 g/day (the intake required to yield

TABLE 85. Measurements of ^{127}I and ^{129}I in Samples of Pig Tissue Collected at Pasco, Washington on November 19, 1973.

SAMPLE TYPE	FRESH WEIGHT (grams)		^{129}I pCi/g (FRESH)	^{127}I milligram/g (FRESH)	ATOM RATIO $^{129}\text{I}/^{127}\text{I}$
	ORGAN	TOTAL BODY			
THYROID	6.4	90,700	2.63 (-2) ⁽¹⁾	1.55	9.5 (-8)
THYROID	12	122,000	4.23 (-2)	2.82	8.3 (-8)
THYROID	8.7	107,000	2.67 (-2)	1.53	9.8 (-8)
THYROID	6.9	99,800	3.15 (-2)	1.77	1.0 (-7)
THYROID ⁽²⁾	8.5	107,000	2.40 (-2)	2.01	6.7 (-8)
THYROID	6.6	107,000	---	---	---
AVERAGE ⁽³⁾	8.2 ± 2.1	$(10.6 \pm 1.0) \times 10^4$	$(3.02 \pm 0.73) \times 10^{-2}$	1.94 ± 0.53	$(8.9 \pm 1.4) \times 10^{-8}$
PORK CHOPS	48,000 ⁽⁴⁾		4.40 (-7)	1.1 (-6)	3.8 (-7)
LIVER	1700 (1 PIG)		1.07 (-6)	3.33 (-5)	1.8 (-7)
LIVER	1800 (1 PIG)		1.67 (-6)	2.85 (-5)	3.2 (-7)
AVERAGE	1750		1.37 (-6)	3.09 (-5)	2.5 (-7)
KIDNEY	840 (6 IN 3 PIGS)		2.09 (-6)	5.57 (-5)	2.1 (-7)
BRAINS	76.2 (3 PIGS)		2.98 (-6)	---	
MAMMARY GLANDS	442 (3 PIGS)		2.24 (-6)	2.33 (-5)	5.4 (-7)

(1) THE NOTATION 2.63 (-2) MEANS 2.63×10^{-2}

(2) AVERAGE OF TWO REPLICATES

(3) $\pm$ ONE STANDARD DEVIATION

(4) ESTIMATED FROM AVERAGE BODY WEIGHT OF 106 kg, AND COMAR, 1966, WHICH STATES THAT A 200 kg SWINE HAS 85 kg OF MUSCLE

the observed transfer factor for stable ^{127}I to thyroid). Although an ingestion rate of 1400 g of soil per day seems unreasonably high, 300 g/day may not be. However, different atom ratios were found in different tissues, thus requiring different soil ingestion rates to yield matching atom ratios. This anomaly implies that factors other than soil ingestion are also involved.

One such factor could have been the high intake ^{127}I provided by feed. Comar, 1966, quotes a typical stable iodine intake rate of 0.6 mg/day for a 200 kg swine. The ~100 kg pigs sampled in this study ingested about 6 mg/day of stable iodine (presumably as mineral iodine in an inorganic form). It is known physiologically that high intake of inorganic iodine may block the further up-

take of inorganic iodine. This mechanism is less efficient in blocking the uptake of organic compounds of iodine (Vaughan, 1974). If ^{127}I (in the feed at least) were in an inorganic form and the ^{129}I were in an organic form, it seems possible that ^{129}I could be taken up more readily than the ^{127}I .

Another factor could be different biological half lives T_b for iodine among the various tissues, coupled with an ^{129}I intake which varied over the life span of the animal. Tissues which had a relatively rapid turnover would more nearly reflect the levels of ^{129}I in the diet at the time of slaughter. Those with longer turnover times would contain ^{129}I levels reflecting integration of the varying amount of ^{129}I available over a longer time period.

TABLE 86. Concentrations of ^{127}I and ^{129}I in Items of Pig's Diet.

SAMPLE TYPE	DATE COLLECTED	pCi $^{129}\text{I/g}$	mg $^{127}\text{I/g}$	ATOM RATIO $^{129}\text{I}/^{127}\text{I}$
HOG FINISHER	10/25/73	5.46 (-6)	1.82 (-3)	1.7 (-8)
HOG FINISHER	11/19/73	2.33 (-6)	1.26 (-3)	1.0 (-8)
AVERAGE		3.90 (-6)	1.54 (-3)	1.4 (-8)
GROUND COVER ⁽¹⁾ TO 3/4" DEEP	12/6/73	2.96 (-4)	2.49 (-4)	6.6 (-6)
SOIL BENEATH GROUND COVER 12" TO 14" DEEP	12/6/73	9.26 (-6)	3.95 (-4)	1.7 (-7)
COLUMBIA RIVER WATER ⁽²⁾	1971-1972 ⁽³⁾	6.0 (-8)	1.1 (-6)	3.1 (-7)
AIR AT RICHLAND, WA ⁽²⁾	4/73	9.0 (-6) pCi/m ³	2.5 (-6) mg/m ³	---
	1/74	1.9 (-5) pCi/m ³	1.5 (-6) mg/m ³	---
AVERAGE		1.4 (-5) pCi/m ³	2.0 (-6) mg/m ³	4.0 (-5)

(1) FROM UNPLANTED 3 ACRE PASTURE USED BY THE PIGS

(2) FROM BRAUER, 1974

(3) NO WATER ANALYSES GIVEN FOR 1973 OR 1974; HOWEVER, THERE WERE NO SIGNIFICANT LONG-TERM TRENDS IN THE AIR CONCENTRATION OVER THE PERIOD 1971-74.

TABLE 87. Estimated Intake Rates of ^{127}I and ^{129}I by Pigs.

ITEM	DAILY CONSUMPTION	IODINE INTAKE RATES		ATOM RATIOS
		pCi $^{129}\text{I/day}$	mg $^{127}\text{I/day}$	$^{129}\text{I}/^{127}\text{I}$ IN DIET
HOG FINISHER	4 kg	1.56 (-2)	6.16	1.4 (-8)
WATER	6 liters	3.6 (-4)	6.6 (-3)	3.1 (-7)
AIR	27 m ³ (1)	2.8 (-4)	4.0 (-5)	4.0 (-5)
SOIL @	0.01 kg	3.0 (-3)	2.5 (-3)	6.6 (-6)
	0.1 kg	3.0 (-2)	2.5 (-2)	6.6 (-6)
	0.3 kg	8.9 (-2)	7.5 (-2)	6.6 (-6)
	1.0 kg	3.0 (-1)	2.5 (-1)	6.6 (-6)
	1.4 kg	4.1 (-1)	3.5 (-1)	6.6 (-6)
TOTAL INTAKE INCLUDING SOIL @	0.0 kg	1.62 (-2)	6.17 ⁽²⁾	1.5 (-8)
	0.01 kg	1.92 (-2)	6.17	1.8 (-8)
	0.1 kg	4.58 (-2)	6.19	4.2 (-8)
	0.3 kg	1.05 (-1)	6.24	9.6 (-8)
	1.0 kg	3.12 (-1)	6.42	2.8 (-7)
	1.4 kg	4.31 (-1)	6.52	3.8 (-7)

(1) ASSUMING 75% RETENTION THIS IS EQUIVALENT TO THE IODINE CONTENT OF 20 m³ OF AIR

(2) COMAR STATES THAT AVERAGE DAILY INTAKE OF IODINE BY SWINE (200 kg) IS 0.6 mg/day

TABLE 88. Estimated Uptake Factors for Iodine Isotopes by Pigs.

ORGAN OR TISSUE	DIET INCLUDES SOIL AT S kg ⁽¹⁾	PERCENT OF DAILY INTAKE/kg ORGAN ⁽²⁾		f _w ⁽³⁾	
		¹²⁹ I	¹²⁷ I	¹²⁹ I	¹²⁷ I
PORK CHOPS (1 SAMPLE)	S = 0.0	2.7	0.11	1.6 (-2)	1.0 (-4)
	0.1	0.96	0.11	5.6 (-3)	1.0 (-4)
	0.3	0.42	0.11	2.4 (-3)	1.0 (-4)
	1.0	0.14	0.10	8.2 (-4)	9.8 (-5)
	1.4	0.10	0.10	6.0 (-4)	9.7 (-5)
THYROID (AVERAGE OF 5 SAMPLES) ⁽⁴⁾	S = 0.0	1.9 (+5)	3.1 (+4)	2.0 (-1)	3.4 (-2)
	0.1	6.6 (+4)	3.1 (+4)	7.0 (-2)	3.4 (-2)
	0.3	2.9 (+4)	3.1 (+4)	3.1 (-2)	3.4 (-2)
	1.0	9.7 (+3)	3.0 (+4)	1.0 (-2)	3.3 (-2)
LIVER (AVERAGE OF 2 SAMPLES)	S = 0.0	8.5	0.50	1.5 (-2)	8.7 (-4)
	0.3	1.3	0.50	2.3 (-3)	8.6 (-4)
	1.0	0.44	0.48	7.6 (-4)	8.3 (-4)
KIDNEY (1 SAMPLE FROM 3 PIGS)	S = 0.0	12.9	0.90	3.6 (-3)	2.5 (-4)
	0.3	2.0	0.90	5.5 (-4)	2.5 (-4)
	1.0	0.67	0.87	1.9 (-4)	2.4 (-4)
MAMMARY GLAND ⁽⁵⁾ (1 SAMPLE FROM 3 PIGS)	S = 0.0	14.0	0.38	2.0 (-3)	5.5 (-5)
	0.3	2.1	0.37	3.1 (-4)	5.5 (-5)
	1.0	0.72	0.36	1.0 (-4)	5.3 (-5)
	1.4	0.52	0.36	7.6 (-5)	5.2 (-5)
BRAINS ⁽⁵⁾ (1 SAMPLE FROM 3 PIGS)	S = 0.0	18.0	---	4.6 (-4)	---
	0.3	2.8	---	7.1 (-5)	---
	1.0	0.96	---	2.4 (-5)	---
	1.4	0.69	---	1.7 (-5)	---

(1) AS DEFINED IN TABLE 87

(2) PERCENT OF EACH DAY'S INTAKE APPEARING IN EACH kg OF ORGAN AT SLAUGHTER

(3) f_w AS DEFINED BY EQUATION 48, PAGE 33, ICRP PUBLICATION 2

(4) AVERAGE OF FIRST 5 THYROID SAMPLES LISTED IN TABLE 85

(5) T_B ASSUMED TO BE 7 DAYS

Unfortunately, biological turnover of iodine in different pig tissues is not known, but in the past the assumption has been made that values of T_B for animals are similar to those for man (Garner, 1966; Soldat, 1974). Values of T_B given for iodine in human tissues and organs by the ICRP are 100 days for thyroid and total body, (Int. Com. Rad. Prot., 1959) 14 days for bone and 7 days for liver, kidney, spleen and testes.

Values of the parameter, f_w, defined as the fraction of the ingested iodine reaching the organ of interest, can be calculated from Equation 48, page 33 of ICRP Publication 2 (Int. Com. Rad. Prot., 1959) if the values of T_B, mass and organ burden are known. The values of f_w for both ¹²⁷I and ¹²⁹I, assuming values of T_B for human organs apply to swine, are also listed in Table 88. The highest value calculated for no soil intake

was 0.2 for ^{129}I , slightly less than the value of 0.3 given for humans. The corresponding value of f_w for ^{127}I was 0.034 and only at a soil ingestion rate of about 0.3 kg/d did the value of f_w for ^{129}I fall to such a low.

Clearly, after-the-fact collection of samples, as performed in the present study lead to data that create more questions than can be answered. The questions are important ones which may not have arisen had a planned experiment been performed. Possible future studies are now being considered.

For further information refer to:

"Pertechnetate, Iodide and Methyl Iodide Retention by Surface Soils," R. E. Wildung et al. pp. 37-40.

Cesium-137 in Anaktuvuk Pass Native Residents

Cesium-137 body burdens in the native residents of Anaktuvuk Pass, Alaska, have been measured semiannually since 1969 as continuation of investigations initiated in 1962. Whole-body measurements were made using a portable sodium iodide crystal connected to a single-channel analyzer scaler. Anaktuvuk Pass residents have historically depended upon caribou meat as a dietary mainstay. Although this dependence has lessened in recent years, preference for caribou meat and fluctuations in employment opportunities keep the resident population closely tied to caribou meat as a food source.

Caribou killed during the spring migration have the highest concentrations of radiocesium resulting from weeks of foraging upon lichens which have relatively high concentrations of ^{137}Cs . These spring-killed caribou comprise nearly all of the fresh meat consumed by Anaktuvuk Pass residents during the summer; thus, the maximum body burdens are attained in mid-summer (Table 89).

Autumn-killed caribou show low levels of ^{137}Cs because summer forage consists in part of vascular plants having generally lower radiocesium concentrations than attendant lichen species. Autumn-killed animals are consumed during the winter months, which results in lower body burdens of the people in mid-winter.

Anaktuvuk residents have been divided into three age categories:

- 1) children, <14 years old; 2) minors, 15-20 years old; and 3) adults, >21 years old.

Results show a steady decline in body burdens of radiocesium since 1969 (Table 89). Although caribou meat still comprises most of the fresh meat consumed, imported foods contribute substantially to the diet as more money enters the village through improving economic prosperity. Imported foods which are low in ^{137}Cs are believed to be the major factor in the observed decline of radiocesium in the resident population. However, at times the people must resort to a caribou diet during periods when air freight services are not available. This occurred during the spring and early summer of 1974 and is believed to be the major factor for the observed increase of ^{137}Cs body burdens during the summer of 1974.

Cesium-137 in a Nesting Population of American Coots

The American coot, *Fulica americana*, is one of the most important nesting birds in waste ponds on the Hanford Reservation. Initial results from a radioecological study designed to determine the role of several different radionuclides in relation to the life history and migration patterns of coots show that mature parent birds in late June and early July had ^{137}Cs values in flesh that averaged 325 ± 100 SE pCi/g dry weight. Young coots, less than 3 weeks of age, had an average value of 186 ± 30 .

A radiochemical analysis of food materials taken from gastrointestinal

TABLE 89. Average ^{137}Cs Body Burdens (nanocuries) in Residents of Anaktuvuk Pass, Alaska, 1969-1974.

Year	Adults		Minors		Children	
	Winter	Summer	Winter	Summer	Winter	Summer
1969		789		503		148
1970	330	740	---	380	40	150
1971	230	383	170	168	32	67
1972	192	391	70	154	27	70
1973	163	312	90	181	22	65
1974	135	578	86	308	52	148

tracts showed average ^{137}Cs values of 353 ± 100 pCi/g dry wt for parent birds and 502 ± 87 for young birds.

Studies are continuing to determine the role of coots as potential vectors for the dispersal of radionuclides from waste ponds.

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- Cesium-137 in Northern Alaska Food Chains
R. G. Schreckhise

The primary objectives of this study are to further define the routes, transfer rates, and levels of fallout radionuclides in natural ecosystems of Northern Alaska over extended periods of time. Special emphasis has been focused on the fate of ^{137}Cs in the lichen-caribou-Eskimo food chains. The changing levels of ^{137}Cs in lichens, caribou, and people of Anaktuvuk Pass, Alaska observed from 1970 through 1974 are illustrated in Figure 65.

In the absence of nuclear weapons tests, the levels of ^{137}Cs in lichen cover would be expected to gradually decline through natural elimination processes and physical decay. Although ^{137}Cs levels tended to decline during the study period, the decline was not as great as anticipated because of the addition of fallout materials into the biosphere by foreign weapons tests.

The levels of ^{137}Cs in caribou flesh have also reflected the introduction of additional fallout materials

FIGURE 65. Levels of ^{137}Cs in Lichens, Caribou, and Native Residents of Anaktuvuk Pass, Alaska from 1970 Through 1974.

into the Alaskan ecosystem. The levels of ^{137}Cs in spring-killed caribou (time of maximum concentration) showed a decrease from 1970 through 1972, but increased slightly in 1973 and again in 1974. However, as previously stated, the increase in 1974 alone was not enough to cause the relatively large increase in ^{137}Cs

body burdens observed in the Eskimos during summer, 1974.

Efforts are now being made to summarize existing data from the Alaskan studies to be utilized in the construction of a model to estimate the radiation exposure from ingested radionuclides to the people of Anaktuvuk Pass, Alaska, over the past decade.

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ORGANIZATION CHARTS

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October 1974

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RESEARCH AND DEVELOPMENT

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Page 109, 2nd paragraph, lines 1-7 should read:

In measuring petroleum effects, two formidable technical problems arise: first, major fluctuations in the multi-phase system of oil, air, and water are caused by energy expenditure and manner of mixing in the influent supply; and second, the selective partitioning of specific toxic fractions may markedly alter mortality and other biological end-points.

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