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

Part 1 Biomedical Sciences February 1980

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

**Pacific Northwest Laboratory
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H. Drucker and Staff Members
of Pacific Northwest Laboratory

February 1980

Prepared for
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under Contract EY-76-C-06-1830

Pacific Northwest Laboratory
Richland, Washington 99352

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PREFACE

Pacific Northwest Laboratory's (PNL) 1979 Annual Report to the Department of Energy (DOE) Assistant Secretary for Environment describes research in environment, health, and safety conducted during fiscal year 1979. The report again consists of five parts, each in a separate volume.

The five parts of the report are oriented to particular segments of our program. Parts 1-4 report on research performed for the DOE Office of Health and Environmental Research. Part 5 reports progress on all other research performed for the Assistant Secretary for Environment, including the Office of Technology Impacts and the Office of Environmental Compliance and Overview. Each part consists of project reports authored by scientists from several PNL research departments, reflecting the interdisciplinary nature of the research effort. Parts 1-4 are organized primarily by energy technology.

The parts of the 1979 Annual Report are:

Part 1: Biomedical Sciences

Program Manager - H. Drucker

D. L. Felton, Editor

Part 2: Ecological Sciences

Program Manager - B. E. Vaughan

B. E. Vaughan, Report Coordinator
C. H. Connally, Editor

Part 3: Atmospheric Sciences

Program Manager - C. E. Elderkin

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Part 4: Physical Sciences

Program Manager - J. M. Nielsen

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

Program Managers - D. L. Hessel

S. Marks

W. J. Bair, Report Coordinator

C. M. Unruh

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

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

Credit for this annual report goes to many scientists who performed the research and wrote the individual project reports, to the program managers who directed the research and coordinated the technical progress reports, to the editors who edited the individual project reports and assembled the five parts, and to Dr. Ray Baalman, editor in chief, who directed the total effort.

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

Previous Reports in this series:

Annual-Report for

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1977	PNL-2500, Pt. 1-5
1978	PNL-2850, Pt. 1-5

FOREWORD

This volume describes progress on biomedical and health effects research conducted at PNL in 1979. The contents of the volume are a reflection of our continuing emphasis on the evaluation of risk to man from existing and/or developing energy-related technologies. The emphasis of the PNL program is consistent with the DOE goal of increasing and diversifying national energy resources without increasing risks to human health.

Most of the studies described in this report relate to process-specific activities for four major energy technologies: nuclear fuel cycle; fossil fuel cycle (oil, gas, and coal process technologies, mining, and utilization; synfuel development); geothermal power production; and fusion (biomagnetic effects). The report is organized under these technologies. In addition, research reports are included on the application of nuclear energy to biomedical problems.

Technically, the energy-related projects presented here all center around a common research format involving multitiered toxicologic evaluation of potentially hazardous by-products, fugitive gases and effluents from process-specific energy activities. The solvent refined coal projects, for example, are illustrative of the multitiered toxicologic concept. Products and process streams are examined by an inexpensive microbial mutagenesis assay. The results of these investigations are used to set priorities for materials to be used in the more expensive animal carcinogenicity and teratogenicity test systems. The initial acute animal studies, in turn, are used to identify the need for examining noncarcinogenic effects, such as damage to respiratory, neurologic, and immunologic systems.

The validity of applying results from animal experimentation to man is firmly based on empirical observations. However, as indicated in reports on animal life-span studies associated with nuclear fuel cycles, some progress is being made in obtaining data which will provide a more quantitative basis for the extrapolation of animal data to man.

Major progress is being made in determining potential health effects of coal liquids relative to other fossil fuels and synfuels such as shale oil. In particular, work in progress is leading to some understanding of the specific chemical entities that might be responsible for the biological effects of the complex mixtures that are coal liquids.

The biomedical and health effects program at PNL is an interdisciplinary effort requiring scientific contributions from practically all research departments at PNL. The personnel in the Biology Department, the principal contributors to the volume, are listed on page 317. Requests for reprints from the list of publications and presentations for 1979 on pages 305 to 313 will be honored, except as noted.

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Coal

COAL

Studies in the area of coal energy technology are relevant to potential occupational and environmental health concerns of coal combustion and coal liquefaction.

In the area of coal liquefaction a tiered testing approach is used, going from cellular systems to mammalian acute and chronic toxicity assays and examining systemic/mutagenic potential of materials that may be of occupational and/or environmental health concern.

Toxicologic studies deal with the potential hazard to miners of elements found in mine atmospheres, and with effects of inhaled heavy metals that may be present in fly ash. More basic studies consider the effect of the anemic state upon the translocation of heavy metals that may be released by coal combustion/processing, pharmacologic analyses of effects of aerosolized sulfuric acid and potential blockers of such effects. In addition, analysis of a basic lung process (phagocytosis of inhaled particulates) is being pursued.

• Inhalation Hazard to Coal Miners

Acceleration in coal mining activities to satisfy present and future energy needs may be constrained by potentially severe health hazards. Coal workers' pneumoconiosis (CWP), a chronic debilitating disease associated with coal mine dust, is one known entity; additional inhalation hazards may occur with the proposed extensive use of diesel engines in coal mines.

The objectives of this project are to evaluate the biological effects of specific mine air contaminants in experimental animals and to study the progression of chronic respiratory pathologic changes that occur with coal dusts and/or diesel exhaust fume inhalation.

BIOLOGICAL EFFECTS OF CHRONIC INHALATION OF COAL MINE DUST AND/OR DIESEL ENGINE EXHAUST IN RODENTS

Investigators:

M. T. Karagianes, R. F. Palmer, R. H. Busch, D. W. Phelps,
M. E. Frazier, B. J. McClanahan and R. A. Pelroy

Rats were killed at 4, 8, 16 and 20 mo after the start of exposures to inhaled high-CWP (coal workers' pneumoconiosis) bituminous coal mine dust separately, and combined with unscrubbed exhaust fumes from a diesel engine operated under load-rpm cycling. General health and hematologic parameters were normal. Lung lesions and accumulations of particulate matter increased with length and type of exposure. In vitro biological tests were performed on the diesel exhaust to determine toxic, mutagenic and/or carcinogenic activity. A general characterization of the diesel soot at three separate locations in the exposure system was also performed.

We described the development and operation of an automated diesel-engine exhaust-fume exposure system, and of a whole-body rodent coal dust inhalation exposure system in a previous Annual Report (1977). These integrated systems have allowed us to study the biological effects of inhaled coal dust, diesel exhaust, and a combination of these aerosols, as shown in Table 1.

In addition to monitoring the chamber parameters described in Table 1, the experimental animals were weighed every 2 wk; hematologic analysis was used to determine general health and specific blood cell response; and six animals per group were killed periodically to observe progression of possible pulmonary disease. Aerosol

particle size distribution and concentrations were monitored in all exposure chambers.

Rats (6 per exposure group; 30 per time interval) were killed at 4, 8, 16 and 20 mo after initiation of exposure. Additional animals were killed at 24 and 28 mo (4 and 8 mo recovery time after 20 mo of exposure) to examine the possibility of further progression of lung lesions or, possibly, of tissue repair.

Relevant results concerning carboxyhemoglobin levels, hematologic and serum clinical chemistry, and body weight curves were described in last year's Annual Report (1978); histopathologic results up to 16 mo

TABLE 1. Experimental Protocol for Effects of Coal Dust and/or Diesel Engine Exhaust in Rodents

Group ^(a)	Exposure Regimen ^(b)
1	Inefficient diesel engine exhaust fumes (50 ppm CO, 8.3 ± 2.0 mg/m ³ soot) ^(c)
2	High-CWP bituminous coal at 5.8 ± 3.5 mg/m ³ plus inefficient diesel engine exhaust fumes at 8.3 ± 2.0 mg/m ³ soot = 13.5 ± 4.0 mg/m ³ total particulate
3	High-CWP bituminous coal at 6.6 ± 1.9 mg/m ³
4	High-CWP bituminous coal at 14.9 ± 6.2 mg/m ³ total particulate
5	Controls (identical exposure times and handling, with room air only)

(a) 48 male SPF rats per group

(b) six hours/day; 5 days/week

(c) Mean $\pm$ SD

Chamber parameters measured:

1. CO (continuous monitoring)
2. Respirable and total coal or soot mass concentrations
3. Aerosol particle size distributions
4. NO, NO₂ and SO₂ in diesel exhaust fume chambers
5. Temperature and humidity in all chambers

postexposure were also described. Briefly, the lungs had exposure-related lesions that included bronchiolization of alveoli, interstitial fibrosis, alveolar emphysema and accumulation of black aggregates of inhaled particles in alveolar histiocytes. Similar lesions, though slightly more severe, were seen in animals killed at 20 mo postexposure. Rats killed and examined at 24 and 28 mo (4 and 8 mo recovery time) had the same exposure-related lesions, no apparent repair, and some additional pathology related to age.

Lesion severity in the 20-mo rats, as in the earlier groups, was most noticeable in animals that inhaled diesel-exhaust fumes only and diesel-exhaust fumes plus coal dust. For these two groups, the lesions were similar in severity. Rats exposed to coal dust showed less severe lesions, those in the high-level coal dust group being only slightly more pronounced than those in the group exposed to low-level coal dust. Neoplasms were first observed after 16 mo exposure: a fibrosarcoma of the heart in an animal exposed to diesel exhaust only, and a fibrosarcoma of the subcutis in a control rat. Tumors found in subsequently killed animals are shown in Table 2.

In order to better define the possible toxic, mutagenic and/or carcinogenic potential of the particular diesel emissions used for these animal studies, exhaust samples were collected from three different locations in the exposure system. These samples, which were from the exposure chambers, the mixing-dilution chamber, and the exhaust line, were examined in vitro

biological test systems. Each of the samples was soxhlet-extracted, first with a nonpolar solvent (methylene chloride, MeCl₂) and then with a polar solvent (acetonitrile, ACN).

Gas chromatography analysis showed little difference in the composition of extracts obtained from the exposure and mixing chambers. Chromatograms of extract from the exhaust-line samples, however, did not have as many peaks. Gas chromatography/mass spectroscopy analysis of the MeCl₂ extract showed the major compounds to be alkyl-hydrocarbons, C₁₅ through C₂₄. Polycyclic compounds of three and four rings (anthracene, phenanthrene, pyrene and fluoranthene) were also present in large amounts.

Microbial mutagenesis assays (Ames system) showed that the MeCl₂ extracts of the samples from the exposure and mixing chambers were more active than those from the exhaust line. These extracts were active, both with and without metabolic activation, as frameshift, rather than as base-pair mutagens.

Limited sister chromatid exchange (SCE) tests in rat blood cultures showed an increase in SCE frequency with the MeCl₂ extracts. The ACN extract elicited an increase at all concentrations tested in the first replicate, but only the highest concentration appeared to evoke an increased incidence in the second replicate. A wider range of concentrations is needed to establish a dose-response relationship for the mutagenic potential for each of the extracts in this cell culture system.

TABLE 2. Tumor Data

Tumor Type	Diesel	Diesel + Coal	High Coal	Low Coal	Control
20 Month Exposure					
Fibroadenoma	1 (mammary) ^(a)				
Bronchiolar Adenoma	1 (lung)	1 (lung)			
Fibrosarcoma					1 (sc) ^(b)
Lymphoma			1 (systemic)	1 (systemic)	1 (kidney)
Basal Cell Tumor		1 (hind leg)			
Pheochromocytoma				1 (adrenal)	
Fibroma			1 (sc) ^(b)		
Malignant Histiocytoma			1 (sc) ^(b)		
20 Month Exposure + 4 Month Recovery					
Lymphoma				1 (systemic)	
C. Cell Tumor			2 (thyroid)		
Bronchiolar Adenoma	1 (lung)	1 (lung)			
20 Month Exposure + 8 Month Recovery					
Fibroma	1 (sc) ^(b)	1 (sc) ^(b)			
Lymphoma					1 (systemic)
Mesothelioma		1 (L. Node)			
Sarcoma (undifferentiated anaplastic)			1 (sc) ^(b)		
^(a) No. of rats ^(b) Subcutaneous					

VERO (monkey kidney) cytotoxicity assays of the MeCl₂ extract from the mixing-chamber sample indicated a toxicity range similar to that of the basic and polynuclear aromatic fractions of shale oil. The ACN extract was less toxic than the MeCl₂ extract, but both were more toxic (by an order of magnitude) than crude or diesel oil.

The ability to cause mutation in Chinese hamster ovary cells was also studied for

both types of extracts. The MeCl₂ extract was more active mutagenically than the ACN. Both extracts were mutagenic with and without metabolic activation. The mutation responses were relatively low, indicating that the mutation frequency was not influenced by a cytotoxic mechanism. Results of both the cytotoxicity and cell-mutation tests are more fully described in an accompanying report.

CYTOTOXIC AND MUTAGENIC EFFECTS OF DIESEL EXHAUST CONDENSATES IN MAMMALIAN CELL CULTURES

Investigators:

M. E. Frazier, T. K. Andrews, Jr., M. J. Hooper and J. E. Samuel

Clonal growth and mutational assays using mammalian cells have been used to evaluate the cytotoxic and mutagenic potential of diesel exhaust condensates versus other fossil fuel materials. The results of these assays indicate that diesel fuel exhaust condensates are more toxic (on a microgram/ml basis) than crude oil or diesel fuel, and contain low but measurable levels of mutagenic compounds. However, these condensates are considerably less toxic than other materials (i.e., metal chlorides) which have been tested in the clonal growth assay. Most of the mutagenic potential in these materials does not require metabolic activation.

In a previous Annual Report (1978) we described several *in vitro* methods for evaluating the potential effects of various complex organic mixtures in biological systems. During the past year we have measured the cytotoxic and mutagenic effects of diesel fuel and diesel exhaust condensates using mammalian cell assays. The clonal growth assay using VERO, a monkey cell line, was used to estimate the cytotoxicity of these materials. The Chinese hamster ovary cell hypoxanthine guanine phosphoribosyl transferase (CHO/HGPRT) mutational assay system was then used to evaluate the genetic activity of these same diesel exhaust condensates.

The cytotoxicity assay microtiter plates received ~50 VERO cells per well and a series of dilutions of the test material (i.e., diesel exhaust condensates) in cell culture medium. Following 4 days of incubation, the relative plating efficiency (RPE) was determined for each dilution of the test compound by counting and recording the number of colony-producing cells in each well that contained a given concentration of test material compared to the total number of colony-producing cells in wells that contained only growth medium and solvent. The mean RPE for each test compound at a given concentration of chemical was determined by adding together the RPEs from replicated plates from no less than two experiments. Using this system we have generated concentration response curves (Figure 1) for Prudhoe Bay crude oil, diesel fuel #2, methylene chloride (MeCl₂)- and

FIGURE 1. Effect of Diesel Exhaust Extracts on the Plating Efficiency of VERO Cells

acetonitrile (ACN)-extractable materials from diesel exhaust particulates, and several reference materials.

The data in the figure show that the ACN and MeCl_2 extracts are nearly one order of magnitude more cytotoxic than either Prudhoe Bay crude oil (RPE_{50} dose = $\sim 350 \mu\text{g/ml}$) or diesel oil #2 fuel (RPE_{50} dose = $\sim 250 \mu\text{g/ml}$). The range of cytotoxicity for the ACN extract is between $200 \mu\text{g/ml}$ and $\sim 9 \mu\text{g/ml}$, with an RPE_{50} dose at about $60 \mu\text{g/ml}$. The MeCl_2 extract was slightly more cytotoxic, ranging between $\sim 50 \mu\text{g/ml}$ and $4 \mu\text{g/ml}$, with an RPE_{50} dose of about $25 \mu\text{g/ml}$. While these materials are fairly toxic when compared with crude oil and diesel fuel, they are not nearly as toxic as many other materials that have been tested previously. For example, Cd^{++} (tested as CdCl_2) has an RPE_{50} dose of $0.3 \mu\text{g/ml}$; other metals are even more cytotoxic than cadmium. The higher cytotoxicity observed in the condensates compared to diesel fuel #2 is not particularly surprising since the metal in the fuel is concentrated and many pyrolytic (organic) products become absorbed by the particles produced in these processes. The particles and their absorbed materials are then trapped in the exhaust filters. In a series of experiments with lyophilized preparations of acetonitrile and methylene chloride, we determined that there were no residual materials (from these solvents) that contributed to the cytotoxicity found in these studies.

The mutation assay using mammalian cells employs the purine analog 6-thioguanine (TG) to quantitate induction of mutation at the hypoxanthine-guanine phosphoribosyl transferase locus. In this system, TG is converted to a nucleotide by the HGPRT activity of normal wild-type cells; this nucleotide (or its metabolites) is lethal for the cells. As a result, only mutants lacking the HGPRT enzyme are able to survive and grow in the presence of TG. The mutants are then scored by counting the number of cell

colonies present. It has been shown that mutagenicity with pure compounds follows a linear relationship in this assay system. (Approximately 150 compounds have been tested to date.)

The compound 2-aminoanthracene (2AA) served as a positive control for testing S9 metabolic activation; ethylmethyl sulfonate (EMS) serves as a standard for direct-acting mutagens. The EMS gave the expected mutation frequency at $10 \mu\text{g}$ (under our experimental conditions) of between three and five times background. The low activity of 2AA in the experiments reported here resulted from the inadvertent use of an old stock solution; however, there was sufficient activity to show that the S9 enzymes were capable of converting promutagens to mutagenic compounds.

The results of a typical experiment are shown in Table 3. Based on these results we have come to the following conclusions: The large populations (1×10^6 cells/plate) of CHO cells used are not as sensitive to the cytotoxic effects of these condensates as are the smaller numbers (50 cells/well) of VERO cells used in the cytotoxicity assay. The ACN and MeCl_2 extracts are not highly active mutagens in the CHO/HGPRT assay system. The methylene chloride extract is more active, mutagenically than the acetonitrile extract, which has mutagenic activity near background. Both condensate extracts seem to have significant direct-acting mutagenic activity; in other words, activation with S9 may not be required for all materials in the extract. The MeCl_2 extract showed a dose response; however, ACN did not. We cannot explain this phenomenon, but feel that part of the difficulty lies with the fact that the ACN material is only marginally active in this assay system. Since little cytotoxicity was observed at $200 \mu\text{g/ml}$ or below (data not shown), there is no need for concern about the mutation frequency being greatly influenced by cytotoxic mechanisms.

TABLE 3. Analysis of Diesel Exhaust Extracts from the Mixing Chamber Condensates for Mutation Induction at the HGPRT Locus in Chinese Hamster Ovary Cells

Sample	Dose ^(a)	Mutation Frequency ^(b)	
		+ Activating Enzymes	- Activating Enzymes
Ethylmethylsulfonate ^(c)	10 µg	56.3	58.4
	5 µg	36.8	
Methylene chloride extract	200	82.6	58.5
	100	38.8	
	50	24.8	29.3
	20	19.4	
	2	21	
Acetonitrile extract	400	13.1	23.6
	200	23	
	100	19.4	
	50	15.4	28.3
2 Aminoanthracene ^(d)	5	37	16
	2.5	25.8	14
	0.5		16.2
Solvent control		12.8	15

(a) µg of sample/culture flask

(b) Number of mutants/10⁶ viable cells. Mutation frequency is determined by averaging the values from six petri dishes seeded with 2 x 10⁵ cells/dish.

(c) Direct-acting mutagen

(d) Mutagen requiring activation

• Effects of Pollutant Metals

Several heavy-metal pollutants from energy conversion sources are known to be detrimental to the hematopoietic and immune systems; some are carcinogenic. However, there is a paucity of information concerning potential synergistic effects of nutritional deficiencies (common in both industrialized and underdeveloped countries) combined with heavy-metal exposure. This project will examine the interactions of specific nutritional deficiencies and heavy-metal exposures, with particular emphasis on gastrointestinal absorption of the metals and subsequent effect on the hematopoietic and immune systems.

INTERACTIONS OF NICKEL AND IRON DEFICIENCY

Investigators:

H. A. Ragan, M. J. Pipes and S. L. Klopfer

Technical Assistance:

E. T. Edmerson, M. C. Perkins, J. K. Sweeney,

K. H. Debban and S. L. Schively

Nickel fed at 250 ppm to iron-deficient rats appeared to have a "sparing" effect on the development of iron-deficiency anemia. Absorption of nickel was not enhanced by concurrent administration of 0.5 ppm of chlorinated water.

Nickel fed at 250 ppm to iron-deficient rats seemed to have a sparing effect on the development of iron-deficiency anemia (Annual Report, 1978). That study was performed in female rats, to whose diet nickel was not added until tissue iron stores had been partially depleted by feeding them an iron-deficient diet. If the "sparing" effect of nickel is real, it should be amplified in rapidly growing male rats since metabolic iron demands are highest during that growth period. To test this hypothesis, male rats were placed on an iron-deficient, iron-deficient + nickel, control, or control + nickel diet at weaning (21 days of age).

A moderately severe, chronic diarrhea developed in all four treatment groups, precluding continuation of the study for as long as the previous one (i.e., males on the nickel diet for only 42 days, compared with 75 days for the females last year). This also prevented investigation of the effects of nickel on iron turnover kinetics. Body weights and clinical pathology parameters after 42 days on the diet (at which time the rats were killed) are shown in Table 4.

There were indications that the anemia of iron deficiency was slightly less severe in those rats receiving nickel in their diet; however, the differences between the two groups were less marked than observed previously. Significantly higher hemoglobin concentrations ($p < 0.02$), mean corpuscular volumes ($p < 0.02$), and mean corpuscular hemoglobins ($p < 0.01$) were observed in the iron-deficient + nickel group as compared to the iron-deficient-only diet group. In other words, the microcytic, hypochromic anemia of iron deficiency was less severe in those rats that received nickel. A replicate of this study, which will include a study of iron kinetics, is presently underway; to date, diarrhea has not been observed in any of the diet groups.

A problem was encountered with blood from iron-deficient rats in using the automated multiparameter hematology instrument. Evaluation of mean corpuscular hemoglobin concentrations revealed erroneous results, i.e., they were invalidly high. This occurred because the most microcytic populations of erythrocytes in iron-deficient rats are not

TABLE 4. Evaluation of Rats on Control, Iron-Deficient or Added Nickel Diets for 42 Days (Mean $\pm$ S.D.; n = 22/group).

Group	Weight, g	RBC, $\times 10^6/\mu\text{l}$	Hgb, g/dl	VPRC, ml/dl	MCV, μ^3	MCH, μg	Serum Iron, $\mu\text{g/dl}$	TIBC, $\mu\text{g/dl}$	Alkaline Phosphatase, IU/l
Control	328 $\pm$ 28 (c)	8.21 $\pm$.52	16.7 $\pm$ 1.2	48.5 $\pm$ 2.1	58.7 $\pm$ 2.3	20.5 $\pm$ 1.6	160 $\pm$ 27 (a)	445 $\pm$ 45	275 $\pm$ 84
Control + Ni	304 $\pm$ 20	8.19 $\pm$.39	16.8 $\pm$ 0.8	48.5 $\pm$ 1.8	59.3 $\pm$ 1.1	20.5 $\pm$ 1.1	144 $\pm$ 20	470 $\pm$ 47	292 $\pm$ 67
Fe Deficient	270 $\pm$ 26 (c)	7.88 $\pm$.63	11.3 $\pm$ 1.2 (b)	29.8 $\pm$ 3.0	37.8 $\pm$ 2.0 (b)	14.4 $\pm$ 1.8 (c)	26 $\pm$ 6	724 $\pm$ 70	257 $\pm$ 83 (c)
Fe Defic + Ni	229 $\pm$ 32	7.83 $\pm$.76	12.9 $\pm$ 2.6	30.9 $\pm$ 2.5	39.7 $\pm$ 2.9	16.6 $\pm$ 3.2	29 $\pm$ 15	787 $\pm$ 75	419 $\pm$ 81

(a) $P < 0.05$
(b) $P < 0.02$
(c) $P < 0.01$

t-test comparisons between control and control + Ni or iron-deficient and iron-deficient + Ni

detected electronically, which results in falsely low red cell counts but accurate hemoglobin concentrations. Values for two parameters computed from these data, i.e., volume of packed red cells and mean corpuscular hemoglobin concentrations are, respectively, falsely low and falsely high. Using a multichannel analyzer, it is possible to make a size distribution plot showing the small cells not detected by the automated instrument (Figure 2). By using semiautomated methods (Coulter Model ZH, microhematocrit, automated hemoglobin, and calculated indices), it is possible to obtain valid hematology data from iron-deficient rats (Table 5).

Since the absorption of nickel has been shown to be enhanced by iron deficiency (Annual Report, 1977), it is of interest to determine the influence of an iron deficit on tumor production after exposure to nickel, which is a known carcinogenic metal. One hundred female Wistar rats, weaned at 21 days of age, were placed on either an iron-deficient or control diet for 80 days, at which time the iron-deficient rats were anemic. Both groups then received 20 mg of nickel oxide by injection deep into the musculature of the thigh. An additional group of 30 male rats were divided and treated in a manner identical to that described for the females. All iron-deficient

FIGURE 2. Erythrocyte Size Distributions in Iron-Deficient and Control Rats

TABLE 5. Comparisons of Blood Values Determined by Fully Automated and Partially Automated Methods in Moderately Iron-Deficient Rats (Mean + S.D.; n = 5)

Method	RBC ($\times 10^6/\mu\text{l}$)	Hemoglobin (g/dl)	VPRC (ml/dl)	MCV (μ^3)	MCH (μg)	MCHC (%)
Automated	5.86 ± 0.42	11.7 ± 0.6	27.5 ± 2.2	47.6 ± 0.5	19.9 ± 0.5	42.6 ± 1.4
Manual	7.90 ± 0.31	11.7 ± 0.7	33.8 ± 1.7	42.9 ± 2.8	14.8 ± 1.1	34.3 ± 0.8
Normal Rats (Automated)	7.34 ± 0.35	17.2 ± 0.7	44.4 ± 1.6	62.0 ± 1.2	23.5 ± 0.4	38.7 ± 0.1

rats were maintained on that diet for an additional 30 days, and were then changed to a control diet to prevent deaths from anemia prior to tumor development. The numbers of grossly visible tumors that have developed by 10 mo after injection of nickel are shown in Table 6. Tumor incidence at this time is too small to be statistically valid, but none of the iron-deficient rats have yet developed tumors at the injection site.

TABLE 6. Tumors in Iron-Deficient and Control Rats Given Nickel Oxide by Intramuscular Injection

	Iron Deficient		Controls	
	Females (n = 50)	Males (n = 15)	Females (n = 57)	Males (n = 15)
Muscle	0	0	2	2
Mammary	6	0	3	0

It has been reported that body burdens of nickel were greater in fish exposed to the metal in chlorinated water than those exposed in nonchlorinated water (Ecosystems Annual Report, 1979). It is of interest to determine if the same effect would occur in a mammalian species. Therefore, twenty female Wistar rats, divided into two groups, were administered 40 μCi ^{63}Ni chloride by gavage; the vehicle for one group was double-distilled water containing 0.004 ppm chlorine; for the other group, water containing 0.5 ppm chlorine. The latter concentration is that contained in some municipal water supplies. Forty-eight hr later, the animals were killed, the GI tract was ligated and removed, and the skin was removed. Carcasses were ashed in nitric acid and the radioactivity was determined by scintillation counting. Mean body burdens of ^{63}Ni were not different between the two groups: in those given chlorinated water, it was 17.4 ± 2.8 nCi; in the distilled water group, it was 17.9 ± 4.1 nCi. It thus appears from this pilot study that chlorination of water does not enhance nickel absorption in the rat.

TISSUE BURDENS OF ^{210}Pb ADMINISTERED TO IRON-DEFICIENT RATS BY INHALATION

Investigators:

H. A. Ragan, M. J. Pipes, E. F. Blanton and S. L. Klopfer

Technical Assistance:

E. T. Edmerson, M. C. Perkins and J. K. Sweeney

Iron deficiency did not enhance the pulmonary absorption of inhaled lead.

We have shown that iron-deficient rats absorb significantly more lead from the gastrointestinal tract than littermate control rats (Annual Report, 1976). Since inhalation is a common route of exposure to lead, it was of interest to determine if an iron-deficient status might permit inhaled lead to pass more readily from the respiratory tract to extrapulmonary sites.

Thirty-six male Wistar rats were weaned at 21 days of age and fed either an iron-depleted or a control diet for 60 days. By that time, the iron-deficient-diet group was anemic and had reduced body-iron stores, when compared to the control-diet rats, i.e., volume of packed red cells was 24.5 ± 2.9 ml/dl vs 48.0 ± 2.8 ml/dl, and serum iron values were 30 ± 13 $\mu\text{g/dl}$ vs 187 ± 30 $\mu\text{g/dl}$.

Rats from both groups were randomly placed in the exposure system and exposed nose-only to an aerosol of ^{210}Pb nitrate. Exposure was for 30 min to a concentration of 1.2 $\mu\text{Ci } ^{210}\text{Pb}$ per liter; mass median aerodynamic diameter, 0.6 μm ; geometric standard deviation, 1.7.

All animals were killed by 20 min after exposure in order to limit the intestinal absorption of ^{210}Pb cleared from the respiratory tract and swallowed. The rats were skinned and decapitated to eliminate contamination from lead on the pelt or in the nose. Tissue samples were taken for radioanalysis.

The mean total body burden of ^{210}Pb in the iron-deficient rats was 1.35 ± 0.29 μCi , compared with 1.61 ± 0.41 μCi in control rats ($p < 0.05$). When tissue burdens of ^{210}Pb are calculated as percent of total body burden, only two differences are noted between the two groups (Table 7). Lead burden in the spleen of iron-deficient rats was about twofold greater than in controls, and that in kidneys of control rats was ~30% more than in the iron-deficient group.

Based on the results of this study, iron deficiency does not appear to enhance the overall movement of inhaled lead from lung to other tissues.

TABLE 7. Tissue Burdens Following Inhalation of ^{210}Pb in Iron-Deficient and Control Rats

	% of Body Burden (mean $\pm$ 1 SD)		
	Iron-Deficient	Control	P <
Lung	36.7 ± 9.3	33.8 ± 7.6	NS
Kidney	1.5 ± 0.6	2.0 ± 0.7	0.02
Liver	1.5 ± 0.5	1.7 ± 0.5	NS
Spleen	0.16 ± 0.08	0.07 ± 0.02	0.001
Femur	0.19 ± 0.09	0.17 ± 0.05	NS
Brain	0.06 ± 0.03	0.05 ± 0.02	NS
Testis	0.04 ± 0.04	0.07 ± 0.07	NS
Femoral Marrow	0.01 ± 0.006	0.01 ± 0.004	NS
Blood (per ml)	0.25 ± 0.06	0.31 ± 0.14	NS
Plasma (per ml)	0.14 ± 0.05	0.14 ± 0.16	NS
Remaining Carcass	59.1 ± 9.1	61.8 ± 7.5	NS

• Alveolar Clearance of Inhaled Metal Oxides

Metal oxides produced during the combustion of fossil fuels constitute a potential hazard to man. The purpose of this project is to determine the role of pulmonary cells in influencing the fate of inhaled metal oxides and the acute and chronic toxicity of inhaled metal oxides in the lungs of rodents.

METABOLISM OF INTRATRACHEALLY DEPOSITED VANADIUM PENTOXIDE

Investigators:

A. W. Conklin, C. S. Skinner, T. L. Felten^(a) and C. L. Sanders

Technical Assistance:

K. Rhoads and C. White

Vanadium pentoxide is rapidly solubilized in lung, translocated to skeleton, and excreted. Uptake of gavaged ^{48}V amounted to 2.6% of administered dose with tissue distribution similar to that with intratracheally instilled vanadium.

Vanadium is a trace metal of variable toxicity, depending on the mode of entry into the body. One source of human exposure is the inhalation of trace metals in fly ash. Lethal doses of vanadium produce severe pulmonary and gastrointestinal effects. Human exposure to 0.1 mg V/m^3 for 8 hr produces pulmonary edema. Since the metabolism of vanadium in the lung is not well characterized, this study was designed to examine the retention and translocation of V_2O_5 deposited in the lungs.

Vanadium-48 was mixed with Na_2CO_3 and NH_4NO_3 to form NH_4VO_3 , which was calcined at $500\text{--}550^\circ\text{C}$ for 2 hr to produce $^{48}\text{V}_2\text{O}_5$ (initial specific activity of $12.4 \mu\text{Ci } ^{48}\text{V/mg } \text{V}_2\text{O}_5$). Young adult, female, Wistar rats were given an intratracheal instillation of $40 \mu\text{g}$ of the vanadium oxide preparation suspended in 1.0 ml of 0.9% sodium chloride. Rats were killed 5, 15 and 30 min and 1, 2, 4, 6, 21, 72, 120, 168 and 336 hr after instillation. Tissues were collected, weighed, and analyzed for ^{48}V content by gamma spectroscopy.

The ^{48}V was rapidly cleared from the lungs, with about 40% of the administered ^{48}V lost by 15 min after instillation (Figure 3). About 9% of instilled ^{48}V remained in the lung at 7 and 14 days. About 27% of the ^{48}V body burden was found in skeleton by 6 hr (Table 8). Maximal ^{48}V content in liver and kidney was 10% and 5% of body burden at 2 and 6 hr, respectively. About 50% of instilled ^{48}V was excreted in 7 days (Table 8).

Uptake of $^{48}\text{V}_2\text{O}_5$ from the gastrointestinal tract was measured following gavage to estimate vanadium absorption following pulmonary clearance via mucociliary transport. A total of $2.6 \pm 1.6\%$ of the gavaged dose was absorbed by 72 hr. The distribution of ^{48}V following gavage was similar to that seen following intratracheal instillation (Table 9).

These data indicate that ^{48}V is rapidly absorbed from lung when administered in the pentoxide form, with major tissue uptake from lung occurring in skeleton, followed by liver and kidney.

(a) Presently employed at Columbia Basin College, Pasco, WA.

TABLE 9. Distribution of $^{48}\text{V}_2\text{O}_5$ at 72 Hours After Gavage or Intratracheal Instillation

Treatment:	Percent of Absorbed Dose	
	Gavage ^(a) (n = 7)	Intratracheal ^(b) (n = 4)
Lung	0.52 ± 0.19	7.3 ± 1.4
Liver	4.5 ± 2.3	4.2 ± 1.7
Kidney	3.9 ± 2.4	3.5 ± 0.5
Spleen	0.45 ± 0.23	0.41 ± 0.06
Heart	0.19 ± 0.1	0.09 ± 0.01
Skeleton	28 ± 12.1	25 ± 3.1
Blood	3.3 ± 0.1	2.0 ± 0.28
Urine	53.8 ± 42.8	29.7 ± 5.1

^(a) Total Absorption, 2.6 ± 1.6%

^(b) 100% Absorbed

Vanadium retention in lungs differs from that of ^{109}Cd , where 20% of instilled dose was retained for periods longer than 24 hr (Annual Report, 1978). Cadmium accumulated in liver and kidney, whereas vanadium accumulated in the skeleton and, to a lesser degree, in liver and kidney. Unlike cadmium, vanadium was rapidly excreted from the body.

FIGURE 3. Distribution of Intratracheally Instilled ^{48}V as V_2O_5 in the Lung and Skeleton. Points are means with standard error bars.

TABLE 8. Distribution of $^{48}\text{V}_2\text{O}_5$ After Intratracheal Instillation

Time Postinstillation	% Body Burden of ^{48}V										
	5 min	15 min	30 min	1 hr	2 hr	4 hr	6 hr	1 day	3 days	5 days	7 days
Lung	91	63	49	42	36	33	32	31	7.3	9.5	9.7
Liver	0.25	3.0	6.3	7.1	10	7.8	5.8	3.9	4.2	4.3	4.1
Kidney	0.08	1.6	3.2	3.9	4.2	4.3	5.1	3.2	3.5	4.5	3.9
Spleen	0.01	0.09	0.15	0.23	0.21	0.22	0.23	0.24	0.41	0.55	0.61
Heart	0.26	0.39	1.02	0.58	0.48	0.45	0.29	0.16	0.10	0.12	0.10
Skeleton	3.1	6.9	9.1	14	17	22	27	22	25	31	29
Blood	1.7	11	20	24	22	18	13	4.9	2.0	3.0	0.94
Urine								22	36	30	33
Feces								2.6	21	15	17

TOXICITY OF NICKEL IN THE EARLY CHICK EMBRYO

Investigator: T. L. Felten^(a)

Nickel caused an increase in mortality and in developmental abnormalities, particularly ocular anomalies, during early chick embryogenesis.

Transmembranous injection of 0.07 µg Cd into chick eggs decreased viability and increased developmental abnormalities (Annual Report, 1979). In additional studies, we have compared the toxicity of nickel with that of cadmium. Fertile chicken eggs were injected transmembranously with either 0.2 ml physiological saline, 0.07 µg Cd (CdCl₂) or 0.07 µg Ni (NiCl₂) in 0.2 ml physiological saline, and incubated at 37°C. An uninjected control group of eggs was also included in the study. At 48 and 72 hr after injection embryos were examined. The incidences of cadmium-caused mortality and developmental abnormalities were consistent with our previous findings (Annual Report, 1978). Mortality was significantly less at 48 and 72 hr after nickel than after cadmium administration. At both times, nickel induced significantly fewer abnormalities than cadmium (Table 10). The predominant abnormalities at 48 hr after nickel administration were underdeveloped brain, unfused

neural tube, dense abnormal peripheral blood vessels, and densely packed, irregularly formed somites. The latter are precursors of vertebral and rib defects. At 72 hr, lack of brain fesculation, of neural tube fusion, and of eye formation were obvious neurological abnormalities. Peripheral vascular system development was abnormal and heart chambers were not formed in some embryos.

Ocular anomalies specific to nickel administration were seen (e.g., anophthalmia and microphthalmia). Ocular teratogenicity in rats has been observed following administration of nickel carbonyl (Sunderman et al., 1979, *Science* 203:550-553) but not following nickel chloride in mice (Lu et al., 1979, *Teratology* 19:137-142). The observed abnormalities in somite development of nickel-exposed chick embryos are similar to effects observed by Lu et al. (1979) in mice. Our results indicate that nickel, but not cadmium, induces specific developmental abnormalities in the eye and in somites of the chick embryo.

(a) Presently employed at Columbia Basin College, Pasco, WA

TABLE 10. Viability and Developmental Abnormalities of Chick Embryos Treated with 0.7 µg Cadmium or Nickel Chloride

Treatment ; Time After Injection, (hr)	% Viable		% Developmental Abnormalities	
	48	72	48	72
Uninjected	100 (14) ^(a)	100 (11)	0 (14) ^(a)	0 (11)
Saline Alone	87 (15)	100 (13)	7 (13)	7 (13)
0.07 µg Cd (CdCl ₂)	71 (15)	64 (11)	80 (10)	71 (7)
0.07 µg Ni (NiCl ₂)	82 (11)	85 (13)	56 (9)	45 (11)

(a) Number of embryos examined

TOXICITY OF CADMIUM CHLORIDE, OXIDE AND SULFATE IN THE EARLY CHICK EMBRYO

Investigators:

T. L. Felten,^(a) R. R. Adee and C. L. Sanders

All forms of cadmium tested resulted in approximately the same increase in mortality and in developmental abnormalities in the early chick embryo. At doses of 0.7 μg Cd or more, the Cd precipitates in a small spot in the albumin, near the yolk membrane.

Transmembranous injection of CdCl_2 into fertile chicken eggs caused increased mortality and incidence of developmental abnormalities at doses as low as 0.07 μg Cd/egg (Annual Report, 1978). The chemical form of cadmium may alter the solubility and toxicity of cadmium to the chick embryo.

Fertile White Leghorn eggs were injected transmembranously with either 0.2 ml physiological saline, or 0.73 μg Cd as CdCl_2 , CdO or CdSO_4 , and incubated at 37°C. An uninjected control group of eggs was also included in this study. Approximately 48, 72 and 96 hr after injection the embryos were examined for viability and the presence of developmental abnormalities (Table 11).

An increased mortality was seen for all three compounds at all observation times. Both CdSO_4 and CdO were as effective as the supposedly more-soluble CdCl_2 . Incidences of developmental abnormalities were increased in all groups, with no significant effect of chemical form (Table 11).

A compact, white, precipitated spot in the albumin adjoining the yolk membrane was found when 0.7 μg or more Cd was injected into the egg. Cadmium-109 was injected transmembranously into fertile chick eggs, and the precipitated spot, shell, albumin, yolk and embryo were analyzed by gamma spectroscopy. About 80% of injected ^{109}Cd was found in the precipitated spot. This indicates a possible cadmium-binding protein in the albumin, perhaps similar to the metallothionein found in mammals.

^(a) Currently employed at Columbia Basin College, Pasco, WA.

TABLE 11. Viability and Developmental Abnormalities of Developing Chick Embryos Treated with 0.73 μg Cadmium Chloride, Oxide or Sulfate.

Treatment	% Viable			% Developmental Abnormalities		
Time After Injection, hr	48	72	96	48	72	96
Uninjected	96 (28) ^(a)	90 (29)	100 (26)	0 (27) ^(a)	4 (26)	4 (26)
Saline-injected	100 (28)	82 (27)	88 (25)	4 (28)	5 (22)	18 (22)
CdSO ₄	83 (29)	72 (29)	68 (29)	68 (25)	71 (21)	85 (20)
CdO	71 (28)	85 (27)	31 (26)	50 (20)	78 (23)	75 (8)
CdCl ₂	74 (31)	59 (29)	62 (29)	70 (23)	65 (17)	56 (18)

^(a) Number of embryos examined

AN INEXPENSIVE METABOLISM CAGE AND MUZZLE FOR TOXICOLOGICAL STUDIES WITH RODENTS

Investigator: C. Skinner

Technical Assistance: W. D. Leach

Efficient metabolism cages can be constructed from materials on hand in most laboratories. Combined with easily built muzzles, the units allow collection of metabolites following dermal or whole-body aerosol exposure free from oral interference.

Simple metabolism cages built from solvent bottles allow recovery of metabolites at minimal cost. Units for rats (Figure 4) can be constructed using a 1-gal. solvent bottle with the addition of a tubular feeder, screens and a 40-ml water bottle. The unit allows separation of feces, urine and volatile metabolites. A Kimax gas washing bottle is connected, in series with a vacuum pump, to the top of the cage. Negative pressure within the bottle is relieved by air flowing through gaps where the lid rests on three plastic spacers. Incoming air provides oxygen and washes exhaled metabolites into the gas trap. A similar cage for mice (Figure 5), can be built from a 1-l bottle. For a feeder, a plastic cylinder is fitted with a #5 1/2 rubber stopper bored with a #2 and #8 cork to hold an alligator clamp on one end and a food pellet on the other. The cylinder is cut from a 5-cm length of a 35-cc disposable syringe case. The water container is a 25-ml Pyrex centrifuge tube with a #0 rubber stopper, and a 2-mm hole ground in the bottom.

Approximately 1 hr is required for cutting, boring and polishing the rat cage and cutting and shaping the feeder. A mouse cage can

be cut in less than half an hour. Costs are approximately one-tenth of the commercial price. In addition, this cage has the advantage of a lower volume and path length for urine and feces than for commercial systems. In long-term experiments with ^{14}C benzo(a)-pyrene, ^{14}C parathion and ^{48}V chloride, more than 90% of excreted radioactivity was collected using these units.

When the systems are used for collection of metabolites from rodents following dermal or whole-body aerosol exposure, muzzles are used to prevent oral exposure and copography. Muzzles for mice are fabricated from 1-ml disposable syringe cases; 60-ml cases are used for rats. Figure 6 illustrates the steps in assembling the muzzles. The flared facepiece which interlocks with the collar, is removable.

These inexpensive metabolism cages, which can easily be assembled from materials readily available in most laboratories, allow collection of metabolites as readily as with more expensive commercially manufactured systems. In combination with muzzles, good definition of all routes of exposure and elimination is possible.

FIGURE 4. Rat Metabolism Units are Cut From 1-Gal Solvent Bottles. The feeder is cut and shaped from 1-in. pyrex glass tubing.

FIGURE 5. Mouse Metabolism Units are Cut From 1-Liter Solvent Bottles. The feeder includes a 1-in.-diameter shield to prevent food pellet scattering.

FIGURE 6. Mouse and Rat Muzzles are Constructed from 1-ml or 60-ml Plastic Disposable Hypodermic Syringe Cases. The plastic is shaped and cut using hot glass tubing of the appropriate diameter.

• Factors Influencing the Cross-Placental Transfer and Teratogenicity of Metallic Pollutants

This project seeks to define the specific factors which affect the qualitative and quantitative aspects of the cross-placental transfer and distribution of heavy metals within the fetoplacental unit. Intravenous, oral, and inhalation routes of administration are used to provide differences in the rate and, possibly, in the chemical form in which metals are presented to the placenta. Data from these studies will provide quantitative estimates of doses to the fetoplacental unit, which can then be used as bases for interpretation of toxicologic and teratologic effects observed following oral or inhalation exposure to heavy metals.

CROSS-PLACENTAL TRANSFER AND DISTRIBUTION OF INGESTED LEAD NITRATE IN RATS

Investigators:

P. L. Hackett, J. O. Hess and M. R. Sikov

Technical Assistance:

K. A. Poston, A. J. Clary, R. L. Music,
B. L. Egbert and J. P. Herring

Pregnant rats, at 9, 15 and 19 days of gestation, were gavaged with two dose levels of lead nitrate to determine the absorption and distribution of lead in maternal and fetal tissues. A tracer lead dose was readily absorbed (11% of the administered dose) and rapidly cleared from the gastrointestinal tract. Gastric clearance of a high lead dose was impaired, and absorption amounted to only 0.5% of the administered dose after 6 hr. Lead deposition in the fetoplacental unit was proportional to maternal absorption and directly related to age at exposure or sacrifice.

We have previously reported (Annual Report, 1976-1978) on studies of the distribution and cross-placental transfer of lead in pregnant rats following intravenous administration or inhalation at specific times during gestation. Since lead ingestion is an important route of lead exposure in the general population, and, in our studies, a large fraction of inhaled lead was found in the gastrointestinal tract (Annual Report, 1978), parallel studies with orally administered lead were initiated.

Pregnant rats, at 9, 15 and 19 days of gestation (dg), received a single dose of an aqueous solution of lead nitrate by gavage. Food was withheld 16 hr prior to the administration of two dose levels of lead: a

"tracer" level (^{210}Pb only), and a "carrier" level ($^{210}\text{Pb} + 312 \text{ mg Pb/kg}$). The carrier dose was found to be minimally toxic (2 of 62 rats died within 24 hr), even though it was 20 times higher than the dose of 15.6 mg Pb/kg, which produced overt fetal effects when administered intravenously. Groups of five animals were killed at 6 and 24 hr postadministration and at 20 dg. Radiolead activity of tissues and excreta was determined by measuring the 47-KeV gamma emission of ^{210}Pb in a scintillation counter. Biologically translocated ^{210}Bi , a ^{210}Pb daughter, was allowed to decay before activity measurements were made.

The carrier dose of lead cleared more slowly from the gastrointestinal tract than

did the tracer dose (Figure 7). At 6 hr postadministration, most of the gastrointestinal ^{210}Pb was still in the stomach of carrier-dosed rats, but in the tracer group the major fraction had reached the large intestine (Figure 8). At 24 hr postadministration, a significant fraction of this burden remained in all sections of the gastrointestinal tract of the carrier group; while in tracer rats, the activity had been excreted in the feces. This prolonged gastrointestinal retention, together with the observations that the slope of cumulative urinary excretion curve was greater in carrier-dose than in tracer rats (Figure 9), and that their retention curve was more constant (Figure 10), strongly indicate that lead absorption took place over a more prolonged period. Estimates of the percentage of dose absorbed were obtained from retention and urinary excretion data. Six-hour absorption values were $11.2 \pm 2.2\%$ of the dose for the tracer rats, and $0.51 \pm 0.06\%$ for the carrier group. These absorption values do not include gastrointestinal loss, which amounted to 10% of the intravenously administered lead dose in the first 6 hr (Annual Report, 1976). The percentage of the administered dose appearing in the tissues after 6 hr was about 20 times higher in tracer-dose rats than in the carrier group, and was proportional to the absorption values (Table 12). However, blood levels in the high-lead dose group were

FIGURE 7. Clearance of Gavaged ^{210}Pb Dose from the Gastrointestinal Tract of Adult Rats

FIGURE 8. Partition of the ^{210}Pb Dose in the Gastrointestinal Tract and Feces of Adult Rats at 6 and 24 hr Postadministration

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FIGURE 7. Clearance of Gavaged ^{210}Pb Dose from the Gastrointestinal Tract of Adult Rats

FIGURE 8. Partition of the ^{210}Pb Dose in the Gastrointestinal Tract and Feces of Adult Rats at 6 and 24 hr Postadministration

FIGURE 9. Cumulative Urinary Excretion of Gavaged ^{210}Pb Dose in Adult Rats

FIGURE 10. Retention of Gavaged ^{210}Pb Dose in Adult Rats

TABLE 12. Percentage of Administered ^{210}Pb Dose in Organs after 6 hr

Organ	Dose Level	
	Tracer	Carrier
Skeleton	3.97 ± 0.74	0.219 ± 0.023
Kidneys	2.12 ± 0.35	0.141 ± 0.024
Liver	1.61 ± 0.48	0.086 ± 0.016
Blood	1.71 ± 0.37	0.031 ± 0.045
Lungs	0.043 ± 0.008	0.0037 ± 0.0018
Spleen	0.035 ± 0.008	0.0010 ± 0.0002
Brain	0.006 ± 0.001	0.0005 ± 0.0001
Ovaries	0.004 ± 0.0006	0.0004 ± 0.0001
Uterus(a)		
9 dg	0.050 ± 0.012	0.0021 ± 0.0008
15 dg	0.311 ± 0.117	0.0136 ± 0.0050
19 dg	1.02 ± 0.55	0.0535 ± 0.0092

(a) Pregnant Uterus at Specific Days of Gestation (dg)

lower than expected, and may also reflect a lower absorption rate. Soft-tissue clearance of the ^{210}Pb occurred throughout the 11-day observation period resulting in retention by the skeleton of the bulk of the retained dose, 3.7% and 0.43% in tracer and carrier rats, respectively.

Lead deposition in the fetoplacental unit (FPU) was directly related to age at exposure or sacrifice (Figure 11). The increased lead deposition in the more-advanced FPU (i.e., 19 dg > 15 dg > 9 dg) has also been observed following inhalation or intravenous administration of lead. By 20 dg, the FPU of rats carrier-dosed at the egg-cylinder stage (9 dg) had accumulated lead burdens almost 13 times greater than the burden measured shortly after dosing. The older FPU (15 dg and 19 dg) did not acquire additional amounts of lead in the interval between dosing and 20 dg, but, by this time, they contained two to nine times the lead burden of the 9-dg FPU.

Partition of the ^{210}Pb dose within the FPU is shown in Figure 12. Larger fractions of the dose appeared in the placenta than in the fetus of the 15-dg FPU, while the fetal fraction was larger in 19-dg FPU. By 20 dg, the placental fraction remained higher than in the FPU exposed earlier in gestation (i.e., 9 dg > 15 dg > 19 dg). In general, carrier-dosed fetuses acquired a larger percentage of the FPU lead burden than the fetuses of the tracer-dosed rats.

Results from this study demonstrate that maternal lead absorption following oral administration is dose-dependent, and that lead burden and rate of acquisition in the FPU are both dose- and age-dependent. Furthermore, despite the fact that a lower percentage of the maternal dose appears in the carrier-treated FPU than in the tracer-treated FPU, a larger fraction of the high-lead FPU burden is accumulated by the fetus.

FIGURE 11. Percentage of the ^{210}Pb Dose in Fetoplacental Units of Various Ages at Exposure

FIGURE 12. Percentage of Fetoplacental Unit ^{210}Pb Burden in the Placenta and Fetus Following Exposure at Various Gestational Ages

FETOPLACENTAL LEAD KINETICS IN RATS AFTER ADMINISTRATION
BY INTRAVENOUS INJECTION, GAVAGE OR INPUT INHALATION TO A MODEL

Investigators:

P. L. Hackett, J. O. Hess, M. R. Sikov, W. C. Cannon,
E. F. Blanton, J. P. Herring and A. C. Case

Technical Assistance:

K. A. Poston, R. L. Music, A. J. Clary and B. L. Egbert

The deposition, distribution, and absorption of lead in pregnant rats was compared following exposure to two dose levels by three routes of administration. Adult absorption was found to be limited by both dose level and route of exposure. Blood lead levels in adult animals were proportional to absorption values and dictated the availability of lead to the fetoplacental unit.

Knowledge of the kinetics of maternal lead absorption and distribution following exposure by inhalation or ingestion is essential for prediction of the extent of metal deposition and distribution within the fetoplacental unit (FPU) and of its subsequent effect on development and behavior of the offspring. We have performed a series of studies in which maternal and fetal deposition and distribution were measured following lead administration by inhalation, gavage, and intravenous injection at various gestational ages. Analysis of these data is providing an estimate of the contribution of gastrointestinal lead absorption following inhalation exposure as well as information on the kinetics of clearance of the absorbed lead which occurs simultaneously. These estimates, along with comparisons of the effect of maternal dose level on the rate of metal presentation to the placenta, are providing bases for defining the factors affecting lead deposition in the fetoplacental unit at different developmental ages.

The procedures used to evaluate the interaction of experimental conditions on the cross-placental transfer of lead in Hill-top Wistar rats are summarized in Table 13. Exposure of the animals to the intravenously administered carrier dose at 9 days of gestation produced teratisms and 100% embryolethality when given at 15 dg. By comparison, when administered, the gavaged carrier lead dose was minimally toxic (3% mortality in

dams). Although the inhaled carrier dose was moderately high, no toxic effects were observed in the dam or FPU by 20 dg.

The aerosol properties for inhalation exposures are outlined in Table 14, which also includes values for deposition, distribution and estimates of absorption. These data show that a high percentage of both tracer and carrier doses appeared in the gastrointestinal tract following inhalation exposure. The increased deposition in the gastrointestinal tract and the decreased deposition in lungs of carrier-dosed rats were attributed to the larger particle size of the aerosol. Absorption of the inhaled tracer dose was twice that of the gavaged dose (Table 14). With either route of administration, absorption of the carrier dose was lower than that of the tracer. Although the amount of carrier lead presented to the animal by gastric gavage was much higher than the amount deposited by inhalation (Table 13), the percentage absorbed following gavage was decreased. Nevertheless, the net lead burden was higher after gavage (1500 μg Pb/kg), than after inhalation (140 μg Pb/kg).

A comparison of the percentage of the tracer and carrier lead doses that appeared in the blood of the adult rats (Figure 13) demonstrates the effect of dose and route of administration on the amount of lead available for fetoplacental deposition at various

TABLE 13. Protocol for Lead Exposure of Pregnant Rats by Three Routes of Administration

	Route of Administration		
	Intravenous	Gavage	Inhalation
No. Days of Gestation at Exposure	9, 15	9, 15, 19	9, 10, 14, 15, 19
Method of Exposure	Single Injection	Food Withheld 16 hr., Single Dose	60 Minute, Nose-Only
Dose Level ($\mu\text{g Pb/kg Body Weight}$)			
Tracer (^{210}Pb)	1.17	0.68	0.86 (a)
Carrier [$^{210}\text{Pb} + \text{Stable Pb}(\text{NO}_3)_2$]	$15.6 \times 10_3$	$312 \times 10_3$	$1.11 \times 10_3$ (a)
Time of Sacrifice			
Hr Postexposure	1.5, 6, 12, 24	6, 24	6, 24, 48
No. Days of Gestation	20	20	20

(a) Values calculated from deposition data

TABLE 14. Lead Deposition and Absorption in Adult Rats After Inhalation or Gastric Gavage

Inhalation	Tracer	Carrier
Aerosol Properties		
Concentration ($\mu\text{g Pb}/\ell$)	2.06 ± 0.17	41.2 ± 5.15
AMAD	1.32	2.20
GSD	2.16	2.19
Deposition		
Total ($\mu\text{g}/\text{rat}$) ^(a)	0.24 ± 0.03	308 ± 14.2
Lung (% of total)	14.0 ± 0.85	3.52 ± 0.42
Gastrointestinal (% of total)	63.7 ± 1.14	79.0 ± 1.28
Absorption (% of total)	22.4 ± 2.42	12.9 ± 1.05
Gavage		
Absorption (% of administered dose)	11.2 ± 2.18	0.51 ± 0.06

(a) Pelt excluded

times following exposure. Figure 14 shows that the percentage of the dose in the FPU following tracer or carrier doses by either inhalation or gavage was less than after intravenous injection, and that it was proportional to that in the blood of the dam. In all cases, the percentage of the dose accumulated in the FPU increased with gestational age and was lower in carrier-dosed than in tracer-dosed rats. Fetal fractions of the FPU lead burden increased with time after exposure (Figure 15). At early times, fetal lead percentages were higher after exposure to carrier doses by

either inhalation or gavage, but lead content was lower than in the intravenously dosed fetuses.

These initial comparisons show that lead absorption in the dam is limited by the route of administration and dose level. Resulting blood levels dictate the availability of lead to the FPU and, in turn, the kinetics of deposition. A model to predict the potential embryotoxicity of lead is presently being developed from these kinetic data.

FIGURE 13. Percentage of ^{210}Pb Dose in Blood of Adult Rats Following Exposure to Tracer or Carrier Lead Doses by Intravenous Injection, Inhalation or Gavage

FIGURE 14. Percentage of ^{210}Pb Dose in Fetoplacental Unit at Various Gestational Ages Following Exposure to Tracer or Carrier Lead Doses by Three Routes of Administration

FIGURE 15. Percentage of Fetoplacental ^{210}Pb Burden in Fetus Following Exposure to Two Dose Levels of Lead by Three Routes of Administration at 15 Days

• Solvent Refined Coal Biostudies

The objective of this program is to establish a broad biomedical data base for solvent refined coal (SRC) materials to: (1) identify possible health effects problems, (2) help identify appropriate control technologies or operating parameters to minimize potential health effects, and (3) help the SRC technology to meet present and future regulatory requirements. We are employing an approach which integrates chemical characterization, cellular and animal bioassay systems, and dosimetric studies. Thus, SRC materials for toxicologic studies selected on the basis of plant operational data are subjected to chemical characterization, determination of mutagenicity in microbial systems, and an evaluation of toxicity and transforming potential for cultured mammalian cells. Animal studies include determinations of: (a) acute and subchronic oral toxicity, (2) developmental toxicity, and (3) consequences of dermal and inhalation exposures. In addition, dosimetric and metabolic studies are being performed to provide data to aid in the extrapolation of animal toxicologic data to man.

IN VITRO TRANSFORMATION OF SYRIAN HAMSTER EMBRYO CELLS

Investigators:

M. E. Frazier, T. K. Andrews, Jr. and B. B. Thompson

Technical Assistance:

J. E. Samuels and M. J. Hooper

A mammalian cell transformation assay is being used to evaluate the biological effects of effluents and products from various fossil fuel processes. The results of this assay, using cultured cell transformation as criteria, predict that both crude petroleum and synthetically produced fuels contain some level of potentially carcinogenic compounds. Further, while most of these compounds require metabolic activation, some direct-acting carcinogens also appear to be present.

During the past year we have continued our efforts to evaluate the carcinogenic potential of complex organic mixtures derived from various synthetic fuel processes using cell culture transformation assays. In these studies, second-passage mammalian cell cultures (Syrian hamster embryo cells) are exposed to the test compound, then monitored to determine if the exposed cells undergo morphological and biochemical changes that result in unrestricted growth in vitro. This is analogous to the growth of cancer cells in vivo. Representative colonies of "transformed" cells are then inoculated into nude mice to evaluate their tumor-forming ability in vivo. The development of tumors in nude mice inoculated with

cells transformed as a consequence of in vitro exposure to these compounds provides evidence that these materials are potential carcinogens.

Figures 16-18 show the respective concentration response curves for benzo(a)pyrene, SRC I process solvent and SRC II heavy distillate in the transformation assay. It should be noted that the SRC materials used in these studies are of pilot plant origin (see this report, Renne et al.) and may not be representative of commercial operations or of all operating conditions for the pilot plant. These particular data indicate that it is possible to obtain reasonable dose-response curves with these

FIGURE 16. Relationship of cell survival (cloning efficiency of benzo (a) pyrene-treated cultures/cloning efficiency of solvent control cultures X 100) and % transformation (number of transformed colonies/number of total colonies X 100) to concentration of benzo (a)-pyrene. The test plates contained secondary cultures of Syrian hamster embryo (SHE) cells, various concentrations of BAP and S9 activating enzymes (7 mg of protein). Cell survival was determined at 7 days postexposure and transformation frequency at 20 days postexposure. Each data point represents the summation of six plates. Colonies were scored blind. ○, Relative survival at listed chemical concentration; ●, transformation frequency at listed chemical concentration.

FIGURE 17. Cell survival and % transformation relative to concentration of SRC I process solvent. The methods are the same as described in Figure 1. The solvent used in these studies was dimethylsulfoxide (DMSO). The DMSO had no effect on Syrian hamster embryo cell survival at the concentrations employed in these tests (0.1% or below). ○, Relative survival at listed chemical concentrations; ●, transformation frequency at listed chemical concentration.

FIGURE 18. Relationship of cell survival and % transformation to concentration of SRC II heavy distillate. The methods are the same as described in Figures 1 and 2. ○, Relative survival at listed chemical concentration; ●, transformation frequency at listed chemical concentration.

complex mixtures comparable to those observed with BAP (Figure 16). However, we wish to stress that problems of solubility and toxicity cause difficulties in reproducibility (and thus in interpretability) of these types of studies.

Table 15 illustrates several points of interest regarding materials from these synthetic fuel processes. First, we have noted a small but consistent transformation frequency (0.3 to 0.6%) in cells exposed to a standard crude oil. Secondly, most of the transforming potential is apparently due to the activation of procarcinogens by (S9) microsomal enzymes. However, we have detected some transforming activity in oil shale and SRC I process solvent that does not require exogenous activation (S9 enzymes). Finally, shale oil and SRC I process solvent appear to have comparable transforming potential on a µg/ml basis, and both of these materials appear to be more active, *in vitro*, relative to benzo(a)-pyrene than they are *in vivo* (see skin painting studies in this Annual Report).

The transformation of these Syrian hamster embryo cells following exposure to one of these materials (e.g., the basic fraction of SRC I process solvent) does, indeed, correlate with the ability of these cells to form tumors in nude mice. Five transformed colonies were selected and grown for 20 passages in culture. Approximately 5×10^6 cells were then inoculated into nude mice. All five cell stocks resulted in the development of undifferentiated sarcomas at the site of inoculation.

TABLE 15. Effect of Microsomal (S9) Activating Enzymes on Transformation Frequency of Syrian Hamster Embryo (SHE) Cells Exposed to Complex Organic Mixtures

Test Material, Concentration in µg/ml	Activating (S-9) Enzymes	Relative Survival	% Transformation
DMSO (Control)	+	91	0
	-	100	0
Prudhoe Bay Crude (200)	+	24	0.5
Shale Oil-LERC (10)	+	39	3.1
	-	92	0
Shale Oil Basic Fraction (50)	+	48	7.1
	-	55	0.5
Shale Oil PNA Fraction (10)	+	52	3.9
	-	88	0
SRC I Process Solvent (50)	+	31	6.8
	-	43	1.1
SRC I Process Solvent Basic Fraction (10)	+	52	12.8
	-	92	0.8
SRC I Process Solvent PNA Fraction (50)	+	52	3.9
	-	68	0
Benzo(a) pyrene (5)	+	75	4.8
	-	84	0

TABLE 16. Comparative Transformation Properties of Complex Organic Mixtures

Material	Number of Transformants per µg of Test Material ^(a)	Range of Linear Portion of Concentration Response Curve ^(b)
DMSO Control (0.1%)	0	--
Prudhoe Bay Crude	~0.002	100-200
Wilmington Crude	~0.002	75-150
SRC I Process Solvent	0.15	5-40
SRC II Heavy Distillate	0.7	5-20
Shale Oil (LERC)	0.3	1-10
SRC I Process Solvent Basic Fraction PNA Fraction	1.2	1-20
	0.3	1-5
Shale Oil (LERC) Basic Fraction PNA Fraction	0.76	1-10
	0.8	5-50
Benzo(a) pyrene	0.76	1-20

^(a) Extrapolated from linear region of the concentration response curve
^(b) µg/ml

Table 16 serves as a summary of our data on the transforming properties of the complex organic mixtures tested to date. Two crude oil preparations and benzo(a)-pyrene have been added for reference. The data in this table are derived by extrapolating from the linear regions of concentration response curves similar to those shown in Figures 16-18.

The major points appear to be: fractions from SRC I and SRC II materials, and from shale oil, are comparable to BAP relative to

activity in this assay; the crude oils show some transforming capabilities; and the PNA fractions of SRC materials and of shale oil show transforming potential in the SHE cell assay. The last two points provide information regarding activity that was not detected in the Ames assay of these same materials (Pelroy, this Annual Report).

Animal skin-painting studies being conducted at this laboratory should help to clarify the validity of these findings.

EPIDERMAL CARCINOGENESIS STUDIES IN MICE OF CRUDE MATERIALS
FROM SEVERAL FOSSIL FUEL TECHNOLOGIES

Investigators:

R. A. Renne, L. G. Smith and D. D. Mahlum

Technical Assistance:

B. J. Holloway, K. G. Braezel and E. C. DeBattista

Repeated epidermal exposure to crude materials from several fossil fuel technologies induced malignant epidermal neoplasms in mice. Tumor incidence reached 100% in mice after 62 applications of 22.8 mg of the heavy distillate fraction (HD) from a solvent refined coal process (SRC II), after a maximum latency period of 147 days. Tumor incidence reached 100% in mice exposed to 0.05 mg/dose of benzo(a)pyrene, a known carcinogen, after a latency of 193 days. Tumor incidence reached 100% in 372 days in mice exposed to 2.3 mg HD/dose, and in 409 days in mice exposed to 21.2 mg/dose of crude shale oil. To date (456 days of exposure), similar tumors have been observed grossly in mice exposed to lower concentrations of these materials or to SRC II light distillate, Wilmington crude petroleum, or 2-aminoanthracene. Further skin-painting studies are now underway on subfractions of the most biologically active of these materials.

There is evidence to suggest that the crude products of coal liquefaction and oil-shale retorting contain potentially carcinogenic materials. The purpose of our research is to determine the carcinogenic potential of some of the materials from these technologies, relative to crude petroleum and two known carcinogens: benzo(a)pyrene (BaP), a polycyclic aromatic hydrocarbon; and 2-aminoanthracene, an aromatic amine. This work is being correlated with ongoing mutagenesis studies of the same materials (see Pelroy, this Annual Report) in an attempt to determine the correlation between mutagenesis and carcinogenesis data for fossil fuel materials and known chemical carcinogens.

Samples of crude shale oil were obtained from the Lawrence Livermore Laboratory (LLL). These samples were collected from run L0-1 of Anvil Points, CO oil shale (24 gal/ton Fischer Assay) obtained in April, 1977, from the LLL experimental 6000-kg-capacity, modified in situ retort. Samples of solvent-refined coal (SRC II) heavy distillate fraction (HD) and light distillate fraction (LD) were obtained from the Pittsburgh & Midway Coal Mining Co.'s solvent refined coal pilot plant in Ft. Lewis,

WA. The HD was collected on January 29, 1978 during processing of Blacksville coal, and the LD on February 8, 1978 during processing of Illinois No. 6 coal. They represent fractional distillates rather than end products of the process. Since the LLL retort and the SRC II pilot plant are both experimental units, data reported on materials from these units should not be considered representative of all operating conditions of these processes nor of future commercial practice. Wilmington, CA crude petroleum (Sample #71052) was obtained from the Bartlesville Energy Technology Center.

The study now in progress consists of application of three dose levels of each material to the shaved dorsal skin of C3HF/HeBd mice three times a week for a period up to 2 yr. The highest dose of each fossil fuel material was selected on the basis of: (a) the maximum concentration of the material in acetone which could be accurately and uniformly applied to the skin, and (b) a lack of acute toxicity when applied at this concentration. The other two doses are 1:10 and 1:100 dilutions, respectively, in acetone of the highest concentration. The highest concentration of BaP for the chronic study was based on previously established data on

its carcinogenicity in this strain of mouse, under the conditions of this test protocol. The amount of material in each individual dose is indicated in Table 17.

The incidence of tumors observed at gross examination and tumor latency for each group in which tumors have occurred are indicated in Table 18. Diagnoses other than those for the tumors listed in Table 19 of this report are based only on gross examination of intact, living animals. Thus, the tumor incidence data in Table 18 should be interpreted as unconfirmed, pending microscopic evaluation of tissues. The incidence of grossly observable tumors with time is indicated graphically in Figures 19 and 20.

Tumor incidence reached 100% in the group exposed to the highest dose of HD on day 147, after 62 applications (a total dose of 1414 mg of HD). A 100% incidence of tumors in mice exposed to the highest dose of BaP was reached on day 193 of the study, after 79 applications (a total dose of 4 mg of BaP).

Tumors were observed in mice exposed to the middle dose of HD (2.3 mg per exposure) within 2 wk after the first tumor had appeared in the group exposed to the highest dose of this material. The tumor incidence in the middle-dose group of HD-exposed mice slowly increased until day 227, when it began to rise rapidly, and reached 100% on

TABLE 17. Exposure Data for Epidermal Carcinogenesis Studies of Crude Fossil Fuel Materials in Mice.

Group	Concentration of Test Material in High Dose, %	Amount of Test Material ^(a) Per Application, mg/50 µl		
		High Dose	Middle Dose	Low Dose
Crude Shale Oil	50	21.2	2.1	0.21
SRC-II Heavy Distillate Fraction (HDF)	50	22.8	2.3	0.23
SRC-II Light Distillate Fraction (LDF)	50	20.0	2.0	0.20
Wilmington Crude Petroleum	40	16.8	1.7	0.17
Benzo(a)pyrene	0.1	0.05	0.005	0.0005
2-Aminoanthracene	0.1	0.05	0.005	--
Acetone (Vehicle)	100	39.4	--	--

^(a) All test materials were diluted in acetone; a total volume of 50 µl was applied at each exposure.

TABLE 18. Skin Tumor Incidence in Mice After 456 Days of Exposure to Crude Fossil Fuel Materials, Fractional Distillates, or Known Carcinogens.

Group	Dose per Exposure, mg/50 µl	No. Mice at Risk	No. Mice With Tumors	% Neoplastic Response ^(a)	Minimum Latency		Median Latency		Maximum Latency	
					No. at Risk	No. Days	No. at Risk	No. Days	No. at Risk	No. Days
Control (Untreated)		49	0	0	(--)	--	(--)	--	(--)	--
Vehicle (Acetone)	39.4	47	0	0	(--)	--	(--)	--	(--)	--
Crude Shale Oil	0.21	45	0	0	(--)	--	(--)	--	(--)	--
	2.1	50	41	82%	(50)	148	(50)	351	(--)	--
	21.2	38	38	100%	(46)	95	(45)	213	(38)	409
Wilmington Crude Petroleum	0.17	46	0	0	(--)	--	(--)	--	(--)	--
	1.7	45	3	7%	(50)	282	(--)	--	(--)	--
	16.8	45	20	44%	(50)	260	(--)	--	(--)	--
SRC-II Heavy Distillate Fraction	0.23	48	6	13%	(50)	282	(--)	--	(--)	--
	2.3	47	47	100%	(50)	72	(47)	294	(47)	372
	22.8	49	49	100%	(49)	56	(49)	95	(49)	147
SRC-II Light Distillate Fraction	0.20	41	0	0	(--)	--	(--)	--	(--)	--
	2.0	46	1	2%	(48)	427	(--)	--	(--)	--
	20.0	44	1	2%	(46)	410	(--)	--	(--)	--
2-Aminoanthracene	0.005	49	0	0	(--)	--	(--)	--	(--)	--
	0.05	32	25	78%	(50)	115	(34)	379	(--)	--
Benzo(a)pyrene	0.0005	50	0	0	(--)	--	(--)	--	(--)	--
	0.005	46	44	96%	(49)	239	(46)	358	(--)	--
	0.05	50	50	100%	(50)	106	(50)	143	(50)	193

^(a) This number represents the following ratio: $\frac{\text{No. of mice with tumors at 456 days of exposure} \times 100}{\text{No. of mice alive at first exposure} - \text{No. of mice dying without tumors}}$

TABLE 19. Histopathology Data from Skin Tumors Examined Microscopically After 456 Days Exposure of Mice to Crude Fossil Fuel Materials, Fractional Distillates, or Known Carcinogens.

Group	Dose per Exposure, mg/50 μ l	No. Animals Examined Microscopically	No. Animals With Malignant Skin Tumors	No. Animals With Metastasis of Skin Tumors	No. Animals With Papillomas Only	Gross/Microscopic Correlation
Crude Shale Oil	21.2	9	7	3	0	7/7 (100)(a)
Wilmington Crude Petroleum	16.8	2	2	0	0	2/2 (100)
SRC-II Heavy Distillate Fraction	0.23	1	1	1	0	1/1 (100)
	2.3	8	3	1	2	5/6 (83)
	22.8	30	29	16	1	30/30 (100)
2-Aminoanthracene	0.05	4	2	0	0	2/3 (67)
Benzo(a)pyrene	0.005	2	2	1	0	2/2 (100)
	0.05	31	31	15	0	31/31 (100)
Total, All Groups		87	77	37	3	80/82 (98)

(a) Percent of grossly observed skin masses which were diagnosed as neoplastic at histologic examination

FIGURE 19. Tumor Incidence Versus Duration of Exposure of Mice to Middle and Low Doses of Fossil Fuel Materials or Known Carcinogens.

FIGURE 20. Tumor Incidence Versus Duration of Exposure of Mice to High Doses of Fossil Fuel Materials or Known Carcinogens.

day 372 after 150 applications (a total dose of 345 mg of HD). Tumor incidence reached 100% in the group exposed to 21.2 mg/dose of crude shale oil after 409 days of exposure, a total of 165 applications and a total dose of 3506 mg.

All epidermal tumors occurred in the area of the application site. Tumors varied in size from less than 1 mm to 5 cm in diameter. The majority of tumors were raised, conical to round or verrucous, with a keratotic surface. Many of the larger tumors were ulcerated and some were discoid, with deeply ulcerated central craters. Many of the animals had more than one tumor at the application site.

Tumors from 80 animals have been examined microscopically. The results of these examinations are indicated in Table 19; 77 of 87 animals examined microscopically had malignant tumors; 37 of 77 had microscopic evidence of metastasis. Several animals had one or more papillomas in areas adjacent to malignant tumors; three animals had papillomas only.

There is a wide range of histologic morphology in these tumors. The predominant appearance is that of a highly invasive squamous-cell carcinoma. In some cases, there is a gradual transition from cells typical of well-differentiated squamous-cell

carcinoma to oval or fusiform tumor cells forming interlacing bundles and whorls suggestive of an anaplastic, spindle-cell variant of squamous-cell carcinoma. However, in other cases there is no obvious transition from malignant squamous epithelium, but rather a tumor-cell morphology typical of a sarcoma, with a few large, highly anaplastic-appearing cells interspersed among whorls of fusiform tumor cells. This wide variation in tumor morphology is present among histologically examined tumors from all exposure groups, with no obvious correlation of tumor morphology with exposure to any particular material. The results of cell culture, transplantation, and electron microscopic studies currently in progress may aid in identifying the cell of origin of the spindle-cell tumors.

Invasion of adjacent soft tissues is present in all malignant tumors. Metastases are present microscopically in the lungs, regional lymph nodes, liver, kidney, and heart. In some cases in which the primary tumor is composed predominantly of spindle cells, metastases have the appearance of squamous-cell carcinoma.

Figure 19 depicts the relationship of tumor incidence with duration of exposure for mice exposed to the highest concentrations of materials. There is a distinct parallelism between the tumor latency curves

for mice exposed to 0.05 mg BaP or 22.8 mg SRC II HD. After observation of the first tumors, tumor incidence rose to 100% in both these groups over a period of approximately 3 mo. This rapid induction of tumors by repeated application of 22.8 mg of HD and the highly malignant nature of the majority of these tumors, when examined histologically, indicate that this material is a strong carcinogen for mouse skin under the conditions of this study. The observation of tumors in 100% of mice exposed to a tenfold-lower concentration of HD for 372 days supports this evidence of carcinogenicity. Further support for the carcinogenic potency of this material is the fact that the median tumor latency for the group exposed to the highest dose of HD (22.8 mg per application) was considerably shorter than the median tumor latency for the group exposed to the highest dose of BaP (0.05 mg per application).

Chemical analysis of the SRC II heavy distillate fraction we used in these studies indicated a concentration of BaP in this material of approximately 114 parts per million (this Annual Report). Based on this value, there is approximately 0.0026 mg of BaP in 22.8 mg of HD, about 20-fold less than that present in the highest dose given the BaP-exposed group. Thus, the carcinogenic effect of HD is apparently not due to the presence of BaP alone, but must be related at least in part to other factors such as the carcinogenic, cocarcinogenic, or tumor-promoting effects of other polycyclic

aromatic hydrocarbons, aromatic amines, or other compounds present in the HD.

The tumor latency for the mice exposed to the highest dose of crude shale oil (21.2 mg per application) was much longer than that for the highest dose of SRC II HD. Nonetheless, this dose of crude shale oil induced tumors in 100% of exposed mice at 409 days of exposure, indicating a definite carcinogenic effect.

The data generated in this study to date have provided important information regarding the biological activity of several fossil fuel materials. We hope that the results of exposures at lower doses will enable us to demonstrate an accurate dose-response curve and predict more clearly the potential biological effect of lower doses. Also, the results of the epidermal exposure of mice to 2-aminoanthracene will enable us to evaluate its potential role in the process of HD-induced carcinogenesis.

Our results correlate well with mutagenesis assays performed on these fossil fuel materials in our laboratory (Pelroy, this Annual Report) and with similar skin-painting studies done on samples from other coal-liquefaction and shale-oil processes. Additional mouse skin-painting studies now in progress are assaying the basic and polycyclic aromatic fraction of HD for epidermal carcinogenicity.

DEVELOPMENTAL TOXICITY OF SRC II-RELATED MATERIALS

Investigators:

F. D. Andrew and D. D. Mahlum

Technical Assistance:

K. A. Poston, R. Music and J. D. Hess

Maternal and developmental toxicity potential of three SRC-II-related materials was evaluated following oral administration to pregnant rats. Dose-related developmental toxicity effects were seen, especially when exposure was from 12-16 days of gestation, usually at maternally toxic doses only. The SRC-II-related materials gave results very similar to materials of similar boiling-point range from SRC I.

We have evaluated the potential for maternal and developmental toxicity of materials from the Solvent Refined Coal II process (SRC II) in rats. The experimental design was very similar to that employed in the evaluation of analogous SRC I materials. It should be noted that materials used in this study were derived from pilot-plant operation and may not be fully representative of demonstrational/commercial plant operation.

Maternal and fetal toxicity was evaluated in both dams and offspring following administration 7-11 (Table 20) and 12-16 (Table 21) days of gestation. Developmental toxicity was evaluated in the offspring of rats exposed by gavage to one of three undiluted materials from SRC II: light, middle, or heavy distillate (LD, MD, or HD, respectively). Dosages ranged from those producing significant maternal toxicity in the dam to those producing no apparent effect (maternal or fetal): 2.0 to 0.3 g/kg/day, respectively. Corn oil alone (3 ml/kg/day) was administered to negative controls, and polychlorinated biphenyls (Aroclor 1254, 3.3% in corn oil) were used for positive control groups. All animals were fasted overnight prior to agent administration. Maternal toxicity was estimated in pregnant and nonpregnant female Wistar rats dosed from 7-11 or 12-16 days of gestation (dg). Rats were sacrificed at 21 dg for evaluation of prenatal mortality (resorptions), intra-uterine growth retardation (IUGR) and teratogenesis (malformations).

Administration of LD did not consistently induce prenatal mortality, major malformations, nor IUGR at doses up to those that were maternally toxic or lethal.

Administration of MD may induce prenatal mortality, but it did not produce major malformations nor IUGR at doses up to those that were maternally toxic as well.

Administration of HD from 7-11 dg induced prenatal mortality at doses that were moderately toxic to the dams. The occurrence of IUGR and of malformations demonstrated dose-related increases only following dosing from 12-16 dg. Cleft palate, hypoplastic lungs and diaphragmatic hernias were the malformations most often seen.

Thus, dose-related manifestations of developmental toxicity were rarely seen at doses lower than those inducing maternal toxicity as well. Dosing from 12-16 dg tended to produce more maternal and fetal toxicity than did dosing from 7-11 dg. Boiling-point analogs from the two SRC processes (SRC I PS and SRC II HD) gave surprisingly similar results.

Results of this study establish developmental toxicity levels in rats after ingestion of a number of SRC-II-related materials. These data may be valuable in designing chronic toxicity studies for SRC II materials, as well as for comparison with analogous data for materials associated with the SRC I process, petroleum refining, and oil-shale retorting. The comparative data base, however, for complex hydrocarbon mixtures is far from complete at this time.

TABLE 20. Maternal and Fetal Toxicity in Rats Following Dosing with SRC II Materials from 7-11 Days of Gestation

Agent	Maternal Considerations					Fetal Considerations				
	Dose, g/kg/day ^(a)	Number Dosed ^(c)	% Dead ^(c)	Number Pregnant at 21 dg	Weight Gain to 21 dg ^(d)	% Implants Resorbed ^(e)	Litters With Resorptions	% Malformed Fetuses ^(e,g)	Litters With Malformed ^(g)	Fetal Weight at 21 dg ^(h)
Corn Oil		14	0	12	154 ± 24	1	1	0	0	5.5 ± 0.7
Aroclor ^(b)	0.11	12	0	9	117 ± 32	14	6	0	0	4.9 ± 0.7
Light Distillate	0.56	5	20	3	142 ± 9	5	1	0	0	5.1 ± 0.5
	0.84	10	20	6	112 ± 31	13	4	0	0	5.1 ± 0.7
	1.12	10	0	4	129 ± 11	5	2	0	0	5.2 ± 0.5
	1.41	8	38	4	143 ± 40	4	1	0	0	4.8 ± 0.4
	1.69	4	75	1	34 ± 0	-- ⁽ⁱ⁾	--	--	--	--
Middle Distillate	0.65	5	0	4	160 ± 23	0	0	0	0	5.8 ± 0.6
	0.98	12	0	4	145 ± 31	3	1	0	0	6.1 ± 0.6
	1.30	10	0	6	127 ± 44	35	5	0	0	5.0 ± 0.8
	1.63	7	57	1	154 ± 0	7	1	0	0	4.3 ± 0.4
	1.96	2	100	0		--	--	--	--	--
Heavy Distillate	0.37	11	0	7	114 ± 43	53	5	2	1	5.7 ± 0.7
	0.73	13	0	7	86 ± 35	68 ^(f)	7	0	0	4.9 ± 0.4
	1.10	12	42	3	74 ± 33	67 ^(f)	3	0	0	5.5 ± 0.5

^(a) Administered by gavage once daily for five consecutive days; if undiluted, 0.1-1.2 ml given per 300 g body weight

^(b) Diluted in corn oil and 1 ml given per 300 g body weight

^(c) Includes both pregnant and nonpregnant adult females

^(d) Body weight gain between 0 and 21 days of gestation (dg); mean ± SE

^(e) Calculated on a per fetus rather than a per litter basis; includes resorbed and dead implants

^(f) One or more litters with all implants resorbed

^(g) Combination of soft tissue and skeletal malformations

^(h) Pooled means of each litter

⁽ⁱ⁾ One or more dams delivered prematurely; implant data incomplete and not included

TABLE 21. Maternal and Fetal Toxicity in Rats Following Dosing with SRC II Materials from 12-16 Days of Gestation

Agent	Maternal Considerations					Fetal Considerations				
	Dose, g/kg/day ^(a)	Number Dosed ^(c)	% Dead ^(c)	Number Pregnant at 21 dg	Weight Gain to 21 dg ^(d)	% Implants Resorbed ^(e)	Litters With Resorptions	% Malformed Fetuses ^(e,g)	Litters With Malformed ^(g)	Fetal Weight at 21 dg ^(h)
Corn Oil	--	14	0	12	148 ± 31	1 ⁽ⁱ⁾	1	0	0	5.4 ± 0.5
Aroclor ^(b)	0.11	13	0	10	101 ± 24	7	5	0	0	4.3 ± 0.6
Light Distillate	0.28	11	9	8	116 ± 36	4	4	2	1	5.5 ± 0.6
	0.56	12	8	9	101 ± 37	23 ^(f)	4	0	0	5.5 ± 0.5
	0.84	14	43	7	113 ± 27	4	3	1	1	5.4 ± 0.5
	1.12	6	67	1	32 ± 0	-- ⁽ⁱ⁾	--	--	--	--
	1.41	7	86	1	50 ± 0	20	1	0	0	4.7 ± 0.2
	1.69	5	80	1	112 ± 0	0	0	0	0	5.1 ± 0.3
Middle Distillate	0.33	9	0	9	127 ± 43	5	5	3	1	5.8 ± 0.6
	0.65	11	9	7	149 ± 32	7	5	0	0	5.8 ± 0.6
	0.98	9	11	8	118 ± 40	20 ^(f)	5	0	0	5.1 ± 0.4
	1.30	6	33	4	126 ± 19	0	0	0	0	4.9 ± 0.5
	1.63	6	67	2	94 ± 36	64 ^(f)	2	0	0	4.5 ± 0.4
	1.96	3	100	0	--	--	--	--	--	--
Heavy Distillate	0.37	15	0	14	129 ± 31	9 ^(f)	7	1	2	5.3 ± 0.5
	0.73	13	0	10	85 ± 23	66 ^(f)	9	74	5	4.3 ± 0.7
	1.10	12	17	9	76 ± 23	69 ^(f)	9	60	6	4.3 ± 0.5

^(a) Administered by gavage once daily for five consecutive days; if undiluted, 0.1-1.2 ml given per 300 g body weight

^(b) Diluted in corn oil and 1 ml given per 300 g body weight

^(c) Includes both pregnant and nonpregnant adult females

^(d) Body weight gain between 0 and 21 days of gestation (dg); mean ± SD

^(e) Calculated on a per fetus rather than a per litter basis; includes resorbed and dead implants

^(f) One or more litters with all implants resorbed

^(g) Combination of soft tissue and skeletal malformations

^(h) Pooled means of each litter

⁽ⁱ⁾ One or more dams delivered prematurely; implant data incomplete and not included

DEVELOPMENTAL TOXICITY OF SRC-I-RELATED MATERIALS

Investigators:

F. D. Andrew and D. D. Mahlum

Technical Assistance:

K. A. Poston, R. Music and L. F. Montgomery

The maternal and developmental toxicity potential of three SRC-I-related materials was evaluated following oral administration to pregnant rats. Dose-related developmental toxicity effects were seen, especially when exposure was from 12-16 days of gestation, usually at maternally toxic doses only.

As reported previously (Annual Report, 1978), the potential for maternal and developmental toxicity of materials from the Solvent Refined Coal I process (SRC I) in rats has been evaluated. Efforts described this year amplify and extend those preliminary results. It should be noted that materials used in this study were derived from pilot-plant operations and may not be fully representative of demonstrational/commercial plant operation.

Developmental toxicity was evaluated in the offspring of rats exposed by gavage to one of three materials from SRC I: light oil (LO), wash solvent (WS), or process solvent (PS) undiluted or after dilution in corn oil. Dosages ranged from those producing significant maternal toxicity to those producing no apparent effect (maternal or fetal): 2.9 to 0.2 g/kg/day, respectively. Corn oil alone (3 ml/kg/day) was administered to vehicle control groups, and a polychlorinated biphenyl (Aroclor 1254, 2.5% in corn oil) was used for positive control groups. All animals were fasted overnight prior to agent administration. Maternal toxicity was estimated in pregnant and nonpregnant female Wistar rats dosed from 7-11 or 12-16 days of gestation (dg). Rats were sacrificed at 21 dg for evaluation of prenatal mortality (resorptions), intra-uterine growth retardation (IUGR), and teratogenesis (malformations).

The detailed data are presented in Tables 22 and 23, while only the most noteworthy observations are indicated in the following paragraphs.

Administration of LO consistently induced IUGR only at doses that were both maternally toxic and lethal. Prenatal mortality was elevated only after dosing with maternally toxic doses from 12-16 dg. Occasional major malformations were seen in some groups dosed from 7-11 dg, but not after dosing from 12-16 dg.

Administration of WS did not induce IUGR nor a significant incidence of malformations at doses that were not also maternally toxic and lethal. The incidence of prenatal mortality was elevated only following dosing at 12-16 dg.

Administration of PS consistently induced prenatal mortality at doses below those that were maternally toxic. The occurrence of IUGR and major malformations increased only following dosing from 12-16 dg. Cleft palate was the malformation most often seen.

Thus, dose-related manifestations of developmental toxicity were rarely seen at doses lower than those inducing maternal toxicity as well. Dosing from 12-16 dg tended to produce more maternal and fetal toxicity than did dosing from 7-11 dg.

The results of this study establish developmental toxicity levels in rats after ingestion of a number of SRC-I-related materials. These data may be valuable in designing chronic toxicity studies for SRC I materials, as well as for comparison with SRC II, petroleum refining, and oil-shale retorting. The comparative data base, however, for complex hydrocarbon mixtures is far from complete at this time.

TABLE 22. Maternal and Fetal Toxicity in Rats Following Dosing with SRC I Materials from 7-11 Days of Gestation

Agent	Maternal Considerations					Fetal Considerations				
	Dose, g/kg/day ^(a)	Number Dosed ^(c)	%, Dead ^(c)	Number Pregnant at 21 dg	Weight Gain to 21 dg ^(d)	%, Implants Resorbed ^(e)	Litters with Resorptions	%, Malformed Fetuses ^(e,g)	Litters with Malformed ^(g)	Fetal Weight at 21 dg ^(h)
Corn Oil	--	14	0	13	154 ± 22	6	5	1	1	5.6 ± 0.4
Aroclor ^(b)	0.08	5	0	4	96 ± 30	41	4	0	0	4.9 ± 0.6
Light Oil ^(b)	0.24	4	0	2	164 ± 1	7	2	0	0	6.2 ± 0.4
	0.72	4	0	3	147 ± 5	0	0	2	1	5.9 ± 0.5
	1.20	4	0	3	178 ± 4	0	0	0	0	5.8 ± 0.3
Light Oil	2.39	9	56	2	110 ± 50	3	1	0	0	4.9 ± 0.7
	2.63	5	80	1	88	8	1	9	1	4.2 ± 0.4
	2.87	5	80	0	--	--	--	--	--	--
Wash Solvent ^(b)	0.85	6	0	6	139 ± 22	5	3	0	0	5.7 ± 0.5
	1.11	5	0	5	144 ± 22	5	3	0	0	5.4 ± 0.4
	1.27	7	0	4	124 ± 41	6	2	0	0	5.9 ± 0.6
	1.43	9	0	5	114 ± 33	16	3	2	1	5.1 ± 0.4
	1.58	6	67	2	154 ± 2	4	1	0	0	5.4 ± 0.2
Process Solvent ^(b)	0.34	8	0	6	150 ± 23	22	5	0	0	5.9 ± 0.5
	0.51	8	0	6	127 ± 29	25	5	0	0	5.6 ± 0.5
	0.69	8	0	5	88 ± 37	75 ^(f)	5	6	1	6.0 ± 0.5
	0.85	5	0	4	52 ± 38	77 ^(f)	4	0	0	5.3 ± 0.7

(a) Administered by gavage once daily for five consecutive days; if undiluted, 0.1-1.2 ml given per 300 g body weight

(b) Diluted in corn oil and 1 ml given per 300 g body weight

(c) Includes both pregnant and nonpregnant adult females

(d) Body weight gain between 0 and 21 days of gestation (dg); mean ± SD

(e) Calculated on a per fetus rather than a per litter basis; includes resorbed and dead implants

(f) One or more litters with all implants resorbed

(g) Combination of soft tissue and skeletal malformations

(h) Pooled means of each litter

(i) One or more dams delivered prematurely; implant data incomplete and not included

TABLE 23. Maternal and Fetal Toxicity in Rats Following Dosing with SRC I Materials from 12-16 Days of Gestation

Agent	Maternal Considerations					Fetal Considerations				
	Dose, g/kg/day ^(a)	Number Dosed ^(c)	%, Dead ^(c)	Number Pregnant at 21 dg ^(c)	Weight Gain to 21 dg ^(d)	%, Implants Resorbed ^(e)	Litters with Resorptions	%, Malformed Fetuses ^(e,g)	Litters with Malformed ^(g)	Fetal Weight at 21 dg ^(h)
Corn Oil	--	22	0	20	141 ± 30	2	4	1	2	5.5 ± 0.5
Aroclor ^(b)	0.08	14	0	11	116 ± 30	12	8	5	2	4.6 ± 0.5
Light Oil	1.44	5	0	3	135 ± 18	7	3	0	0	5.2 ± 0.4
	1.69	10	20	7	109 ± 34	5	2	0	0	5.1 ± 0.4
	1.93	13	0	7	58 ± 57	29	6	0	0	3.9 ± 0.8
	2.17	10	30	5	84 ± 40	8	4	0	0	4.6 ± 0.7
	2.41	11	27	5	83 ± 81	35 ^(f)	3	0	0	4.7 ± 0.7
Wash Solvent ^(b)	0.95	13	0	10	118 ± 43	14	4	0	0	5.4 ± 0.5
	1.11	10	0	7	98 ± 39	10	3	2	1	4.7 ± 0.7
	1.27	9	67	2	88 ± 56	50	1	0	0	5.0 ± 0.3
Process Solvent ^(b)	0.34	5	0	3	128 ± 3	10	2	6	1	5.0 ± 0.4
	0.52	5	0	4	100 ± 28	24 ^(f)	4	4	1	4.8 ± 0.5
	0.69	13	8	10	86 ± 32	28	7	13	6	4.4 ± 0.6
	0.85	12	25	6	55 ± 41	70 ^(f)	6	13	2	4.3 ± 0.8
	1.03	4	50	1	72	75	1	33	1	3.4 ± 1.0
Process Solvent	0.34	6	0	4	134 ± 41	5	2	0	0	5.2 ± 0.4
	0.69	6	0	6	93 ± 41	36	3	4	2	4.8 ± 0.7
	1.03	3	67	1	53	54	1	50	1	3.4 ± 0.4

(a) Administered by gavage once daily for five consecutive days; if undiluted, 0.1-1.2 ml given per 300 g body weight

(b) Diluted in corn oil and 1 ml given per 300 g body weight

(c) Includes both pregnant and nonpregnant adult females

(d) Body weight gain between 0 and 21 days of gestation (dg); mean ± SE

(e) Calculated on a per fetus rather than a per litter basis; includes resorbed and dead implants

(f) One or more litters with all implants resorbed

(g) Combination of soft tissue and skeletal malformations

(h) Pooled means of each litter

RESPONSE OF AWAKE GUINEA PIGS TO INHALED HISTAMINE AEROSOLS

Investigators:

S. M. Loscutoff, B. W. Killand, and R. L. Buschbom

Guinea pigs were repeatedly exposed to histamine aerosols, while allowing progressively shorter recovery times between exposures, to determine the repeatability of the histamine response in individual animals and to assess residual effects caused by previous exposure. Repeated measurements of histamine responses in the same animal showed substantial variability, which was not significantly different from that measured in the group. Fourteen-month-old guinea pigs had significantly higher values of pulmonary compliance during initial exposure to histamine compared with all subsequent exposures. Nine-month-old guinea pigs showed no substantial differences between their initial response to histamine and responses following 12, 6 and 3 days recovery from a previous histamine exposure. Because of difficulty interpreting the dose response relationships, we propose to conduct future studies with animals exposed to a single histamine dose for a constant time interval.

An increased responsiveness to inhaled histamine aerosols is a sensitive means of detecting effects of breathing low concentrations of air pollutants in dogs and humans. Because of the sensitivity of this test (compared with more standard measures of pulmonary function, such as pulmonary resistance and dynamic pulmonary compliance), we were interested in using this test in non-anesthetized guinea pigs. The response of guinea pigs to inhaled histamine aerosols has been reported to be highly variable: approximately a 50- to 100-fold difference in the concentration of histamine producing a particular response. We therefore examined each animal repeatedly, allowing progressively shorter recovery periods between exposures, to determine if the response of an animal was consistent over several days, and to determine if there were residual effects of previous histamine exposures. Two groups of guinea pigs were examined: 12 old animals (approximately 14 mo old, weighing 800 g) and 13 young animals (9 mo old, weighing 500 g). Both groups were mature adults. The "old" and "young" designation is used to differentiate between the two exposure groups.

The characteristics of inhaled histamine aerosols are shown in Table 24. The aerosol was generated in a Model 51 B deVilbiss ultrasonic nebulizer from a 0.2% histamine dihydrochloride solution. Airflow to the

exposure chamber was 10 l/min; the histamine concentration was increased in steps by increasing air flow through the nebulizer. There was an approximately linear relationship between nebulizer air flow and histamine concentration in the chamber. Particle size distributions at four nebulizer flow rates were between 0.6 and 0.8 μ m mass median aerodynamic diameter (MMAD). At the high nebulizer flow rates, a fraction of the aerosol had a very large particle size (7-20 μ m). However, this fraction comprised only 1 to 2% of the total aerosol concentration unless the high flow rate was maintained for 15 to 20 min (the last sample at 1200 ml/min), when 12% of the aerosol had an MMAD of 7 μ m. Exposures were conducted by placing guinea pigs in the exposure chamber and obtaining stable values of pulmonary resistance, compliance and breathing pattern at zero nebulizer flow. Nebulizer flow was then increased in 100-ml/min increments, and pulmonary compliance values were allowed to stabilize for 5 to 10 min before increasing flow to the next level. The exposure was stopped when compliance dropped to less than the 0.8 ml/cm H₂O.

The principal change measured during these exposures was a decrease in dynamic pulmonary compliance, with little or no change in breathing pattern or resistance until histamine concentrations reached 8 to 10 mg/m³. At these high concentrations,

TABLE 24. Characteristics of Inhaled Histamine Aerosol

Flow Rate Through Nebulizer, ml/min	Histamine Concentration, mg/m ³	%	Particle Size Distribution	
			MMAD (a) (μm)	GSD (b)
100	1.81	--	--	--
200	2.58	--	--	--
300	3.35	100	0.64	1.9
300	3.35	100	0.55	1.8
400	4.13	--	--	--
500	4.90	--	--	--
600	5.67	100	0.64	1.5
600	5.67	100	0.64	1.5
700	6.44	--	--	--
800	7.22	--	--	--
900	7.99	100	0.70	1.6
900	7.99	98 2	0.71 20.1	1.5 2.1
1000	8.76	--	--	--
1100	9.54	--	--	--
1200	10.31	99 1	0.72 23.4	1.5 2.1
1200	10.31	88 12	0.76 7.1	1.5 1.7

(a) Mass Median Aerodynamic Diameter
(b) Geometric Standard Deviation

respiration rate tended to increase and tidal volume decreased, with minimal change in resistance. Lee, et al. (J. Appl. Physiol. 43: 626-631, 1977), studying responses to inhaled histamine, found an increase in resistance along with a decrease in compliance. However, their work was done with aerosols of approximately 2 to 4 μm MMAD. We feel that the small histamine aerosols generated by the ultrasonic nebulizer are not depositing in, and are therefore not affecting, the large airways (the primary site of resistance changes).

Each animal was tested four times; Figures 21 and 22 show the mean responses of the two groups. The 0-line represents the initial response to histamine exposure; 12, 6 and 3 indicate the number of days allowed for recovery before the next exposure. Figure 1 shows a significantly higher compliance on day 0 compared with all other

exposures for the older animals ($p < 0.05$, one-way analysis of variance). This response was not seen in the young animals. The two cases where responses on one day were significantly different from those on all other days did not follow a pattern and were very likely due to chance.

When examining the responses of individual animals, variability of repeated measurements made on the same animals was not significantly different from variability of the group. Because of the variability of the response, we were not able to define a parameter or group of parameters useful in comparing responses of an individual animal or among animals. Figure 23 shows a fairly consistent response to inhaled histamine measured in one guinea pig on four separate days. Depending on the parameter examined, relationships between the four exposures could be interpreted very differently.

FIGURE 21. Histamine Concentration vs Dynamic Pulmonary Compliance in 14-Month-Old Guinea Pigs (N = 12)

FIGURE 22. Histamine Concentration vs. Dynamic Pulmonary Compliance in 9-Month-Old Guinea Pigs (N = 12)

FIGURE 23. Histamine Concentration vs. Dynamic Pulmonary Compliance in an Individual Guinea Pig, Measured on 4 Different Days.

For example, on days 0, 12 and 3 there was a marked drop in compliance as histamine concentration increased from 1 to 6 mg/m³; the response pattern on day 6 was completely different. On days 0, 12 and 6 the histamine concentration which caused compliance to fall to 0.8 ml/cm H₂O were similar; on day 3, the concentration was different. Therefore, comparing the histamine concentrations causing a 50% decrease in compliance with the concentration decreasing compliance to 0.8 ml/cm H₂O results in different interpretations of the data. Because of this problem in interpreting dose-response curves,

in future experiments we plan to expose all animals to a single dose of histamine for a constant time interval. Easily defined parameters, such as the maximum change in compliance, the time required to reach this maximum and, perhaps, some aspect of recovery can then be easily measured and compared. The histamine dose selected will be one which produces at least a 25% decrease in compliance in 90% of the animals. This protocol should allow more consistent interpretation of the results than those we have obtained by examining cumulative dose response curves.

PULMONARY EFFECTS OF LONG-TERM SULFURIC ACID AEROSOL EXPOSURE IN NORMAL AND EMPHYSEMATOUS GUINEA PIGS

Investigators:

S. M. Loscutoff and R. L. Buschbom

Technical Assistance:

M. B. Bair, R. G. Bennett and B. W. Killand

Groups of normal and elastase-treated guinea pigs (the latter had experimentally induced pulmonary emphysema) were exposed to 0, 2 and 10 mg/m³ sulfuric acid aerosols, 6 hr/day, 5 days/wk for 16 wk. Pulmonary function measurements showed changes primarily due to elastase treatment. Acid exposure significantly increased residual volume and functional residual capacity. There appeared to be a significant interaction between acid exposure and elastase treatment, resulting in increased gas trapping in the lung. However, elastase-treated animals were not markedly more sensitive to sulfuric acid aerosols compared with normal animals.

Humans with pulmonary emphysema are more likely than normal individuals to suffer adverse health effects during severe air pollution episodes. The purpose of this study was to determine if guinea pigs with experimentally induced pulmonary emphysema caused by intratracheal elastase instillations, were more sensitive than normal animals to daily sulfuric acid (H₂SO₄) aerosol exposures.

Guinea pigs were exposed to 0, 2 and 10 mg/m³ sulfuric acid aerosols, 6 hr/day, 5 days/wk, for 16 wk. To produce emphysema, half the animals exposed to each acid concentration were pretreated with intratracheally instilled elastase (5 units/100 g body wt, crystalline porcine pancreatic elastase, Sigma Chemical Co.) 10 days before exposure. Characteristics of the aerosols and the number of animals per group are shown in Table 25. Pulmonary function measurements made on urethan-anesthetized animals to evaluate effects of aerosol exposure included: quasi-static compliance, multi-breath N₂ washout maneuvers and all divisions of lung volume. After testing, the animals were killed and excised lung weights and volumes were measured.

Analysis of body weight data showed that both normal and elastase-treated animals exposed to 2 mg/m³ H₂SO₄ weighed approximately 10% less than those of the other four exposure groups. These animals were generally exposed from 3:30 p.m. to 9:30 p.m.

TABLE 25. Characteristics of Inhaled Sulfuric Acid Aerosols and Number of Animals/Exposure Group

		Mass Concentration, mg/m ³	Particle Size Distribution MMAD ^(a) (µm)	GSD ^(b)	No. Animals Group
Control		0	--	--	--
		--	--	--	18 Normal
		--	--	--	18 Elastase
Low	$\bar{X}$	2.3	0.70	2.3	17 Normal
	SE	0.3	0.18	0.4	18 Elastase
	N	14	5	5	
High	$\bar{X}$	9.7	1.0	1.8	18 Normal
	SE	1.4	0.25	0.24	17 Elastase
	N	19	6	6	

^(a) MMAD - Mass Median Aerodynamic Diameter
^(b) GSD - Geometric Standard Deviation

daily; the high-dose and control animals were exposed from 8:30 a.m. to 2:30 p.m. This difference in the time of exposure probably caused the difference in weight due to the daily activity and eating patterns of the animals. In presenting and discussing the data, we have omitted values for the low-dose animals because many of the experimental parameters were significantly correlated with body weight. Including these animals in the analysis (with or

without correcting for weight) would have confused the interpretation, masking significant effects found when only the control and high-dose animals were compared.

The major effects observed in this study were due to elastase treatment. A two-way analysis of variance was used to evaluate differences between normal and elastase-treated animals, and between control and sulfuric-acid-exposed groups. Table 26 shows body weights, excised lung weights and excised lung volumes. Body weights and lung weights were not significantly different among the different groups. Excised lung volumes and lung volumes divided by lung weights were significantly higher in elastase-treated compared with normal animals, indicating an increased volume of trapped gas.

Values of quasi-static compliance, the slope and "y" intercept of the multi-breath N_2 washout maneuver plotted as log end expired N_2 vs breath number are listed in Table 27. Each of these parameters showed significant effects of elastase treatment, but no effect of aerosol exposure, and no interaction between effects of elastase and aerosol.

Values for all divisions of lung volumes are shown in Table 28 and, except for functional residual capacity, all volumes were measured during deflation after inflating the lungs with either air or oxygen. All volumes were significantly higher in elastase-treated compared with normal animals. In addition, acid-exposed animals had significantly higher values of residual volume (following inflation with O_2) and functional

TABLE 26. Body Weights, Excised Lung Weights and Lung Volumes in Guinea Pigs Exposed to Sulfuric Acid Aerosols

			Body Weight, g	Lung Weight, g	Lung Volume, ml	Lung Volume/ Lung Wt., ml/g
Normal Control	$\bar{X}$		784	4.4	6.1	1.4
	SE		22	0.2	0.2	0.004
	N		13	13	13	13
Elastase Control	$\bar{X}$		775	4.6	7.3	1.6
	SE		17	0.1	0.3	0.005
	N		12	12	12	12
Normal 10 mg/m ³ H ₂ SO ₄	$\bar{X}$		771	4.3	6.0	1.4
	SE		17	0.1	0.1	0.01
	N		13	13	13	13
Elastase 10 mg/m ³ H ₂ SO ₄	$\bar{X}$		751	4.6	8.0	1.8
	SE		27	0.1	0.3	0.006
	N		12	12	12	12
Significant Effect p < 0.05 two-way ANOVA			--	--	Elastase	Elastase

TABLE 27. Quasi-Static Compliance and Multibreath N_2 Washout in Guinea Pigs Exposed to Sulfuric Acid Aerosols

		Quasi-Static Compliance, ml/cm H ₂ O	Slope N_2 Washout, Log N_2 /breath	Y-Intercept N_2 Washout, Log N_2
Normal Control	$\bar{X}$	2.5	-0.033	1.82
	SE	0.2	0.002	0.01
	N	13	13	13
Elastase Control	$\bar{X}$	3.3	-0.029	1.78
	SE	0.1	0.002	0.01
	N	12	12	12
Normal 10 mg/m ³ H ₂ SO ₄	$\bar{X}$	2.5	-0.033	1.83
	SE	0.1	0.002	0.01
	N	13	13	13
Elastase 10 mg/m ³ H ₂ SO ₄	$\bar{X}$	3.4	-0.029	1.76
	SE	0.2	0.002	0.01
	N	12	12	12
Significant Effect P < 0.05 two-way ANOVA		Elastase	Elastase	Elastase

residual capacity than control animals. The only significant ($p < 0.05$) interaction between elastase treatment and aerosol exposure was in measurements of vital capacity following inflation with O_2 . In normal animals, acid exposure tended to increase vital capacity; while in elastase-treated animals, acid exposure tended to decrease vital capacity.

Although a significant interaction was measured between elastase treatment and sulfuric acid aerosol exposure, elastase-treated animals are not markedly more sensitive to sulfuric acid aerosols. These results suggest that tests of forced expiration to maximize any tendency for gas trapping may be very useful in evaluating effects of aerosol exposure in guinea pigs. In addition, exposure to other agents which produce more extensive lesions in small airways than sulfuric acid aerosols may be more effective in exacerbating effects of elastase-induced emphysema.

TABLE 28. Lung Volumes in Guinea Pigs Exposed to Sulfuric Acid Aerosols

		Residual Volume Air, ml	Residual Volume O ₂ , ml	Functional Residual Capacity Air, ml	Vital Capacity Air, ml	Vital Capacity O ₂ , ml	Total Lung Capacity Air, ml	Total Lung Capacity O ₂ , ml
Normal Control	$\bar{X}$	3.7	3.8	7.7	20.6	20.5	24.3	24.3
	SE	0.3	0.3	0.4	0.6	0.6	0.8	0.8
	N	13	13	13	13	13	13	13
Elastase Control	$\bar{X}$	6.2	6.7	10.4	23.7	23.3	29.9	29.9
	SE	0.5	0.5	0.5	0.6	0.7	0.6	0.6
	N	12	12	12	12	12	12	12
Normal 10 mg/m ³ H ₂ SO ₄	$\bar{X}$	4.5	4.6	8.7	22.1	22.0	26.0	26.6
	SE	0.3	0.3	0.3	0.4	0.4	0.6	0.4
	N	13	13	13	13	13	13	13
Elastase 10 mg/m ³ H ₂ SO ₄	$\bar{X}$	7.2	8.1	11.6	23.0	22.2	30.0	30.3
	SE	0.7	0.8	0.6	0.7	0.6	1.2	1.1
	N	12	12	12	12	12	12	12
Significant Effect P < 0.05 two-way ANOVA		Elastase	Elastase H ₂ SO ₄	Elastase H ₂ SO ₄	Elastase	Elastase Interaction	Elastase	Elastase

ORAL TOXICITY OF SRC II HEAVY DISTILLATE IN THE MOUSE

Investigators:

D. D. Mahlum, J. Loera and L. P. Montgomery

Charles River Swiss-Webster mice were administered one dose of SRC II heavy distillate (HD) by gavage for determination of acute toxicity. For subchronic toxicity studies, the material was administered once each day for 5 consecutive days. The animals were observed for death, and weight changes were determined. The acute LD₅₀ value for mice (3.65 g/kg) was somewhat higher than that found for rats (2.98 g/kg). The subchronic LD₅₀ value for mice (approximately 0.8 g/kg/day) was approximately 25% lower than that found for rats.

We wanted to study the effect of SRC II heavy distillate (HD) on the production of dominant lethal mutations in the mouse. In preparation, it was necessary to establish an acute and a subchronic LD₅₀ value for the strain that we were using.

Swiss-Webster mice obtained from Charles River Breeding Laboratories, Inc., were administered undiluted HD by gavage. For acute studies, HD was administered one time only; for subchronic studies, HD was administered once daily for 5 consecutive days. Deaths were recorded, as well as symptoms of toxicity, and weights were determined frequently throughout the experiment.

Figure 24 is a probit plot for the acute toxicity studies. From this plot, a value of approximately 3.65 g/kg body weight was derived for the acute LD₅₀ value. This value is somewhat higher than the value of 2.98 g/kg established for rats.

For the determination of subchronic toxicity, groups of mice were administered daily doses of HD corresponding to 100, 75, 50, 25 or 10% of the acute LD₅₀ value for 5 consecutive days. The doses corresponding to 100, 75 or 50% of the acute LD₅₀ resulted in death of all of the animals. About 50% of the animals that received 25% of the acute LD₅₀ dose, and about 30% of those which received 10% of the acute LD₅₀ dose died during this experiment. It thus appears that the approximate subchronic LD₅₀ dose was 0.9 g/kg/day. The subchronic toxicity value appears to be about 25% lower for mice than the 1.2 g/kg/day found for rats. However, errors associated with the small volumes of HD required for exposing mice might account for part of the difference in values between the two species.

FIGURE 24. Acute Toxicity of Heavy Distillate in Swiss-Webster Mice

ORAL TOXICITY OF SOLVENT-REFINED-COAL-RELATED HYDROCARBONS IN RATS

Investigators:

D. D. Mahlum and F. D. Andrew

Wistar rats were exposed to solvent refined coal (SRC) materials or to other fossil-derived materials, including shale oil, diesel oil, and crude petroleum. Acute toxicity was determined by administering the materials one time only by gavage. Subchronic studies were performed by administering the material once daily for 5 consecutive days. The SRC-I wash solvent was the most toxic in the acute studies, having an LD₅₀ of about 0.6 g/kg body weight. Toxicities of the other SRC materials were similar to one another with acute LD₅₀ values of 2.3 to 3.8. Acute LD₅₀ values for shale oil, diesel oil, and crude petroleum were about three- to fourfold higher, ranging from 9 to more than 12 g/kg body weight. Subchronic values for light distillate, process solvent, heavy distillate, and middle distillate were considerably lower than for the acute dose of these materials. Acute and subchronic values were similar for wash solvent and light oil. Acute LD₅₀ values for process solvent and heavy distillate were substantially lower in 14-day-old rats than in adults; however, the values for 21-day-old rats (weanlings) were not greatly different from those for the adult.

Experiments were performed to extend the work presented in last year's Annual Report (1978) on the toxicities of solvent refined coal (SRC) materials in rats. The toxicities of other fossil-derived materials (including crude shale oil, diesel oil, and a coal solvent) have also been determined for comparative purposes. The toxicities of SRC I process solvent and SRC II heavy distillate have also been studied in 14- and 21-day-old rats to determine if the immature animal is more sensitive than the adult. SRC materials used in this study are of pilot plant origin and may not be representative of commercial operation or all operating conditions of the pilot facility.

The material to be studied was administered undiluted by gavage. For the study of acute toxicity, the material was administered once; for the subchronic studies, administration was once daily for 5 consecutive days.

The LD₅₀ values in adult rats after acute or subchronic administration of several materials are compared in Table 29. Shale oil, diesel oil, and crude petroleum had similar acute LD₅₀ values, ranging from 9.2 to more than 12 g/kg body weight. Wash

TABLE 29. Comparison of the Acute and Subchronic Toxicities of SRC Materials in the Female Wistar Rat^(a)

Material	Acute LD ₅₀ ^(b)	Subchronic LD ₅₀ ^(b)
Wash Solvent	0.57 (1.66) ^(c)	(1.50) ^(c)
Light Distillate	2.30	0.96
Process Solvent	2.81	1.04
Light Oil	2.90	2.41
Heavy Distillate	2.98	1.19
Middle Distillate	3.75	1.48
Shale Oil	9.22	--
Diesel Oil	11.8	--
Crude Petroleum	>12	--

(a) The materials were administered one time by gavage for the acute toxicity studies. For the subchronic studies, materials were gavaged once daily for five consecutive days.

(b) LD₅₀ is defined as the dose in grams per kilogram of body weight required to kill 50% of the animals. For the acute toxicity study, this value represents a single dose while for the subchronic studies, the value represents a daily dose.

(c) The LD₅₀ values in parentheses are for materials diluted in corn oil. All other values are for undiluted material.

solvent was the most toxic (LD_{50} , approximately 0.6 g/kg body weight). The acute LD_{50} values for the other materials ranged from 1.8 for coal solvent (coal-derived material used as start-up solvent for SRC processes; not shown in table) to 3.8 for middle distillate. The subchronic toxicity for wash solvent and light oil were not greatly different from the acute LD_{50} value. In the case of light distillate, process solvent, middle distillate, and heavy distillate, the subchronic LD_{50} was substantially lower than the acute LD_{50} value.

When process solvent or heavy distillate was administered to weanling animals, the acute LD_{50} values were only slightly lower than for adults (Figures 25 and 26). However, when these materials were administered to 14-day-old rats, the LD_{50} values were 1.74 and 1.41 g/kg, respectively, for process solvent and heavy distillate. Thus, 14-day-old rats appeared to be about twice as sensitive to process solvent and heavy distillate as were adults.

Our tentative conclusion after completion of last year's studies was that the SRC materials were moderately toxic; this still appears to be valid.

FIGURE 25. Influence of Age and Sex on the Acute Toxicity of Process Solvent. Each point represents 10-25 rats.

FIGURE 26. Influence of Age and Sex on the Acute Toxicity of Heavy Distillate. Each point represents 10-27 rats.

DOSIMETRIC AND METABOLIC STUDIES WITH SOLVENT REFINED COAL (SRC)

Investigators:

F. G. Burton, R. E. Schirmer and D. D. Mahlum

Tissues of rats which had received oral doses of SRC I process solvent were analyzed to determine changes in distribution of the process solvent components in the organs with time. Absorption of all the compounds from the stomach contents is slow, the rate decreasing with increasing molecular weight. Amounts of a given compound recovered from the rat also vary with the compound and with time.

Two studies have been made of the absorption and distribution of process solvent components in rats receiving oral doses of SRC I process solvent. Tissue levels were quantitated by gas chromatography on capillary columns, using the procedure developed during FY 1978.

In the first study, female rats were dosed by gavage with 0.5 ml of process solvent from the SRC I process. Animals were sacrificed at 2, 4, 8, 24 and 48 hr after dosing and tissues taken for analysis. Blood was taken at 0.5, 1.0, 1.5 and 16 hr, as well as at sacrifice. As indicated in Figure 27, the loss of compounds from the stomach contents appears to be a linear function of time, but the absorption rate constant varies between compounds. Absorption of all the compounds studied was slow, and the rate appears to decrease with increasing molecular

weight (Figure 28). The total recovery of individual components from all the analyzed tissues also varies with the compound and with time (Figure 29). The differences in recoveries for the different compounds are presumably due to differences in the rates at which they are metabolized and eliminated from the body.

A second study was designed to estimate inter-animal variability and rate constants for absorption and elimination of the major process solvent components. Animals were dosed by gavage with 0.7 ml process solvent; blood samples were taken at 1, 2, 4, 7 and 24 hr; and animals were sacrificed and tissue samples taken at 4, 7 and 24 hr. Samples were taken from three animals at each time point. Recovery of compounds from the red blood cells is shown in Figure 30. Plasma concentrations of all components were much

FIGURE 27. Loss of Compounds from Stomach Contents

lower than concentrations in red blood cells. Phenanthrene, for example, was present at less than 1 $\mu\text{g}/\text{ml}$ in plasma at all time points. Concentration of process solvent components in liver, kidney, and adipose tissue of rats are shown in Figures 31, 32 and 33. The error bars in the figures are standard deviations estimated from the analysis of samples from three animals. They are a measure of the inter-animal variability in absorption and disposition of each compound.

In all work done to date on animals sacrificed at periods up to 48 hr after dosing, the highest levels of unmetabolized aromatic constituents of process solvent were found in the gastrointestinal tract; significant amounts were also found in liver

FIGURE 28. Half Life of a Compound in Stomach Contents as a Function of Its Molecular Weight

FIGURE 29. Total Recovery from Analyzed Tissues with the Compound and with Time.

and fat. Trace amounts were found in lung, ovary, brain, kidney and heart. The relative distribution of different aromatic constituents varied according to the tissue and time after administration.

It should be noted that the process solvent used in this study was derived from pilot-plant operation and may not be fully representative of demonstrational/commercial plant operation.

FIGURE 30. Recovery of Compounds from Red Blood Cells of Rats

FIGURE 31. Recovery of Compounds from Liver of Rats

FIGURE 32. Recovery of Compounds from Kidney of Rats

FIGURE 33. Recovery of Compounds from Fatty Tissues of Rats

INHALATION STUDIES WITH SRC I PROCESS SOLVENT

Investigators:

D. L. Springer, E. H. Willard, J. R. Laidler and D. D. Mahlum

A system has been devised for generating aerosols of solvent refined coal process solvent (PS) with concentrations of up to 6.5 mg/l and a mass median aerodynamic diameter of $\leq 2.2 \mu\text{m}$. Exposure of adult rats to PS for 6 hr/day for 5 days/wk for 1-3 wk resulted in loss of weight for a significant fraction of the animals. The weight was regained on termination of the exposure.

Exposure of occupational personnel in a solvent refined coal (SRC) plant might occur by skin contact or inhalation. We have begun studies to evaluate the effects of exposure by inhalation to one of the process stream materials from the SRC-I process. This report will present the results of efforts to derive an aerosol generation technique for the exposure of rodents to SRC-I process solvent (PS). It will also present the results of preliminary toxicity studies in rats.

Process solvent is a complex mixture of organic compounds with a boiling-point range of 450-850°F. To generate an aerosol of PS in the desired concentration range (1-10 mg/l) without changing its composition, we chose a Retec nebulizer with a glass reservoir (PS attacks the usual polystyrene reservoir). The aerosol was generated into a 1-m³ plexiglass chamber and periodic samples were taken from several areas of the chamber for determination of concentration. Other samples were taken with a Mercer cascade impactor to determine the range of particle sizes.

Since quantitative analysis by gas chromatography is a long and cumbersome procedure, a more rapid method was sought. We found that PS showed an absorbance peak at 254 nm; therefore, measurements of aerosol concentrations were performed at this wavelength after dissolution of PS from filters into chloroform. Gas chromatographic analyses are being performed, however, to insure that all individual components of the original PS are present in the aerosol.

Initially, the production of aerosol with the Retec nebulizer was highly variable. This variability was found to be related to

the volume of PS in the reservoir and to clogging of the siphon tube of the nebulizer. These problems were eliminated by filtering the PS through a glass-fiber filter, and maintaining larger amounts of PS in the reservoir. Aerosol concentrations of 1-2 mg/l were consistently achieved when these actions were taken. The mass median aerodynamic diameter of the aerosols ranged from 1.4-2.1 μm .

In the first animal experiment, 12 rats that were exposed for 6 hr/day for 5 days to an average aerosol concentration of 6.3 mg/l showed signs of toxicity. The most pronounced symptoms were profuse tearing of the eyes and loss of hair, which sometimes fell out in patches. By the end of the exposure period, each animal had lost an average of 30 g of body weight, which represented a 10% reduction in size.

In the second experiment, 12 animals were exposed for 3 wk, 6 hr/day, 5 days/wk. The average concentration of PS in the aerosol over the 3-wk period was 1.2 mg/l, with an average mass median aerodynamic diameter of 2.2 μm . The toxic symptoms, which were similar to those observed in the first experiment, included reduced body weight, eye irritation and loss of hair. During the early portion of exposure, the animals as a group showed a very rapid weight loss that plateaued in the second week (Figure 34). On the last day of exposure, individual animals had lost from 2-29% of their pre-exposure weights. The group mean was significantly reduced ($p < 0.01$). The animals fell into three arbitrary, but seemingly distinct, weight loss groups: <6% loss, three animals; 9-13% loss, five animals; >18% loss, four animals. By 30 days after completion of exposure, all

FIGURE 34. Effect of Inhaled SRC Process Solvent on Mean Body Weight of Wistar Rats ($\pm$ S.D.)

animals were within 3% of their beginning weights. No lesions were detected when the animals were necropsied 1 mo after treatment ended. The lungs contained dark, diffuse spots that were presumably SRC-derived material; this observation awaits histological confirmation.

It should be noted that materials used in the study were derived from pilot-plant operations, and may not be fully representa-

tive of demonstrational/commercial plant operation.

These data indicate that process solvent administered at an aerosol concentration of 6.3 mg/l for 5 days, or at 1.2 mg/l for 3 wk, was toxic (but not lethal) to Wistar rats; and that the toxic aerosol concentration for PS is quite high when compared to many organic materials.

• Perinatal Effects of Pollutants

The objective of this project is to establish an improved set of criteria for evaluating the potential of agents to induce one or more manifestations of developmental toxicity. A general approach is being developed that is applicable for testing physical, chemical, and nutritional hazards in appropriate animal models. Our approach focuses on subtle functional impairments at various times during maturation, rather than on the more traditional emphasis on structural malformations. Four principal areas of investigation are being integrated into one test system to provide a set of maturation indices, including: 1) postnatal appearance of a standard series of anatomic landmarks, 2) sequential acquisition of metabolic homeostasis for a battery of essential tissue enzymes during the perinatal period, 3) neonatal ontogeny of neuromuscular and neurobehavioral capabilities, and 4) development of the immune system with a full complement of humoral and cellular elements. Adverse effects detected in this test system are analogous to those expressed in human neonates as stunted growth, congenital errors of metabolism, neurological or mental retardation, and immunodeficiencies. Current studies are correlating the effects of prenatal exposure to complex chemicals, X-irradiation, and dietary deficiencies on the development of the progeny with respect to the maturation indicators cited.

EFFECTS OF SRC-RELATED MATERIALS ON ONTOGENY OF IMMUNOCOMPETENCE IN RATS EXPOSED IN UTERO

Investigators:

T. M. Graham and J. E. Morris

Technical Assistance: M. J. Hooper

Maturation of the immune system was evaluated at 40 and 60 days of age in rats exposed in utero to a PCB (Aroclor 1254; Ar) or to distillates from the Solvent Refined Coal-II (SRC II) process. While most of the effects of exposure were seen at both ages, the responses were more obvious by the older age. Both Ar and heavy distillate (HD) groups exhibited depressed growth of males and females. Thymus weights of the HD group were also depressed. Ar exposure markedly suppressed response of peripheral-blood lymphocytes (PBL) to both T- and B-cell mitogens and the thymus cell response to T-cell mitogens, while the response of PBL from the middle distillate group was minimally affected by both mitogen groups. These effects are consistent with a depressed immune system and/or an immature system.

Responses of the developing immune system were measured to monitor effects of distillates from the Solvent Refined Coal-II process on rats exposed in utero by gavage of the dam. This study included measures of both the humoral and cellular components of the immune mechanism, including serum immunoglobulin levels (humoral) and mitogen-

induced lymphocyte activation (in vitro correlate of cell-mediated immunity). Three SRC II distillates diluted in corn oil were used: light distillate (LD, 0.6 g/kg/day), middle distillate (MD, 0.7 g/kg/day) and heavy distillate (HD, 0.7 g/kg/day). Control groups included: untreated rats (non-exposed controls), corn-oil-dosed rats

(vehicle controls), and Aroclor 1254-treated rats (Ar, 0.1 g/kg/day, positive controls). Rats were exposed daily on days 12-16 of pregnancy; immunocompetence in their offspring was evaluated at 40 or 60 days postpartum. The SRC materials used in this study are of pilot plant origin and thus may not be representative of commercial operation or all conditions of the pilot plant facility.

Peripheral-blood lymphocytes (PBL) and cells isolated from spleen, mesenteric lymph nodes, and thymus were used in the mitogen-induced lymphocyte activation assays. Mitogens can generally be characterized by their degree of lymphocyte-population specificity, i.e., whether they stimulate T and/or B lymphocytes. The mitogens tested in our assays included concanavalin-A (Con-A) and phytohemagglutinin (PHA), which stimulate T-cell populations, and pokeweed mitogen (PWM), which is primarily specific for B-cells, although it also stimulates a small fraction of the T-cell population. Bacterial lipopolysaccharide (LPS), a B-cell activating agent, was used in the tissue cell culture work; however, it does not induce PBL to undergo DNA synthesis.

To prepare lymphocytes for the mitogen-activation assays, up to 10 ml of blood was collected by cardiac puncture of rats anesthetized with ether. Total leukocyte and differential counts were determined. RPMI 1640 culture medium was used to dilute the heparinized blood to a 1:10 suspension of approximately $4-6 \times 10^5$ lymphocytes per ml. Spleens, lymph nodes and thymuses were removed aseptically, trimmed, weighed, and placed in culture medium. Total and differential cell counts, as well as viability with trypan blue, were determined on these cell suspensions. The cells were diluted with supplemented RPMI 1640 to give a final density of 2.2×10^6 viable cells per ml. Cell activation was measured by assessing the incorporation of ^{125}I -iododeoxyuridine (^{125}I -IUDR) into newly synthesized DNA during the final 20 hr of the culture period.

The observations discussed here deal primarily with data collected from the 60-day-old offspring; reference to results from 40-day-old rats is made if they varied substantially from that observed in 60-day-old rats. Between-litter variability (litter effect), and the small number of litters examined per variable, limit the conclusions that can be drawn from this range-finding study.

Ar- and HD-exposed male and female rats at 60 days of age had consistently reduced body weights when compared to the corn-oil-exposed group (Figure 35). A decrease in body weight was also observed in male LD-exposed rats but not in females (Figure 35).

FIGURE 35. Body Weights and Relative Spleen and Thymus Weights (percent of Body Weight) of 60-Day-Old Rats Exposed in Utero to SRC II Distillates (N in Parentheses)

Weights of exposed and control rat spleens and thymuses showed some differences when expressed in either total weights (absolute) or percent of body weight (relative). Spleens from Ar- and HD-exposed rats were increased relative to body weight (Figure 35), while thymus weights were depressed in HD-exposed rats. (Ar-dosed rats at 40 days of age also had reduced relative thymus weights.) Relative weight values may only be a reflection of reduced or increased body weights, thus making an evaluation of absolute tissue weights necessary (Table 30).

In conjunction with the whole body and tissue weight study, mitogen-induced activation of PBL and cells isolated from the spleen, lymph node, and thymus was measured and expressed as a stimulation index (SI):

(vehicle controls), and Aroclor 1254-treated rats (Ar, 0.1 g/kg/day, positive controls). Rats were exposed daily on days 12-16 of pregnancy; immunocompetence in their offspring was evaluated at 40 or 60 days postpartum. The SRC materials used in this study are of pilot plant origin and thus may not be representative of commercial operation or all conditions of the pilot plant facility.

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In conjunction with the whole body and tissue weight study, mitogen-induced activation of PBL and cells isolated from the spleen, lymph node, and thymus was measured and expressed as a stimulation index (SI):

TABLE 30. Tissue and Body Weights of 60-Day-Old Rats Exposed in Utero

Group	Sex	N	Weight, g ^(a)		
			Body	Spleen	Thymus
Light Distillate	M	8	212 ± 25	0.58 ± 0.04	0.54 ± 0.03
	F	8	194 ± 9	0.66 ± 0.05	0.50 ± 0.03
Middle Distillate	M	6	322 ± 8	0.86 ± 0.07	0.87 ± 0.05
	F	10	211 ± 5	0.58 ± 0.02	0.59 ± 0.02
Heavy Distillate	M	10	253 ± 5	0.88 ± 0.11	0.49 ± 0.05
	F	2	159 ± 19	0.60 ± 0.06	0.31 ± 0.01
Aroclor 1254	M	2	167 ± 17	0.55 ± 0.07	0.37 ± 0.01
	F	2	119 ± 9	0.50 ± 0.04	0.11 ± 0.04
Corn Oil Vehicle	M	8	296 ± 14	0.84 ± 0.02	0.71 ± 0.04
	F	8	213 ± 7	0.62 ± 0.02	0.55 ± 0.03

(a) Mean ± Standard Error

$\frac{\text{Total cpm incorporated by stimulated cells}}{\text{Total cpm incorporated by unstimulated cells}}$

Con-A-induced stimulation of PBL from Ar-dosed rats was approximately 5% (40 days) and 39% (60 days), respectively, of control values; PHA and PWM responses were depressed also (Figure 36). There was also a possible decrease in the PBL response to PWM of MD-exposed rats. Enhanced PBL activation was observed in 40-day-old animals exposed to LD.

The mitogen response of spleen cells showed no differences between exposed and control rats at 60 days of age for any of the mitogens tested (Figure 37). However, spleen cells of 40-day-old Ar-exposed rats had Con-A and PHA activation values approximately 50% greater than those of controls; PWM induced an enhanced response also.

The lymph node cell SI value for HD-exposed rats was higher than that of the controls (Figure 37) for all mitogens tested.

The response of thymus cells to Con-A and PHA from Ar-dosed rats appears to be depressed (Figure 37). An enhanced Con-A response by thymocytes from LD-dosed rats was seen at 40 but not at 60 days of age.

These preliminary data will require additional studies in order to confirm and extend the observations noted. Nevertheless, there is an indication that exposure to complex chemicals (such as Aroclor) in utero may block and/or delay immune system maturation in rats. It is very possible that other complex mixtures such as petroleum-derived materials might also cause effects on immune competence. Research is not yet in place, however, to address this possibility.

FIGURE 36. Stimulation Indices (SI) of Mitogen-Activated Peripheral Blood Lymphocytes from 60-Day-Old Male and Female Rats Exposed in Utero to SRC II Distillates (SI = Total cpm Incorporated by Stimulated Cells/Total cpm Incorporated by Unstimulated Cells)(N in Parentheses)

FIGURE 37. Stimulation Indices (SI) of Mitogen-Activated Cells from Spleens, Mesenteric Lymph Nodes and Thymuses of 60-Day-Old Male and Female Rats Exposed In Utero to SRC II Distillates (SI = Total cpm Incorporated by Stimulated Cells/Total cpm Incorporated by Unstimulated Cells)

EFFECTS OF SRC-RELATED MATERIALS ON NEUROMUSCULAR REFLEX ONTOGENY IN RATS EXPOSED IN UTERO

Investigators:

F. D. Andrew and K. A. Poston

Technical Assistance:

R. Music, B. Egbert, L. F. Montgomery and L. D. Montgomery

Postnatal maturation and growth was monitored in offspring of rats exposed to SRC-related materials during midgestation. Growth was depressed in some groups from birth through the end of the observation period. Effects on postnatal functional development were observed in four groups for three neurobehavioral tests.

Solvent Refined Coal Processes I and II (SRC I and II) are coal liquefaction processes currently being evaluated as possible sources of alternative fuels. Careful evaluation of the process streams and end-products are needed to determine any potential hazards associated with their production or use. Behavioral teratology studies are one of many assessments currently being conducted by this laboratory.

It should be noted that materials used in this study were derived from pilot-plant operation and may not be fully representative of demonstrational/commercial plant operation.

Pregnant Wistar rats were exposed by gavage to one of several materials once daily on days 12-16 of gestation. Materials tested included light oil (LO), wash solvent (WS), or process solvent (PS) from SRC I and light, middle or heavy distillate (LD, MD, or HD, respectively) from SRC II. Doses ranged from the maximum tolerated by the offspring to those causing no apparent effects on the offspring. Corn oil was used as a negative control and a polychlorinated biphenyl (PCB; Aroclor 1254) was used as a positive control. The offspring were evaluated using a wide battery of tests at 1, 7, 14, 21, and 35 days of age to detect behavioral and/or maturational abnormalities (Table 31).

The positive controls and rats from four of the six SRC groups [including PCB, 3.33 ml/kg/day (solution concentration, 2.5 or 3.3%); PS, 0.33 and 0.67 ml/kg/day; HD, 0.33 ml/kg/day; LO, 2.3 and 2.7 ml/kg/day;

TABLE 31. Postnatal Maturation Evaluation Battery

Physical Landmarks	Scoring Criteria	Observation Days				
		1	7	14	21	35
Body Weight	grams		x	x	x	x
Incisors Erupted	± (a)		x			
Eyes Opened	0-1-2			x		
Organ Weights	grams					x
Test Measures	Scoring Criteria	Observation Days				
		1	7	14	21	35
Movement	±	x	x	x	x	
Upright	±	x	x	x	x	
Headlift	±	x	x	x	x	
Gait	±			x	x	
Surface Righting	60 sec	x	x			
Grasp Reflex	±	x	x			
Retraction	±	x	x			
Vibrissal Placing	±		x	x		
Cliff Avoidance	±		x	x	x	
Negative Geotaxis	60 sec		x	x	x	
Air Righting	±; 3 trials		x	x	x	
Swimming	0-4 (b)		x	x	x	
Visual Placing	±				x	
Forelimb Strength	60 sec				x	
Audiogenic Seizure	60 sec					x

(a) Rating of - = no response; + = response

(b) Rating per Shapiro et al., Science 168: 147 (1970)

and WS, 1.2 and 1.3 g/kg/day) exhibited evidence of intrauterine growth retardation, as seen by their low weight when compared to the corn oil controls (Table 32) at day 1. Subsequent postnatal growth of the PCB, PS, and HD groups was retarded up through 35 days of age (end of observation period).

TABLE 32. Postnatal Growth

Groups	Dose, ml/kg/day ^(a)	No. of Litters	Body Weights, g (Mean of Litter Means $\pm$ SD)				
			1 Day	7 Days	14 Days	21 Days	35 Days
Corn Oil	3.33	11	7.5 $\pm$ 0.6	19 $\pm$ 2	37 $\pm$ 3	59 $\pm$ 4	151 $\pm$ 9
Cage Controls	--	6	7.9 $\pm$ 0.4	19 $\pm$ 1	36 $\pm$ 2	58 $\pm$ 3	148 $\pm$ 3
Aroclor 1254 ^(b,c,d)	3.33	10	5.7 $\pm$ 0.4 ^(e)	11 $\pm$ 2 ^(e)	24 $\pm$ 3 ^(e)	37 $\pm$ 5 ^(e)	86 $\pm$ 20 ^(e)
Process Solvent ^(d)	0.33-0.67	8	6.7 $\pm$ 0.8 ^(e)	16 $\pm$ 2 ^(e)	34 $\pm$ 4 ^(e)	54 $\pm$ 6 ^(e)	136 $\pm$ 11 ^(e)
Heavy Distillate	0.33	4	6.4 $\pm$ 0.9 ^(e)	15 $\pm$ 2 ^(e)	32 $\pm$ 3 ^(e)	51 $\pm$ 7 ^(e)	140 $\pm$ 2 ^(e)
Light Oil ^(d)	2.33-2.66	4	6.5 $\pm$ 0.3 ^(e)	18 $\pm$ 2	37 $\pm$ 4	61 $\pm$ 7	143 $\pm$ 11
Wash Solvent ^(c,d)	3.33	7	6.7 $\pm$ 0.5 ^(e)	17 $\pm$ 2 ^(e)	35 $\pm$ 3	58 $\pm$ 5	142 $\pm$ 5
Light Distillate ^(f)	0.67	4	6.7 $\pm$ 0.98	15 $\pm$ 4 ^(e)	33 $\pm$ 10	48 $\pm$ 15	133 $\pm$ 23 ^(e)
Middle Distillate	0.67	4	7.4 $\pm$ 0.1	18 $\pm$ 1	33 $\pm$ 4	58 $\pm$ 4	150 $\pm$ 11

(a) Administered by gavage for 5 consecutive days; 12-16 days of gestation

(b) Number of litters decreased from 10 to 8 with time

(c) Material given diluted in corn oil; Aroclor, 2.5 or 3.3%; Wash Solvent, 35 or 40%

(d) Data from two doses combined

(e) $p < 0.05$ by Dunnett's test

(f) Includes data from litter 636; natural mother replaced with foster mother at 6 days

The LO and WS groups, although smaller at 1 and 7 days, subsequently weighed approximately the same as the controls throughout most of this study. The LD-exposed group displayed an unusual and variable growth pattern with significantly low values at two ages. However, most of the variability in group weight was attributed to the data from one of the four litters. The natural mother of litter 636 had to be replaced with a foster mother at 6 days postnatally due to ill health (necropsy revealed a traumatic injury of the esophagus, presumably acquired during gavage).

Rats from the HD group (0.33 ml/kg/day) demonstrated deficits in air righting and

swimming at day 7 but only in air righting at day 14 (Table 33). The LD and MD groups (0.69 ml/kg/day, each) also performed more poorly than CO in the swimming test at 7 days. The PCB group (3.33 ml/kg/day) showed a significantly greater incidence of audiogenic seizures at day 35. No other consistent effects on postnatal functional development were observed in these preliminary studies.

Automated data acquisition and analysis systems are still being developed to handle the voluminous behavioral and neuromuscular effects data. Additional studies, using more animals and a modified test battery, are in progress to confirm and extend these results.

TABLE 33. Postnatal Maturation

Groups	Dose, ml/kg/day ^(a)	No. of Litters	Test Battery ^(e) , Mean of Litter Means											
			Placing				Air Righting			Swimming			Forelimb Strength	Audiogenic Seizure ^(f)
			Vibrissal		Visual		7	14	21	7	14	21	21	35
Days tested			7	14	14	21	7	14	21	7	14	21	21	35
Corn Oil	3.33	11	0.85	1.00	0.90	1.0	0.50	1.8	3.0	1.4	3.0	3.3	25	11
Cage Controls	--	6	0.80	0.94	0.79	1.0	0.42	1.5	3.0	1.6	3.0	3.5	32	0
Aroclor 1254 ^(b,c,d)	3.33	10	0.76	0.63	0.60	1.0	0.64	1.5	2.9	1.1	2.8	3.2	26	63
Process Solvent ^(d)	0.33-0.67	8	0.95	0.91	0.95	1.0	0.68	1.9	3.0	2.0	3.0	3.3	28	0
Heavy Distillate	0.33	4	0.75	0.83	0.81	1.0	0.08 ^(g)	0.9 ^(g)	2.9	0.3 ^(g)	3.0	3.3	32	--
Light Oil ^(d)	2.33-2.66	4	0.72	0.96	0.85	1.0	0.47	1.1	3.0	1.5	3.0	3.3	23	0
Wash Solvent ^(c,d)	3.33	7	0.78	1.00	0.93	1.0	0.54	1.3	3.0	1.5	3.0	3.2	22	0
Light Distillate	0.67	4	0.62	0.92	0.62	1.0	0.50	1.4	2.9	0.3 ^(g)	3.0	3.1	35	--
Middle Distillate	0.67	4	0.79	1.00	1.00	1.0	0.16	1.2	3.0	0.3 ^(g)	3.0	3.1	26	--

(a) Administered by gavage for 5 consecutive days; 12-16 days of gestation

(b) Number of litters decreased from 10 to 8 with time

(c) Material given diluted in corn oil; Aroclor, 2.5 or 3.3%; Wash Solvent, 35 or 40%.

(d) Data from two doses combined

(e) Selected tests from battery tested on days indicated. See Table 1 for scoring

(f) Percent pups responding

(g) $p < 0.05$ by Dunnett's test

APPLICATION OF PERINATAL ENZYME PATTERNS AS BIOCHEMICAL INDICATORS OF DEVELOPMENTAL TOXICITY

Investigator: F. D. Andrew

Technical Assistance:

P. Lytz, R. Music and M. Harty

Young rats are being evaluated for signs of biochemical immaturity by assaying enzyme levels in various tissues, utilizing a small number of selected enzymes representing several metabolic pathways. Quantitative analysis of enzyme patterns in developing tissues can provide sensitive and objective criteria for tissue maturation and function comparisons. This study is attempting to demonstrate that quantification of even a small battery of enzymes in a limited group of tissues can provide a baseline for distinguishing differences in degree of developmental toxicity.

One aspect of developmental toxicity testing that has heretofore received much less attention than anatomic impairment (structural malformations) is that of physiological impairment (functional deficiencies). Criteria such as impaired viability in the postnatal period, retarded weight gain of progeny, and deviations in the function of certain organs and/or systems are of primary concern for the establishment of threshold levels for embryotoxicity and neonatal survival. Moreover, tests are needed that will reveal latent defects in the ability of progeny to adapt to changes in their environment that accompany extrauterine development. There is a need to stress the fact that the effects of some toxic agents are likely to be expressed as subtle functional impairments only over a long period of time.

As it is not possible to predict the type and severity of functional or structural changes, a variety of tests may need to be performed during the entire life span, although particular emphasis should be placed on the immediate postnatal period and the time of weaning. Theoretically, functional tests in animal subjects with a short life span (e.g., rodents) should be conducted for an extended period, from conception through gestation and postnatal maturation until maturity, or even old age. For the present, testing in this laboratory has been restricted to the late fetal, early neonatal, and late suckling periods.

Evaluation of perinatal enzymology represents one approach for improved testing of postnatal functional development. Considerable attention has been given recently to basic studies of the developmental patterns of enzymes which catalyze essential steps in carbohydrate metabolism, phospholipid metabolism, and processes in nitrogen excretion (Figure 38). The general assumption, however, that detectable changes in the developmental patterns of the key enzymes in the main metabolic pathways in newborns may account for postnatal growth inhibition, mental retardation, or decreased viability has received little consideration in experimental teratology. Deviations in the activity of organ-characteristic enzyme systems may be sensitive and reliable criteria for estimating threshold levels for deleterious effects. In this respect, temporal or quantitative deviations of individual enzyme patterns, as well as the study of specific target tissues, may be of practical predictive value. Because enzymes represent one of the largest and most varied classes of specific chemical constituents of cells, detection of metabolic lesions, particularly in the absence of morphological abnormalities, would greatly extend the sensitivity of standard teratological procedures. In addition, organ-specific enzyme profiles continue to evolve and undergo drastic changes long after these organs have become morphologically distinct, so that they can distinguish mature tissues from one another and from immature tissues.

FIGURE 38. Typical Rat Liver Enzyme Profiles During Perinatal Development

Studies have been initiated to evaluate the effects of nutritional deficiencies (low protein), physical insult (X-irradiation), and complex chemicals (SRC-related materials and polychlorinated biphenols). We are characterizing control and agent-exposed progeny at the various developmental periods indicated previously. A limited battery of four enzymes has been selected (Table 34) for initial studies in rats, including: aspartate aminotransferase (AST-2.6.1.1), alanine aminotransferase (ALT-2.6.1.2), alkaline phosphatase (AKP-3.1.3.1), and isocitrate dehydrogenase (ICDH-1.1.1.42). Baseline values for these enzymes are being established for brain, liver, kidney, small intestine, placenta and blood. Enzymes are assayed in vitro in supernatant fractions of homogenates centrifuged at $16,000 \times g$ for 20 min. Standard spectrophotometric kinetic analyses are performed on fresh tissue preparations by batch assays, using commercially available reagent kits and a centrifugal fast analyzer (GEMSAEC) for rapid, accurate, and precise results. Activities, which are proportional to the amount of tissue added, are held constant during the incubation times selected. The results are expressed as units per gram of wet weight of tissue and as units per gram of soluble protein. Enzymes are quantified in assay systems supplemented with saturating amounts of the appropriate substrates and cofactors,

TABLE 34. Perinatal Enzyme Data Matrix - Control^(a)

Enzymes	DAM'S TISSUES					
	Liver		Kidney		Brain	
	Units/g of tissue	Units/g of protein	Units/g of tissue	Units/g of protein	Units/g of tissue	Units/g of protein
ALT	53 ± 4	345 ± 10	3 ± 1	39 ± 4	2 ± 1	17 ± 6
AST	77 ± 17	496 ± 80	44 ± 6	521 ± 61	40 ± 2	898 ± 29
ICD	23 ± 4	154 ± 38	26 ± 4	302 ± 60	1 ± 0	33 ± 6
AKP	1 ± 1	5 ± 2	134 ± 21	1573 ± 205	3 ± 1	70 ± 1
	OFFSPRING'S TISSUES					
ALT	14 ± 2	88 ± 13	1 ± 0	16 ± 2	2 ± 1	41 ± 7
AST	113 ± 14	692 ± 85	23 ± 3	288 ± 32	24 ± 9	660 ± 57
ICD	28 ± 9	185 ± 21	13 ± 2	141 ± 44	2 ± 1	43 ± 7
AKP	2 ± 0	11 ± 5	64 ± 18	785 ± 187	4 ± 1	57 ± 19

^(a) Typical data for adults and progeny sacrificed and analyzed at 23 days post partum.

^(b) Units per liter for blood (serum) samples.

^(c) $n = 2$, otherwise $n = 4$ for adults and $n = 8$ for progeny.

where reaction rate is limited only by the amount of enzyme in the tissue extract added. The results, although expressed in units of "activity," provide a measure of the relative "concentration" of the enzyme, rather than of its catalytic effectiveness in vivo. These units are used for comparison of enzyme concentrations in different

tissues or for the same tissue at various stages of development.

In conjunction with the validation of these biochemical procedures, both anatomical and physiological indicators of maturation are being measured on the same animals. The reports by Morris and Graham (immunocompetence) and by Andrew and Poston (neuro-behavioral), elsewhere in this Annual Report, provide outlines of the specific experimental designs and some of the data. Functional

evaluations currently in use include the following aspects: morphological and physical characteristics (e.g., general appearance, posture, and malformations), maturational landmarks (e.g., eyes opening, incisors erupting), growth (e.g., weight gain), specific reflexes (e.g., hind limb retraction), locomotor activity (e.g., swimming), neuromuscular ability (e.g., coordination, endurance), sensory perception (e.g., vision, audition), and ontogeny of immunity (e.g., cellular and humoral).

• Mutagenicity of Synfuels

The high-molecular-weight distillates obtained by solvent refined liquefaction of coal were analyzed by enzymatic, chemical and physical means to determine the nature of the mutagens contained in these complex mixtures. The Ames/*Salmonella* assay was used as the means for detecting the mutagens formed from the coal liquids. In all cases, fractions active in the Ames assay were found to contain primary aromatic amines, suggesting that compounds of this type are the major contributors to the mutagenicity of the coal liquids.

MUTAGENICITY OF COAL LIQUEFACTION MATERIALS

Investigators:

R. A. Pelroy, J. T. Cresto and B. W. Wilson

The Ames/*Salmonella* mutagenicity assay system was used in combination with thin layer chromatography (TLC), enzyme analysis and chemical methods to determine the most probable source of mutagenicity in the higher-molecular-weight solvent refined coal (SRC) liquefaction materials. The data obtained support the hypothesis that a single chemical class, comprised of primary aromatic amine compounds, contains the major share of the mutagenicity observed in these chemically complex mixtures.

Solvent refined coal (SRC) materials were analyzed by microbial, biochemical and chemical methods to determine the types of compounds that account for the mutagenicity of these materials. The microbial analysis was carried out using the Ames/*Salmonella* mutagenicity assay as described in previous studies (Annual Report, 1978).

Aromatic polycyclic amines were implicated as one of the determinants of mutagenicity of these SRC materials by several lines of evidence. First, specific metabolic activation of aromatic amines to proximal mutagens was achieved with an enzyme, the mixed-function amine oxidase (MFAO) isolated from pig liver (Figure 39). The enzyme appears to be specific for bioactivation of aromatic amines (in this case, polycyclic amines) showing no affinity for the other compounds tested; e.g., benzo(a)-pyrene; isomers of dimethylbenzacridines; and the secondary amine, 2-acetylaminofluorene. Conversely, the 3- and 4-ring primary aromatic polycyclic amines were activated to mutagens by MFAO. Thus, the biological

specificity of MFAO provided a possible means of showing that primary aromatic amines were present and potentially mutagenic as constituents in the complex chemical mixtures represented by the coal distillates and their chemical fractions. Use of MFAO to bioactivate mutagens in the crude coal distillates or their complex chemical fractions proved impractical, probably due to inactivation of the enzyme. However, separation of the chemical constituents of these chemical mixtures by thin layer chromatography (TLC; Table 35), yielded subfractions enriched in mutagenic components.

Mutagenically active SRC materials were chromatographed, and the resultant subfractions were analyzed in the Ames/*Salmonella* assay (Figure 40). From the basic fraction of the SRC II heavy distillate, TLC yielded mutagens in a defined region of the TLC plates, localized near the origin and corresponding to a single peak of mutagenic activity. Metabolic activation was provided either by crude microsomal enzyme (S9) preparations that activate essentially all

classes of mutagens (including the primary amines); or by MFAO. By comparing the data obtained by the two methods of bio-activation, we concluded that: a) aromatic amines of some type (e.g., polycyclic or heterocyclic) were present in the basic fraction and b) that no mutagenic activity (as detected with S9) was found in any region except those TLC regions activated by MFAO.

Similar results via MFAO and S9 activation of TLC subfractions were obtained for the crude higher-molecular-weight distillates and their mutagen containing chemical fractions. These observations are consistent with the fact that primary aromatic amines are major contributors to the mutagenicity of the high-molecular-weight SRC materials. However, they lack a quantitative component, since we do not know how much of the total mutagenic activity (S9-activated) was accounted for by the MFAO specific mutagenic activity. To deal with this problem, nitrous acid treatment (nitrosation) of the mutagenically active SRC fractions was used in an attempt to provide a quantitative estimate of the contribution of aromatic amines to the mutagenicity of the coal distillates (Figure 41, Table 36). As shown by others (Yoshida, et al., 1978),

FIGURE 39. Dose Response of *S. typhimurium* TA98 to Several Aromatic Amines and Benzo (a) pyrene Using the Mixed-Function Amine Oxidase for Metabolic Activation. Each assay plate contained 2.5×10^8 cells and 10.65 units of the amine oxidase.

TABLE 35. Mobility of Polycyclic Compounds in TLC System Used to Separated SRC Compounds

Compound	Amount (µg)	Fluorescence in Near UV	R _F (a)
2-Aminoanthracene (1)	520	yellow-green	0.10
(2)	520	yellow-green	0.16
9-Aminophenanthrene (1)	514	blue-violet	0.10
(2)	514	blue-violet	0.18
1-Aminopyrene (1)	520	blue-violet	0.12
(2)	520	blue-violet	0.18
Benzo(a)pyrene	96	yellow-green	0.76
7,9 Dimethylbenz(c)-acridine	260	orange-brown	0.76
7,10 Dimethylbenz(c)-acridine	260	orange-brown	0.70
Dibenz(a,c)acridine	260	green	0.26
Quinoline	480	--	0.36
Region 2(b)	--	blue-violet	$0.123 \pm 0.01^{(b)}$
Region 3(c)	--	blue-violet	$0.23 \pm 0.07^{(b)}$

(a) Relative mobility

(b) Average $R_f \pm SE$ for regions 2 and 3 from several experiments similar to the one in Figure 2

(c) Average $R_f \pm SE$ for region 3 as shown in Figure 2

BASIC II

FIGURE 40. Ames Mutagenicity Analysis of Materials Eluted from TLC Regions (1-9) After Chromatography of 4000 µg of Basic Tar II

FIGURE 41. Ames Analysis of HD and the Mutagenically Active Chemical Fractions of HD. Medium used for pre-incubation of SRC materials assayed with metabolic activation. DMSO (●); hydrochloric acid (○); nitrous acid (▲); nitrous acid samples assayed without metabolic activation (△). Metabolic activation was provided by 810 µg Aroclor-induced S9 protein from rat liver. Each assay plate contained 1.2×10^6 cells *S. typhimurium* TA98.

TABLE 36. Revertant *S. typhimurium* TA98 Per Assay Plate per μg After Incubation of SRC Material in Medium

Medium Sample	Incubation Medium (a)				Percent Activity Remaining After Nitrous Acid Treatment	
	DMSO	Hydrochloric Acid	Nitrous Acid w/o S9	Nitrous Acid w/S9(d)	(w/o S9)	(w/S9)
Process Solvent (PS)	4.56 ^(a) (3-33) ^(b) (.93) ^(c)	4.00 (1-33)(.51)	1.24 (5-100)(.97)	0.42 (5-100)(.88)	27	9
PS Basic Fraction	70.9 (1-10)(.98)	71.1 (0.5)-10)(.99)	0.7 (5-200)(.96)	0.6 (5-200)(.98)	1	1
PS Basic Tar Fraction	6.7 (3-27)(.94)	10.19 (1-13)(.85)	1.07 (5-110)(.91)	0.45 (5-110)(.54)	16	7
PS Neutral Tar Fraction	13.84 (3-27)(.94)	18.2 (1-27)(1.0)	1.3 (5-116)(.99)	0.4 (5-116)(.91)	9	3
Heavy Distillate (HD)	14.8 (16-16)(.99)	10.8 (2-70)(.95)	1.5 (17-332)(.99)	0.4 (17-332)(.96)	10	3
HD Basic Fraction	193.73 (08-33)(.98) (08-33)(.98)	116.88 (1-10)(1.0) (1-10)(1.0)	7.8 (1-40)(1.0) (1-40)(1.0)	5.9 (1-40)(1.0) (1-40)(.99)	4	2
HD Basic Tar Fraction	23.43 (1.1-9.3)(.88)	34.69 (1.4-14)(.97)	0.9 (10.8-216)(.96)	0.5 (10.8-216)(.86)	4	2
HD Neutral Tar Fraction	34.2 (0.8-6.7)(1.0)	34.4 (1-20)(.96)	5.0 (5.8-116)(.97)	1.9 (5.8-116)(1.0)	15	6

(a) Revertants per μg plate estimated by linear regression.

(b) Concentration range used to estimate regression lines.

(c) Correlation coefficient.

(d) Metabolic activation provided by 1215 μg Aroclor included S9 homogenate protein per assay.

and by previous work in our laboratory, nitrous acid drastically reduces the mutagenicity of primary aromatic amino compounds. Conversely, it does not appear to reduce (or even increases) the mutagenicity of other types of compounds. The data in Figure 41 demonstrate that the highly mutagenic basic fraction derived from SRC II heavy distillate lost most of its mutagenic potency after nitrosation. This was also observed for each of the mutagenic materials derived from the high-molecular-weight SRC distillates, and for the unfractionated distillates themselves. These data are strong

evidence that the only class of mutagen of quantitative importance in the Ames/Salmonella assay is that of the polycyclic or heterocyclic primary amines.

Data presented (see Physical Sciences Annual Report, 1979) will describe the quantitative contribution of the various primary aromatic amines to the SRC molecular weight distillates. Likewise, the effect of hydrotreating SRC materials (i.e., chemical reduction of oxygen, sulfur and nitrogen heteroatoms and/or side chain constituents of polycyclic compounds) will also be described.

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Conservation

CONSERVATION

This section reports on a project which is part of the Pacific Northwest Laboratory's bioelectromagnetic program. It seeks to determine whether electric fields of large magnitude, either static (dc) or time-varying (ac, 60-Hz), can produce genetic effects in simple and familiar biological systems. This project is complemented by ongoing projects on 60-Hz electric field effects in laboratory rodents, sponsored by the DOE Division of Electrical Energy Systems, and in Hanford Miniature Swine, sponsored by the Electric Power Research Institute.

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• Mutagenic Effects of Electric Fields

Microbial and *Drosophila* test systems are being used to determine if large ac or dc electric fields may induce mutations. These organisms were chosen to enable exposure of large numbers with relatively simple apparatus, to minimize the time required for replications and dose-effect studies, and to offer the opportunity for investigating mechanisms, should effects be observed. This project complements ongoing mammalian studies with ac electric fields at PNL, sponsored by the Division of Electrical Energy Systems and the Electric Power Research Institute.

GENETIC EFFECTS OF ELECTRIC FIELDS: EFFECTS OF LARGE DC ELECTRIC FIELDS ON MUTATION FREQUENCIES

Investigators:

F. P. Hungate, S. C. Causey and D. R. Kalkwarf

Using a parallel-plate exposure system, a substantial number of microbial and bacteriophage test systems were evaluated for their response to 500- or 750-kV/m dc fields. Several strains of *E. coli* were tested for forward or reverse mutation; plasmids were tested for both mutation and curing; bacteriophage was tested for reverse mutation and lysogen induction; and *B. subtilis* was examined for evidence of forward mutation. In none of these tests was there a significant increase in mutation frequency of exposed cells as compared to simultaneously tested controls. A consistent change in the plating efficiency of *E. coli*-containing plasmids conferring drug resistance could not be ascribed to mutation or to loss of the plasmids (curing), and the lowered plating efficiency is presumed to be due to a transient effect on intracellular information transfer. The repair-deficient strain of *Drosophila*, mei-41, showed a doubling of the frequency of sex-linked recessive mutations following exposure of pupae to a 360-kV/m dc field, as compared to controls. These data, however, are not to be considered statistically significant. Attempts to determine if secondary oxidants produced by the field might be involved were not successful.

Earlier studies using the Ames *Salmonella* strains, TA100 and TA98, indicated that large dc electric fields (up to 800 kV/m) caused increased frequencies of reversions in the TA100, but not in the TA98 strain. This year, we have investigated a number of alternative microbial systems for their response to dc fields in order to obtain information about the mechanisms that may be involved, and to seek a system more responsive to the electric fields. Strains of *E. coli* and *B. subtilis*, and bacteriophages

have been examined for evidence of forward and reverse mutation responses to dc fields.

In addition to tests with prokaryotic systems, the Mei-41 repair-deficient strain of *Drosophila* was also tested for increased frequencies of sex-linked recessive lethal mutations following exposure to dc fields. These frequencies of sex-linked lethals were evaluated using the standard Muller-5 tester strain.

The exposure system is essentially the same as that described in previous annual reports. The top and bottom copper electrode plates have edges curved apart to prevent corona. They are separated by suspending the top plate from the top of the lucite box which contains each pair of plates. The lucite box is 34.8 x 34.8 x 12.7 cm high (ID), with a hinged lid to allow access, and a side port by which moisture-saturated, sterile air is introduced. When the air within these chambers is monitored for ozone, air from the box is passed into a Bendix Model 8002 ozone analyzer; otherwise, the air escapes from the box through the crack at the joint between top and bottom. Ozone is not usually monitored, since levels are consistently at the detection limit of the instrument, i.e., 0-2 ppb. This level is well below that of room air (15-30 ppb) because the incoming room air is scrubbed with water.

Table 1 shows the biological test systems used, the end point or criterion of effect for each, and the results obtained. A strain of *E. coli* that contains a plasmid conferring resistance to antibiotics was exposed to the electric field and plated on medium containing the specific antibiotics for which it had resistance. Fewer colonies were formed than by nonexposed cultures of the strain. However, when the same cells were plated onto a medium containing no antibiotic and allowed to grow, the drug resistance of exposed and controls were not

different. These results suggest that the changed plating efficiency was due to an effect of dc electric field exposure on transcription, translation, or on membranes controlling drug permeability; however, it was not a permanent effect such as mutation or loss of plasmid.

Tests for lysogen induction with two strains of *E. coli* gave such low frequencies of induction that this test did not appear to offer a suitable system for further study.

Rifampicin is an inhibitor of RNA polymerase, an enzyme required for transcription of genetic information. A single base change in the genes coding for this enzyme is sufficient to alter the properties of the RNA polymerase molecule so that it binds less of the drug, thus permitting cells with such a mutation to become resistant. Experiments using *E. coli* strains CSH 51, 60 and 62 (gram-negative, with a more-complex cell wall), and *B. subtilis* 168 (gram-positive, with a less-complex cell wall) did not suggest any enhancement of forward mutation frequency by exposure to a dc electric field of 500 or 750 kV/m.

Nalidixic acid is an inhibitor of DNA synthesis. Mutation of the *E. coli* Nal-A gene enables organisms to grow on otherwise restrictive concentrations of the drug. When tested, *E. coli* strains CSH 23, 41, 60 and 62 showed no enhancement of forward

TABLE 1. Microbial Systems Tested for DC Field Effects

Organism	Field Strength, kV/m	End Point	Observation
<i>E. coli</i> J53 (RP4)	500	Plating Efficiency	} 15-25% Reduction (No Plasmid Curing)
<i>E. coli</i> C600 (R6K)	500	Plating Efficiency	
<i>E. coli</i> J53 (R388)	500	Plating Efficiency	
<i>E. coli</i> CSH 23 (F'LAC, Pro A,B)	500	Plating Efficiency	No Change
<i>E. coli</i> CSH 45 (λ)	500	Lysogen Induction	} Possible
<i>E. coli</i> CSH 49 (ϕ 80)	500	Lysogen Induction	
<i>E. coli</i> CSH 23	500/750	Drug Resistance	} No Forward Mutation
<i>E. coli</i> CSH 51		Nalidixic Acid	
<i>E. coli</i> CSH 60	500/750	Rifampicin	
<i>E. coli</i> CSH 62	500		
<i>B. subtilis</i> 168	750	Drug Resistance Rifampicin	No Forward Mutation
Phage			
T4D	750 (Nonreplicating)	Plating Efficiency	} No Effect
T4AM N82	750 (Nonreplicating)	Reversion Frequency	
T4AM N122	750 (Nonreplicating)	Reversion Frequency	

mutation frequency following exposure to 500- or 750-kV/m dc fields.

Selected strains of T₄ bacteriophage (virus) were cultured in their host, *E. coli* and, following lysis of the bacteria, the phage was exposed in the field. Thus, the phage were not replicating during the exposure or sham exposure. Both strains of phage mutate in such a way that they are unable to synthesize a required protein. However, the effects of the mutation can be reversed by a suppressor mutation. If the suppressor mutation is carried in the host, *E. coli* (identified as a permissive host), phage is readily replicated. If the phage is caused to infect a host strain not carrying a suppressor (a nonpermissive host), only those phage having their own suppressor mutation can replicate and produce identifiable plaques. Following exposure of the phage to the dc electric field at 750 kV/m, the frequency of mutation to a suppressor was identified by use of a nonpermissive host and the number of phage units exposed by identifying the plating efficiency using a permissive host. In 11 experiments using T4 am N82, and in 11 experiments using T4 am N122, cells exposed to the dc electric field as nonreplicating particles showed no significant difference in reversion frequencies from those of controls.

Tests of mutation to sex-linked recessives in *D. melanogaster* concentrated on a strain having the postreplication repair-deficient mutation, mei-41. This strain was obtained from Dr. Melvin Green, Davis, CA. Pupae (0- to 1-day-old) were exposed for 3 days to a 360-kV/m dc field and, following emergence of the adults, the males were individually mated to virgin Muller-5 females. The F₂ generation was observed for the presence or absence of normal-eyed males. The absence of any normal-eyed male in individual F₂ cultures having more than 15 flies was an indication of a sex-linked recessive lethal. Multiple mutations from an individual exposed male were identified by relating all F₂ cultures with the initial P₁ male. Table 2, which shows the data from five separate tests, indicates an overall mutation frequency of 0.47% in the exposed group compared to 0.21% in the control group, excluding one instance of multiple mutation in the exposed group. These data are consistent with those from last year's tests using a lower field strength. However, neither set of data is sufficient to conclude that the effect is statistically significant.

TABLE 2. Sex-Linked Recessive Mutation Frequencies in *D. melanogaster*, mei-41, Exposed 3 Days as Pupae to a 360-kV/m DC Field

	Exposed, Mutants/total	Control, Mutants/total
	5/600 ^(a) (4/599)	1/609
	2/171	2/420
	1/224	0/406
	0/327	1/280
	1/376	0/182
Total	8/1697 (0.47%)	4/1897 (0.21%)

^(a) 2 mutations from one P₁ male

To test the possibility that effects of dc electric-field exposure may be due to air ions, we used the dye erioglaucine, which loses color (as measured photo-metrically in the 631-nm wavelength) in the presence of oxidizing agents or emitted electrons. Initial tests suggested that some bleaching occurred. However, in more carefully conducted tests using a 750-kV/m field, no evidence of dye change was found. Variations used were: 1) battery-generated field with the top plate grounded; 2) battery-generated field with the bottom plate grounded, and with current and ozone levels monitored; 3) Hypot-generated field with the bottom plate grounded; 4) control, which received the same (parallel) air supply as the exposed group, but was shielded from the dc field by a Faraday cage. The earlier suggestions of dye change appear to have resulted from differences in evaporation of solution from the dishes in the various exposure systems, despite their common supply of prehydrated air. Current flow during the tests was $1-3 \times 10^{-10}$ A; ozone in the air of the exposure chamber was 0.5-1.5 ppb, i.e., at the lower limit of the Bendix ozone sensor--an order of magnitude lower than that measured in room air.

Because of negative data from the extensive series of tests for mutagenic effects in alternative microbial systems, and in the absence of a theoretical basis for observations of biological effects from dc fields, we are currently retesting the *Salmonella* TA100 strain with which we obtained earlier data showing a small but significant effect from exposure in dc fields up to 800 kV/m. These tests are not complete at this time.

● Electric Field Dosimetry

Living organisms may be exposed to extremely low-frequency (ELF, frequency range 0-100 Hz) electric fields produced by electric power systems or specialized communications systems. The purpose of this project is to characterize the electric fields produced at the surface and inside models of humans and in experimental animals exposed to such fields.

DOSIMETRY OF EXTREMELY LOW-FREQUENCY ELECTRIC FIELDS

Investigators:

W. T. Kaune and A. W. Guy^(a)

The electric fields at the surface and those induced inside the bodies of uniformly conducting human and animal models, and in live animals exposed to ELF electric fields will be measured. These measurements are crucial if data obtained with laboratory animals are to be used for the assessment of possible hazards to humans resulting from exposure to ELF electric fields.

Exposure of living organisms to extremely low-frequency (ELF) electric fields is conventionally specified in terms of the field strength present before the organism enters the field. However, the actual electric fields to which an organism is exposed--the fields at the surface and inside the body--are different from the unperturbed field because of perturbations caused by the conducting body of the organism. Theoretical estimates and recent measurements have demonstrated that field perturbations depend strongly on body shape, and that the actual fields experienced by four-legged animals exposed to an (unperturbed) vertical, electric field will be much less than the fields experienced by a human exposed to the same (unperturbed) field. Knowledge of these fields is crucial if data presently being developed in projects investigating the biological effects on animals exposed to ELF electric fields are to be used for the assessment of possible hazards to humans exposed to such fields.

The purpose of this new project is to determine, by measurement, the actual fields experienced by electrically grounded and ungrounded humans and animals exposed to electric and magnetic fields. Two parallel lines of investigation will be followed. At the Pacific Northwest Laboratory (PNL), direct measurements will be made of the fields induced inside models and live animals exposed to 60-Hz electric fields. At the Bioelectromagnetic Research Laboratory, University of Washington, scaled models, whose sizes are small relative to wavelength, will be exposed in resonant cavities to 58-MHz electric or magnetic fields. The induced current and field distribution will be measured thermographically, and extrapolated to 60 Hz. The techniques used in the project will be validated with uniformly conducting hemispheroidal and spheroidal models, where the induced electric fields inside the body can be calculated by theory and compared with measured values.

(a) Bioelectromagnetics Research Laboratory, University of Washington, Seattle, WA.

• In Vivo Mutagenesis—Electric Fields

These studies are concerned with the mutagenic potential of ac electric fields such as those which might be associated with the transmission of electric power. The project will provide indications of mutagenicity or primary DNA damage by electric field exposure in adult male, female and fetal rats. Electric-field-induced sensitization to mutagens will be evaluated by measuring the subsequent susceptibility of selected cell populations from exposed animals to chemical mutagens. This project will supplement the DOE Electrical Energy Systems Division (EES) study of the effects of electric-field exposure on rats and mice.

FREQUENCY OF SISTER CHROMATID EXCHANGES IN BONE MARROW CELLS FROM RATS EXPOSED TO A 60-HZ ELECTRIC FIELD

Investigator: B. J. McClanahan

Technical Assistance:

R. L. Music and L. I. Benton

Exposure of rats to a uniform, 60-Hz electric field at an unperturbed field strength of 100 kV/m has been completed. Cytogenic evaluation of bone marrow cells is in progress.

This new project is concerned with mutagenic effects and damage to DNA that may result from exposure of rats to 60-Hz electric fields. The initial experiments are determining the frequency of sister chromatid exchanges (SCEs) and chromosomal aberrations occurring in rats during and after exposure as a function of duration of exposure.

We have completed an experiment conducted in two replicates, in which female Sprague-Dawley rats were exposed for 5 wk to a vertical, uniform 60-Hz electric field at an unperturbed field strength of 100 kV/m. At 20 to 22 hr before sacrifice they were removed from the field and injected with 5-fluorodeoxyuridine to inhibit the action of thymidylate synthetase. The animals then received a series of six injections, at

hourly intervals, of 5-bromo-2'-deoxyuridine (BUDR) which is incorporated into newly synthesized DNA. Immediately after receiving the last BUDR injection they were returned to the field. The exposure was briefly interrupted at 2 to 2.5 hr before sacrifice to permit administration of colchicine, which arrests dividing cells in metaphase. Bone marrow cells obtained from both femora were processed and stained to determine the incidence of SCEs and chromosomal aberrations. Use of this experimental protocol allows examination of cells whose second cell cycle following BUDR administration occurred while the animals were in the electric field, except for the brief period (~15 min) when the mitotic arrester was injected. Cytogenic evaluation of the marrow cells is in progress.

• In Vitro Effects of Electric Fields

This project seeks to determine and quantify cytotoxicity, DNA damage and/or specific gene mutation resulting from exposure of mammalian cells in suspension to 60-Hz electric fields. In addition, these cells will be exposed to either direct-acting or indirect-acting mutagens in combination with electric fields in order to determine whether exposure to electric fields can alter (increase or decrease) the responses of mammalian cells to known mutagens.

LETHALITY AND MUTAGENICITY STUDIES OF ELECTRIC FIELD EFFECTS ON MAMMALIAN CELLS: EXPERIMENTAL DESIGN

Investigator: M. E. Frazier

A study has been initiated to measure DNA damage and/or specific gene mutation resulting from exposure of mammalian cells (in vitro) to 60-Hz electric fields. This report describes the experimental goals and methods of this project.

Chinese hamster ovary (CHO) cells, and the purine analog 6-thioguanine (TG), are being used to quantify mutation induction at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus. The CHO/HGPRT assay is based on the fact that TG is converted to the nucleotide by the HGPRT activity of wild-type cells. The nucleotide thus produced (or its metabolites) is lethal, and thus kills all the cells retaining the HGPRT enzyme system. The optimal conditions for selection of the mutant phenotype (including phenotypic expression time, concentration of TG, medium composition for the selection step, and optimal cell density to permit maximum recovery) are well defined. The CHO/HGPRT assay has been shown to yield a quantitative dose-response curve with known chemical mutagens. Further, the assay is capable of quantifying the mutagenicity of various physical agents (i.e., UV light, white light, black light, blue light, sunlight, and X-rays). Test results from the assay also appear to correlate with the reported carcinogenicity of chemical and physical agents tested in animals; of 83 agents known to produce carcinogenesis, the assay yielded a positive correlation for 76 of these agents.

To derive further information of potential mutagenic effects of electric field exposure, sister chromatid exchange (SCE) is being used to provide an accurate indicator of primary DNA damage. This technique involves the differential staining of sister chromatids with Hoechst 33258 fluorochrome and Giemsa after electric field exposure. The addition of 5-bromodeoxyuridine identifies SCE present in chromosomes of CHO cells.

Initial experiments involve the exposure of CHO cells at different field strengths for varying periods of time. In these experiments, we are using an exposure system which is capable of providing current densities to the cells of up to 10 mA/cm² and electric fields of 4 V/m. These levels are extremely high, relative to animal exposures to ac electric fields with coupling via air. As a result, if genetic effects occur they should be observed in this system. Once the level of effect (cell death, mutation, and/or DNA damage) is achieved, we will attempt to obtain a dose-effect relationship.

Additional studies will be conducted with cells exposed to electric fields prior to, during, and following exposure to various

doses of the direct-acting mutagen, ethyl methanesulfonate (EMS). Experiments will be conducted to determine whether exposure to electric fields can alter (increase or decrease) the mutagenic response to EMS of CHO cells.

The present exposure system consists of a polystyrene culture vessel with platinum electrodes covering the inside surfaces of two sides. In this system, some platinum will be released into the medium by electrolysis. We are using several controls in order to detect and evaluate potential toxic effects of platinum, and to differentiate between any toxic effects and any electric

field effects. Three approaches are being used. First, we are determining the availability of platinum in the exposed culture medium (both in the presence and absence of CHO cells), using X-ray fluorescence techniques. We will then compare the effects of unexposed medium, medium containing added platinum ions, and unexposed medium (obtained both with and without cells present during exposure) with the effects of cells exposed directly in electric fields. Finally, we will compare these data with those obtained in an alternative exposure system in which platinum is not present. (The new exposure system is currently being tested.)

Fission

FISSION

• Biomedical studies in the fission energy technology area are mostly concerned with the evaluation of long-term effects from low-level exposure to radioactive pollutants. These include studies in progress for many years in dogs that have inhaled various plutonium compounds. The inhalation toxicity of a wider variety of actinide compounds is being studied in rodents. Both dogs and rodents are employed in continuing studies of the effects of chronic exposure to simulated uranium mine atmospheres.

• More recently instituted studies are concerned with the possible synergistic effects of inhaled plutonium and cigarette smoke, with specific reactor technology problems, such as the toxicity of sodium that might be accidentally released from an LMFB (liquid metal fast breeder reactor), or the toxicity of mixed aerosols of plutonium and sodium. The toxicity of chronically inhaled krypton-85, released in small amounts from nuclear reactors, is also being studied. Because of recent interest in nonproliferative fuel cycles, special hazards that might be associated with reactors employing a thorium fuel cycle are being investigated.

More basic studies are concerned with the effects of age on the toxicity of incorporated radionuclides, and the effect of age, and other factors, on the gastrointestinal absorption of radionuclides. Viral and biochemical factors that may be involved in radiation carcinogenesis are also under study.

In the more strictly medical areas, procedures are being developed that may reduce the probability of radiation effects by hastening removal from the body of internally deposited radionuclides. In other studies, advantage is taken of radionuclides incorporated in a device designed to irradiate the blood, which may be a useful treatment for leukemia or for the prevention of transplant rejection.

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• Aerosol and Analytical Technology

We are attempting to improve aerosol exposure techniques by investigating: generation, characterization and monitoring of inhalation atmospheres, relation of aerosol properties to respiratory deposition and clearance, and control of inhalation exposure dose.

DEVELOPMENT OF A MODIFIED RODENT EXPOSURE CHAMBER

Investigators:

W. C. Cannon and E. F. Blanton

Studies of aerosol chamber flow patterns are suggesting ways to improve aerosol distribution in rodent exposure chambers.

Using an equivalent hydraulic-flow system in a half-scale model, we are studying flow characteristics in small-animal aerosol exposure chambers, both for the original design and with modifications intended to improve aerosol distributions. Water flows in the model are adjusted so that the Reynolds number in the model approximates that of air flows in the actual chamber. We have taken into account the differences in dimensions of the chambers, as well as the viscosity and density of the two media.

Flow patterns in the half-scale model are studied by injection of suitable dyes into the water. For visual and photographic studies, KMnO_4 is used. Fluorescein dye is used for sampling with a continuous-flow colorimeter to obtain buildup and decay curves at various points in the chamber.

Figure 1A is a sketch of the original chamber; Figure 1B shows the chamber modified by a system of mixing baffles. Buildup

curves for the third animal level are compared in Figure 2 for the chamber with and without baffles. The time scale in the model differs from that in the actual chamber by a factor of 4.4; both time scales are shown in Figure 2. The oscillations in curve (a) (Figure 2) are due to swirls of denser dye solution passing the sampling port. The absence of these oscillations in curve (b) indicates that mixing is more uniform with the baffles present. If the oscillations of curve (a) were smoothed out it would resemble curve (b), shifted in time. If mixing were perfect, some dye would reach the sample port immediately; the greater the shift, the poorer the mixing.

Our results predict that improved aerosol distribution and more-uniform animal exposures may be achieved by modification of the exposure chamber. Other model configurations will be studied, and the optimum modification will be made on a full-scale chamber to verify test results.

FIGURE 1A. Original Chamber Design

FIGURE 1B. Chamber with Baffle

FIGURE 2. Concentration Build-Up Curves

ELECTRICAL ATOMIZATION OF AEROSOLS

Investigators:

M. L. Clark and O. R. Moss

A method of generating and characterizing aerosols by electrical atomization in conjunction with a charge spectrometer is described.

The purpose of this research is to investigate an alternative method of producing monodisperse aerosols for use during absolute filter testing. Presently, filter testing is performed by employing the Q127 aerosol generator, which creates monodisperse clouds of dioctylphthalate (DOP), a suspected carcinogen. The approach described may lead to the replacement of DOP as a filter challenge aerosol.

In this experiment, a nonconducting liquid solution is fed into a capillary tube at a rate controlled by pressure head. A positive dc potential is applied to the liquid reservoir and gradually increased to 15 kV, resulting in the production of charge carriers in the solution. The charged liquid is emitted at the tip of the capillary tube as a fine-mist spray of positively charged droplets. These droplets are swept past a negative electrode held at -2600 VDC into an aging/drying chamber. From this chamber the aerosol enters a ^{85}Kr charged-particle neutralizer. An activated-charcoal-packed column inserted behind the charge neutralizer serves to remove ethanol vapors (the solvent currently used), which otherwise might interfere with the classification of submicron particles. Particles pass through the column and into a charge spectrometer (Figure 3).

The charge spectrometer consists of a rectangular box, the largest faces of which are made of brass, and are electrically insulated from one another. The box is

operated with its longest axis vertical. Aerosol is introduced into the spectrometer through a grounded copper nozzle at the upper end, midway between the plates. Filtered air is passed along the surface of the brass plates, thereby confining the aerosol to a thin sheet extending across the width of the spectrometer. A positive potential of 2500 VDC is applied to one plate, and an equal but negative potential is applied to the other.

The spectrometer is used to classify particles by their mobility within an electric field. After charge neutralization within the ^{85}Kr environment, the aerosol charge distribution should approach the Boltzmann distribution centered at zero, but with a proportion of particles possessing single, double, triple, etc. charges. By the proper adjustment of such parameters as flow velocity through the spectrometer and applied potential to the plates, it is possible to collect these particles as bands, each band representing a particular colony of like-charged particles.

Sprays of methanol, methanol and deionized water, and methanol and DOP have been generated using this technique. However, the most promising investigations currently center on aerosols of a saturated solution of stearic acid in ethanol, or a 0.01% solution of sodium chloride in ethanol. Size classification of aerosols using the mobility spectrometer have not been successful to date, but research is continuing.

FIGURE 3. Electro-Aerosol System

A FLEXIBLE-BRUSH DUST-FEED MECHANISM

Investigators:

E. M. Milliman, D. P. Y. Chang and O. R. Moss

No single commercially available device has been found capable of aerosolizing all types of finely divided solid particulate matter. A major problem area is associated with feeding bulk powder, in a uniform continuous fashion, at very low rates, to a de-agglomerating device. This project involves development and testing of a novel, flexible-brush, screw-type dust-feed mechanism to achieve uniform and constant output at very low feed rates (0.5 mg/min) for extended periods (up to 2 days) of continuous or discontinuous operation without adjustment.

A dual flexible-brush dust-feed mechanism has been developed to meter finely divided solids at low mass output rate. The dust-feed mechanism is composed of a cylindrical hopper, a feed tube, and two flexible brushes (Figure 4). A large "hopper brush" delivers material to an opening in a small feed tube. A compressed, spirally wound "feed brush" is designed to act as an auger to convey material to an inlet of a de-agglomerating

device (such as a Trost Jet-Mill). Operating experience with a western fly ash indicates an approximately linear dependence of brush rotational speed and feed rate. The device has been operated continuously for several days at a steady rate (coefficient of variation <7% for 5-min averages; <4% for hourly averages) after a short equilibration period, as indicated in Table 1.

OPENING FOR HOPPER SUPPLY, SEALED BY SYRINGE PLUNGER

FIGURE 4. Dual Flexible-Brush Dust-Feed Mechanism

TABLE 1. Output Rate of Flexible-Brush Dust-Feed Mechanism

Mean Feed Rate	Coefficient of Variation	Averaging Time
0.489	3.9%	60
-5.28	1.0%	30
-6.14	6.8%	5
-10.3	1.5%	30
-21.2	2.0%	5
-91.8	2.4%	10

MODIFICATION OF A COMPRESSED-AIR NEBULIZER

Investigators:

H. DeFord and M. L. Clark

A major problem associated with using compressed-air nebulizers is the increase in concentration of the parent solution with time. This is a significant problem when associated with animal exposure studies, where the goal is to maintain a given particle size and aerosol concentration for the exposure duration.

Compressed-air nebulizers of the DeVilbiss and Retec variety operate by aspirating a solution into the air stream, where shear forces atomize the solution as liquid droplets. Droplet evaporation occurs due to the elevation of vapor pressure from curved surfaces, and due to the reduction in the dew point of the air stream upon expansion to atmospheric pressure from pressures typically used to drive these nebulizers. Evaporative losses result in a slow but continuous increase in solution concentration as partially evaporated droplets impact on the nebulizer surfaces and return to the solution reservoir.

Increasing the concentration of a solution leads to a gradual increase in particle size. Depending on the amount of evaporative loss, it is conceivable that over a 6-hr exposure, particle size may double. However, simple and inexpensive modifications to the reservoirs of these nebulizers can yield a stable aerosol output for a duration limited only by the capacity of the solution reservoir.

In this study, a Retec Model PN 7002 compressed-air nebulizer was modified as described below. The modifications have resulted in a steady output of 1 mg/m^3 of a $0.4\text{-}\mu\text{m}$ -diameter salt aerosol for up to 30 hr.

The modified Retec nebulizer (Figure 5) consists of two reservoirs: a large reservoir containing fresh makeup solution, and a small reservoir serving as the source of the solution to be atomized. The reservoirs are separated from one another by a 19-mm-diameter lucite tube attached at the top to the Retec nebulizer (the standard reservoir has been

removed), and closed at the bottom with a one-hole stopper. The aspirating tube for the Retec is lengthened to within $\sim 1 \text{ cm}$ of the length of the lucite tube in order to reach the atomizing solution. This assembly is inserted through a rubber stopper fitted to a 1-l filtering flask, forming an airtight seal. The flask provides the reservoir for the makeup solution.

A vent tube, which is open to the atmosphere and extends to the desired liquid level within the aspirating solution reservoir, is also inserted through the rubber stopper. A rubber bulb is fitted to the filtering-flask hose nipple, and a gentle vacuum is applied until bubbles appear from the end of the vent tube. During the generation of aerosol from this device, the level of solution within the aspirating reservoir is maintained constant by the vacuum above the makeup solution. As solution is aspirated, it is continually replaced from the makeup supply through the one-hole stopper. Though some concentration of solute in solvent occurs initially within the aspirating reservoir, an equilibrium concentration is approached. In this manner, it is possible to generate a constant aerosol, limited only by the capacity of the makeup solution reservoir.

The performance of a modified Retec nebulizer was compared to a standard Retec using 0.1% NaCl in distilled water as the aerosol source. Conductivity vs time measurements are shown in Figure 6 for the solutions held within the makeup and aspirating reservoirs of the modified Retec, and for the solution in the unmodified Retec. As can be seen from Figure 7, the conductivity of the solution in the unmodified

FIGURE 5. Modified Retec Continuous Aerosol Generator

FIGURE 6. Conductivity of Solutions from Retec Generators

Retec nebulizer continuously increases throughout the duration of the experiment.

Figure 7 shows a plot of generator output vs time from an experiment in which identical solutions of $(\text{NH}_4)_2\text{SO}_4$ were aerosolized by each generator type, and vented to a standard Battelle exposure chamber. Generator output

was monitored as sulfate concentration by a total sulfur analyzer. Again, the results demonstrate the continuous increase in concentration for the unmodified Retec, compared to the modified Retec, which reaches stability. Modified Retec nebulizers are now being used for the exposure of rodents to $(\text{NH}_4)_2\text{SO}_4$ aerosols.

FIGURE 7. Aerosol Output of Retec Generators

● Inhaled Plutonium Oxide in Dogs

This project is concerned with long-term experiments to determine the life-span dose-effect relationships of inhaled $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$ in beagles. The data will be used to estimate the health effects of inhaled transuranics.

DOSE-EFFECT STUDIES WITH INHALED PLUTONIUM OXIDE IN BEAGLES

Investigators:

J. F. Park, A. C. Case, D. L. Catt, S. L. Klopfer,
G. E. Dagle, T. C. Kinnas, R. M. Madison, G. J. Powers,
H. A. Ragan, S. E. Rowe, R. E. Schirmer, D. L. Stevens,
C. R. Watson, and E. L. Wierman

Technical Assistance:

D. H. Akin, C. L. Baldwin, E. F. Edmerson, V. T. Faubert,
R. E. Flores, F. S. Gerber, F. M. Gordon, T. A. Hooker,
S. L. Krum, V. L. Madden, B. G. Moore, M. C. Perkins,
M. J. Pipes, and L. R. Peters

Beagle dogs given a single exposure to $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$ aerosols are being observed for life-span dose-effect relationships. The ^{239}Pu body burden of the nine dogs that died of pulmonary-fibrosis-induced respiratory insufficiency during the first 3 yr after exposure was 1 to 12 μCi ; one of these dogs had a pulmonary tumor. Seventeen additional dogs, with body burdens of 0.2 to 1.8 μCi , died due to pulmonary neoplasia 3 to 8 yr after exposure. Ten of the dogs exposed to ^{238}Pu have died during the first 5 1/2 yr postexposure due to bone and/or lung tumors; the body burden at death ranged from 1.5 to 10 μCi . Lymphopenia was the earliest observed effect after inhalation of $^{239}\text{PuO}_2$ or $^{238}\text{PuO}_2$, occurring 0.5 to 2 yr after deposition of ≥ 80 nCi plutonium in the lungs.

To determine the life-span dose-effect relationships of inhaled plutonium, 18-mo-old beagle dogs were exposed to aerosols of $^{239}\text{PuO}_2$ (mean AMAD 2.3 μm , mean GSD 1.9), prepared by calcining the oxalate at 750°C for 2 hr; or to $^{238}\text{PuO}_2$ (mean AMAD 1.8 μm , mean GSD 1.9), prepared by calcining the oxalate at 700°C and subjecting the product to H_2^{16}O steam in argon exchange at 800°C for 96 hr. This material, referred to as pure plutonium oxide, is used as fuel in space-nuclear power systems.

One hundred thirty dogs exposed to $^{239}\text{PuO}_2$ in 1970 and 1971 were selected for long-term studies; 22 will be sacrificed to obtain plutonium distribution and pathology data, and 108 were assigned to life-span dose-effect studies (Table 2). One hundred thirteen dogs exposed to $^{238}\text{PuO}_2$ in 1973 and 1974 were selected for life-span dose-effect studies (Table 3). Twenty-four additional dogs were exposed for periodic sacrifice. The appendix (following the entire Annual Report) shows the status of the dogs on these experiments.

TABLE 2. Life-Span Dose-Effect Studies with Inhaled $^{239}\text{PuO}_2$ in Beagles^(a)

Dose Level Group	Number of Dogs		Initial Alveolar Deposition ^(b)	
	Male	Female	nCi ^(c)	nCi/g Lung ^(c)
0	10	10	0	0
1	10	10	3.5 ± 1.3	0.29 ± 0.011
2	10	10	22 ± 4	0.18 ± 0.04
3	10	10	79 ± 14	0.66 ± 0.13
4	10	10	300 ± 62	2.4 ± 0.4
5	10	10	1100 ± 170	9.3 ± 1.4
6	3	5	5800 ± 3300	50 ± 22
	63	65		

^(a) Exposed in 1970 and 1971

^(b) Estimated from external thorax counts at 14 and 30 days post-exposure and estimated lung weights (0.011 x body weight)

^(c) Mean ± 95% confidence intervals around the means

TABLE 3. Life-Span Dose-Effect Studies with Inhaled $^{238}\text{PuO}_2$ in Beagles^(a)

Dose Level Group	Number of Dogs		Initial Alveolar Deposition ^(b)	
	Male	Female	nCi ^(c)	nCi/g Lung ^(c)
0	10	10	0	0
1	10	10	2.3 ± 0.8	0.016 ± 0.007
2	10	10	18 ± 3	0.15 ± 0.03
3	10	10	77 ± 11	0.56 ± 0.07
4	10	10	350 ± 81	2.6 ± 0.5
5	10	10	1300 ± 270	10 ± 1.9
6	7	6	5200 ± 1400	43 ± 12
	67	66		

^(a) Exposed in 1973 and 1974

^(b) Estimated from external thorax counts at 14 and 30 days post-exposure and estimated lung weights (0.011 x body weight)

^(c) Mean ± 95% confidence intervals around the means

During the first 8 yr following exposure to $^{239}\text{PuO}_2$, eight dogs in the highest-level dose group, seventeen dogs in Dose Level Group 5, three in Group 4, two in Group 2 and one in Dose Level Group 1 were euthanized when death was imminent. Fourteen dogs were sacrificed for comparison of plutonium tissue distribution. Table 4 and Figure 8 show the primary causes of death and the distribution of ^{239}Pu in the tissues of these animals. One 80-mo-old control dog was euthanized because of an oral tumor.

As survival time increased, the fraction of plutonium in the lung decreased to ~25% of the final body burden by 7-8 yr post-exposure. During the first postexposure year, plutonium was translocated primarily to the thoracic lymph nodes, with little plutonium translocated to other tissues. Plutonium content of the thoracic lymph nodes was ~40% of the final body burden at 7-8 yr after exposure; the abdominal lymph nodes, principally the hepatic nodes, contained ~10%. The fraction of plutonium in liver increased, accounting for 35% of the final body burden at 7 to 8 yr after exposure in the higher (0.4 to 0.5 μCi body burden) dose-level groups. The organ distribution of plutonium in the periodically sacrificed dogs was generally similar to that of the high-dose-level dogs euthanized when death was imminent during the first 2 yr after exposure. The lower-dose-level dogs (0.001 to 0.1 μCi final body burden) sacrificed or euthanized during the 4th to 8th postexposure years had a much smaller fraction of the final body burden in the

liver, with a larger fraction retained in the lungs and/or thoracic lymph nodes. About 3% of the final body burden was in the skeleton at 7 to 8 yr after exposure.

The dogs euthanized because of respiratory insufficiency during the 3-yr postexposure period had increased respiration rates, and hypercapnia and hypoxemia associated with lesions in the lungs. Intermittent anorexia and body weight loss accompanied the respiratory insufficiency. Histopathologic examination of the lungs showed radiation pneumonitis characterized by focal interstitial and subpleural fibrosis, increased numbers of alveolar macrophages, alveolar epithelial hyperplasia, and foci of squamous metaplasia. Autoradiographs showed activity primarily composed of large stars, more numerous in areas of interstitial and subpleural fibrosis. Dog 804 M also had a pulmonary tumor, classified as a bronchiolar-alveolar carcinoma.

Eighteen of the twenty-three dogs euthanized 3 to 8 yr after exposure had lung tumors. Radiographic evidence of pulmonary neoplasia was observed before respiratory insufficiency developed. However, respiratory insufficiency was frequently observed prior to euthanasia due to neoplasia in the lung. All of the dogs with lung tumors were in Dose Level Groups 4, 5 and 6. Dogs 803 M, 835 F, 754 M, 769 F, and 875 M in Dose Level Groups 4, 2, and 1 died during the 7-8 yr postexposure period, of causes not thought, at the present time, to be due to plutonium exposure.

TABLE 4. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{239}\text{PuO}_2$

Dog Number	Time After Exposure, Mo	Final Body Burden, μCi	Percent of Final Body Burden					Cause of Death
			Lungs	Thoracic Lymph Nodes ^(a)	Abdominal Lymph Nodes ^(b)	Liver	Skeleton	
478M	0.25	0.293	98	0.15	0.02	0.24	0.08	Sacrifice
435F	0.25	3.841	99	0.11	0.01	0.00	0.03	Sacrifice
816M	0.50	0.399	99	0.12	0.01	0.00	0.03	Sacrifice
918M	1	0.074	99	0.82	0.02	0.11	0.08	Sacrifice
920F	1	0.011	94	0.47	0.03	0.08	0.61	Sacrifice
913M	1	4.849	98	1.1	0.00	0.03	0.05	Sacrifice
702F	5	1.682	94	5.7	0.00	0.01	0.09	Sacrifice
709M	5	1.726	97	2.2	0.00	0.00	0.05	Sacrifice
734M	5	0.914	96	3.4	0.00	0.01	0.05	Sacrifice
739F	5	1.511	95	4.7	0.03	0.00	0.00	Sacrifice
910M	11	12.229	84	15	0.01	0.06	0.05	Radiation Pneumonitis
747F	12	5.434	71	29	0.03	0.07	0.07	Radiation Pneumonitis
906F	12	6.154	88	12	0.00	0.03	0.05	Radiation Pneumonitis
849F	13	0.0007	80	15	0.20	0.04	1.6	Sacrifice
896F	15	4.115	81	15	0.92	0.23	0.12	Radiation Pneumonitis
817M	21	3.794	64	34	0.13	1.4	0.19	Radiation Pneumonitis
815M	25	0.074	64	32	--	0.08	0.10	Sacrifice
829M	26	3.198	75	19	0.79	4.2	0.45	Radiation Pneumonitis
760M	31	0.978	71	23	0.57	3.7	0.28	Radiation Pneumonitis
890F	31	2.012	55	28	2.2	13	0.26	Radiation Pneumonitis
804M	37	1.101	62	29	0.19	7.9	0.36	Radiation Pneumonitis, Lung Tumor
798F	43	0.0056	55	44	0.02	0.17	0.43	Sacrifice
772M	53	1.821	42	22	0.88	29	0.69	Lung Tumor
759M	53	0.707	43	27	12	15	0.65	Lung Tumor
796F	55	0.671	40	31	4.1	21	1.0	Lung Tumor
783M	59	1.377	59	11	1.8	26	0.67	Lung Tumor
873M	62	1.746	45	27	6.4	16	0.76	Lung Tumor
753F	69	1.171	35	31	0.09	24	0.64	Lung Tumor
761M	69	1.064	36	37	6.3	19	0.53	Lung Tumor
727M	72	0.585	39	24	12	23	0.78	Lung Tumor
762M	72	0.0017	51	42	0.34	0.71	0.66	Sacrifice
837M	72	1.034	42	38	0.70	14	0.46	Lung Tumor
863F	76	0.617	33	12	1.3	47	1.4	Lung Tumor
852F	77	1.067	33	35	0.88	26	0.94	Lung Tumor
803M	79	0.415	20	46	11	20	1.4	Interstitial Pneumonitis
875M	83	0.0026	24	66	0.34	0.64	6.3	Malignant Lymphoma, Kidney
754M	84	0.0046	29	66	0.23	0.39	1.2	Status Epilepticus
835F	86	0.099	27	65	0.95	3.1	1.7	Reticulum Cell Sarcoma
880F	86	0.468	19	31	13	34	0.37	Lung Tumor
769F	90	0.019	36	57	0.32	1.7	1.8	Ovarian Tumor
888M	93	0.179	32	40	10	12	2.1	Lung Tumor
856F	94	0.306	40	45	0.78	9.0	3.9	Lung Tumor
889F	94							Lung Tumor
787M	95	0.473	24	19	12	39	2.7	Lung Tumor
820F	96	0.387	14	40	7.6	29	1.4	Lung Tumor

(a) Includes tracheobronchial, mediastinal and sternal lymph nodes

(b) Includes hepatic, splenic and mesenteric lymph nodes

FIGURE 8. Tissue Distribution of Plutonium in Beagles After Inhalation of PuO_2 (● = Low-Dose-Level $^{239}\text{PuO}_2$ Dogs)

In twelve of the dogs, the lung tumors were classified as bronchiolar-alveolar carcinoma; in four dogs as adenosquamous carcinoma; in one dog, epidermoid carcinoma; and in another dog, epidermoid and bronchiolar-alveolar carcinoma. The epidermoid carcinoma metastasized to the skeleton; the bronchiolar-alveolar carcinomas metastasized only to the thoracic lymph nodes in six dogs, and to several organs (including the thoracic lymph nodes, mediastinum, kidney, skeleton, adrenal, aorta, and axillary, prescapular and hepatic lymph nodes) in two other dogs. Two of the adenosquamous carcinomas metastasized to thoracic lymph nodes, mediastinum and thoracic pleura. Three of the dogs had lesions of secondary hypertrophic osteoarthropathy. Sclerosing lymphadenitis was associated with the high

concentration of plutonium in the thoracic and hepatic lymph nodes. There was also a generalized lymphoid atrophy that may be related to debilitation in the dogs with respiratory insufficiency, or to lymphocytopenia. Livers of the dogs euthanized during the 4- to 8-yr postexposure period showed moderate diffuse centrilobular congestion. Liver cells in these areas contained fine granular yellow pigment resembling lipofuscin and were frequently vacuolated. Focal aggregation of vacuolated lipofuscin-containing cells in the sinusoids was associated with alpha stars on autoradiographs.

Lymphopenia developed after inhalation of $^{239}\text{PuO}_2$ in the dose level groups with mean initial alveolar depositions of 79 nCi and greater (Figure 9). Through 91 mo

FIGURE 9. Mean Leukocyte, Neutrophil and Lymphocyte Values in Dogs After Inhalation of $^{239}\text{PuO}_2$

postexposure, mean lymphocyte values were significantly lower ($p < 0.02 - 0.001$) than the control group. The reduction in lymphocytes was dose-related, both in time of appearance and magnitude. At mean alveolar depositions of 3.5 and 22 nCi, lymphocyte values were within ranges observed in control dogs. A reduction in total leukocytes was evident in the higher dose group, which was also lymphopenic. No effects have been observed on red cell parameters following $^{239}\text{PuO}_2$ inhalation.

Serum chemistry assays have been performed to detect organ-specific damage from plutonium that translocated from lung to extrapulmonary sites. There have been no consistent dose-related alterations in serum constituents, including glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, alkaline phosphatase, urea nitrogen, and serum protein fractions, of dogs exposed to $^{239}\text{PuO}_2$.

During the first 5 1/2 yr following exposure to $^{238}\text{PuO}_2$, eight dogs in the highest-level dose group and six dogs in Dose Level Group 5 were euthanized when death was imminent. Twenty-one were sacrificed for comparison of plutonium tissue distribution. Table 5 and Figure 8 show the causes of death and the distribution of ^{238}Pu in the tissues of these animals. One 97-mo-old control dog died due to pyometritis.

Of the fourteen euthanized dogs, seven were euthanized due to bone tumors (osteosarcoma), three due to lung tumors, three due to Addison's disease and one due to pneumonia. Three of the dogs euthanized due to bone tumor also had lung tumors. One of the dogs euthanized due to bone tumor also had Addison's disease.

The lung tumors were classified as bronchiolar-alveolar carcinomas. In one dog, three lung tumor types were observed:

TABLE 5. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{238}\text{PuO}_2$

Dog Number	Time After Exposure, Mo	Final Body Burden, μCi	Percent of Final Body Burden					Cause of Death
			Lungs	Thoracic Lymph Nodes (a)	Abdominal Lymph Nodes (b)	Liver	Skeleton	
1032M	0.25	0.150	97	0.34	0.20	1.7	0.16	Sacrifice
921F	1	0.0044	93	0.65	0.04	0.38	2.1	Sacrifice
930F	1	0.052	99	0.63	0.01	0.07	0.35	Sacrifice
931F	1	0.347	96	1.9	0.01	0.05	0.36	Sacrifice
929F	2	0.017	91	7.5	0.002	0.26	0.58	Sacrifice
932F	2	0.382	96	2.5	0.01	0.18	0.39	Sacrifice
923F	2	0.0023	88	9.4	0.03	0.09	0.44	Sacrifice
925M	3	0.0064	91	4.1	0.04	0.04	1.2	Sacrifice
926M	3	0.078	87	11	0.23	0.65	1.1	Sacrifice
934M	3	0.902	92	4.8	1.7	0.45	0.95	Sacrifice
1318M	12	0.030	45	27	0.08	10	15	Sacrifice
1319M	12	0.077	41	26	0.03	11	20	Sacrifice
1214M	13	0.014	52	9.2	0.32	6.2	16	Sacrifice
1310M	25	0.026	19	36	0.08	15	28	Sacrifice
1317M	25	0.041	20	33	0.16	17	26	Sacrifice
1315M	25	0.047	22	31	0.04	17	28	Sacrifice
1191F	35	0.658	26	32	0.13	18	22	Pneumonia
1215M	36	0.011	21	43	0.17	13	21	Sacrifice
1311M	37	0.036	13	31	0.22	21	32	Sacrifice
994F	42	5.024	17	45	0.50	18	18	Addison's Disease
970F	48	0.0022	20	34	0.36	16	24	Sacrifice
1312M	49	0.035	6.8	29	0.26	25	35	Sacrifice
1143M	49	6.331	11	43	2.0	15	22	Bone Tumor, Lung Tumor
1025M	50	10.033	16	27	7.1	24	23	Lung Tumor
1064M	51	8.427	13	48	1.9	15	20	Bone Tumor, Lung Tumor
1175F	52	3.641	14	31	0.08	25	26	Lung Tumor
1079M	56	2.182	9.8	40	4.3	13	25	Addison's Disease
1096F	59	1.204	4.3	22	2.7	36	24	Addison's Disease
1189M	60	0.044	8.9	25	0.16	37	25	Sacrifice
1115F	61	1.534	5.0	32	2.3	26	33	Bone Tumor
1162F	61	3.663	12	32	5.9	21	25	Bone Tumor, Addison's Disease
1009M	62	4.360	15	25	2.4	31	23	Lung Tumor
974F	64	1.465	5.1	24	5.9	33	29	Bone Tumor
1092M	65							Bone Tumor
975F	66							Bone Tumor, Lung Tumor

(a) Includes tracheobronchial, mediastinal and sternal lymph nodes

(b) Includes hepatic, splenic and mesenteric lymph nodes

bronchiolar-alveolar, adenocarcinoma and fibrosarcoma. Lung tumor metastases were not observed. Bone tumor metastases were found in the lungs of three dogs; and in one dog, the bone tumor metastasized to liver, lung, and tracheobronchial lymph node. The four dogs with Addison's disease had adrenal cortical atrophy.

In addition to the lesions associated with the cause of death, lesions in the lungs of the high-dose-level dogs included focal alveolar histiocytosis, alveolitis, alveolar epithelial cell hyperplasia, alveolar emphysema, pleural fibrosis, and interstitial fibrosis. Numerous alpha stars were observed, mainly in foci of fibrosis, and single alpha tracks were scattered throughout sections in foci of alveolar histiocytosis and in alveolar septa. The tracheobronchial and mediastinal lymph nodes were completely obliterated by necrosis and scarring, associated with high concentrations of plutonium observed as alpha stars. Similar but less severe lesions were seen in the hepatic lymph nodes. There were extensive alterations in bone, including multiple areas of focal atrophy of bone; endosteal, trabecular and peritrabecular bone fibrosis; and osteolysis of cortical, endosteal, and trabecular bone. One dog had lesions of secondary hypertrophic osteoarthropathy. Radioactivity in the bone was present as single tracks, generally scattered throughout the bone, cartilage and bone marrow. The liver contained foci of hepatocellular fatty change, where small clusters of single tracks were seen. There was also

mild focal nodular hyperplasia of hepatocytes. Elevated serum glutamic pyruvic transaminase levels, suggestive of liver damage, were observed in the high-dose-level dogs (Figure 10).

Dose-related lymphopenia was observed in groups with mean alveolar deposition of 77 to 5200 nCi, as in the dogs exposed to $^{239}\text{PuO}_2$ (Figure 11). However, the lymphocyte depression was more pronounced, both as to magnitude and time of appearance, than that observed in dogs exposed to similar doses of $^{239}\text{PuO}_2$. As was found with ^{239}Pu , lymphocyte values in the two lowest exposure groups, i.e., 2.3 and 18 nCi, were not different from control values. A dose-related reduction in total leukocytes was evident, primarily due to the lymphopenia, except in Groups 5 and 6, in which neutropenia was observed. No difference in monocyte values was seen in relation to dose levels. A significant and progressive reduction in eosinophils was evident only in Group 6 dogs following ^{238}Pu inhalation. No chronic effects have been observed in the red cell parameters.

With $^{238}\text{PuO}_2$, serum chemistry assays showed only glutamic pyruvic transaminase values elevated above those of the control group, and only in Group 6 dogs (5200 nCi), after about 46 mo following exposure (Figure 10). Elevations in this "liver-specific" enzyme are consistent with histopathologic findings and radiochemical analyses of ^{238}Pu translocation to the liver. Alkaline phosphatase was elevated in some of the dogs with bone tumors.

FIGURE 10. The Serum Glutamic Pyruvic Transaminase Values in Control Dogs and Those Exposed to Initial Lung Burdens of 5200 nCi $^{238}\text{PuO}_2$

At 5 1/2 yr postexposure, the fraction of the final body burden in the lungs of the ^{238}Pu -exposed dogs was about 10%, compared to 40% in the ^{239}Pu -exposed dogs (Figure 8). Twenty-five to 30% of the plutonium was found in the thoracic lymph nodes and about 30% in the liver, at 5 1/2 yr after exposure, for both ^{239}Pu and ^{238}Pu . About 25% of the final body burden was found in the skeleton of the ^{238}Pu -exposed dogs, at 5 1/2 yr postexposure, compared to less than 1% in the ^{239}Pu -exposed dogs. During the 5 1/2-yr

postexposure period there were no obvious differences in the ^{238}Pu tissue distribution in low-dose-level dogs compared to high-dose-level dogs.

After inhalation of $^{239}\text{PuO}_2$ or $^{238}\text{PuO}_2$, lymphopenia was the earliest observed effect, occurring after deposition of >80 nCi plutonium in the lungs. On a concentration basis, the 80-nCi dose level is about 40 times the 16-nCi maximum permissible human lung deposition, based on 0.3 rem/wk to the lung.

FIGURE 11. Mean Leukocyte, Neutrophil and Lymphocyte Values in Dogs After Inhalation of $^{238}\text{PuO}_2$

DATA STORAGE AND RETRIEVAL FOR LONG-TERM DOG STUDIES

Investigators:

C. R. Watson, G. M. Trauger, J. M. McIntyre,
A. L. Slavich and J. F. Park

Technical Assistance:

R. O. Budd, E. L. Kelley and J. D. Zimmerman

Over half of the 500,000 records collected on dogs in the last 20 years in our laboratory have been converted from sequential storage on magnetic tape to direct-access disk storage on a PDP 11/70 minicomputer. An interactive storage and retrieval system, based on a commercially available query language, has been developed to make these records more accessible. Data entry and retrieval are now performed by scientists and technicians rather than by keypunch operators and computer specialists. Further conversion awaits scheduled computer enhancement.

In the last 20 years several approaches have been used to store, retrieve, and analyze information about the status of the dogs in our laboratory. Initially, individual folders were kept in file cabinets. Update was slow and the possibility existed that records could be lost or inadvertently destroyed, but retrieval of a medical history on a single dog was efficient. However, this manual retrieval and summary was inefficient for data from groups of dogs.

In the late 1960s and early 1970s, the manual records were converted to a punched-card input, batch-updated, magnetic-tape-based computer system. The computer programs were developed around the concept of clustering all information about a dog in one location. Again, update was slow, but retrievals for information on individual dogs were efficient. Through the use of archived tape copies, physical integrity of the records was greatly improved over the former system. The legibility of the printed information was much greater than that of handwritten comments. However, it was still difficult to compare information on groups of dogs, unless computer programs were written for each specific analysis. A further disadvantage was that some types of information, the extent of which was not predetermined (such as pathologist's observations and radioanalysis results), did not lend themselves to this approach, and had to be stored separately in somewhat incompatible computer systems.

In 1977, a PDP 11/70 minicomputer was obtained for biometrics applications, and in 1978 DATATRIEVE,* an interactive query language, was purchased. The beagle colony biomedical records were converted to this interactive system, which is based on clustering the data in disk files by record type, rather than grouping all information for a dog. The information is stored in multi-key index-sequential files. Through DATATRIEVE, in seconds, each record is equally accessible from any computer terminal in the laboratory. The DATATRIEVE operations are enhanced by a series of COBOL programs written for interactive input and routine report generation. Scientists and technicians now perform on-line update, error correction and retrieval in a matter of minutes, as opposed to the former time-consuming cycle of form collection, keypunching, batch-updating, proofreading, and error correction.

As shown in Table 6, about 50% of the dog colony information is archived to tape pending scheduled enhancement of the computer.

* The PDP 11/70 and DATATRIEVE are products of Digital Equipment Corp., Maynard, MA.

TABLE 6. Beagle Colony Biomedical Records Accessible Via DATATRIEVE

Number of Records	Type of Information	Description
1,903	Vital Statistics	One record per dog containing dates of birth, death, and experimental assignment, current weight, location, and diet, etc.
19,718 ^(a)	Weights	For each live dog: one or more records per month. Records for dead dogs are temporarily ARCHIVED to tape and are not readily accessible.
79,827 ^(a)	General Observations	Narrative descriptions of colony events, clinical conditions, physical examinations, diagnostic radiography, breeding, whelping, etc. recorded after 1/1/78. Earlier records temporarily ARCHIVED.
17,651	Hematology A	Numerical values for each blood sample analyzed.
3,764	Hematology B	Narrative observations about many of the blood samples.
15,863	Chemistry A	Numerical values for each serum analysis.
2,863	Chemistry B	Narrative observations about many of the serum samples.
(a)	Respiration A	Numerical values for each test conducted - ARCHIVED.
(a)	Respiration B	Narrative observations about many of the tests - ARCHIVED.
43,445	Radioanalysis A	Quantitative descriptions of each excreta or tissue sample.
39,512	Radioanalysis B	Results of each radiochemical determination.
35,414	Radioanalysis C	Remarks about many of the samples.
5,488 ^(b)	Pathology A	SNOP-coded observations on tissues examined since 6/1/78, conversion of other records awaits SNOP coding by pathologist.
111 ^(b)	Pathology B	Narrative observations about some of the tissues.
(b)	Whole Body Counts	These records have never been stored in the computer, this manual system will be converted in FY 80.
265,559		Total number of records available interactively.

^(a) Incomplete files pending computer enhancement

^(b) Additional conversion necessary

PULMONARY DISTRIBUTION OF INHALED $^{239}\text{PuO}_2$ IN DOGS

Investigators:

J. F. McShane, G. E. Dagle, J. F. Park and D. L. Catt

Dogs exposed to $^{239}\text{PuO}_2$ by inhalation were killed from 1 to 69 mo postexposure. The distribution of plutonium in the lungs, which was studied by analyzing autoradiographs, changed relative to time postexposure. This study investigates methods of measuring these changes.

Beagle dogs were given single, nose-only exposures to aerosols of $^{239}\text{PuO}_2$ (see Dose-Effect Studies with Inhaled Plutonium Oxide in Beagles, this Annual Report). Eight of the dogs were selected for this study; they had estimated initial pulmonary burdens of 1 to 7 μCi , and were killed from 1 to 69 mo postexposure.

At necropsy the lungs were inflated and fixed by intratracheal perfusion with 10% neutral buffered formalin. Autoradiographs were prepared from histologic sections. Photomicrographs were prepared from contact negatives of the autoradiographs of right apical lobe cross-sections from each dog. The position of each alpha star was marked on the print, resulting in a "map" of the plutonium distribution.

To determine the distribution of plutonium in lungs, a transparency containing a grid of 1-cm squares was placed over each plutonium map. The percentage of squares that overlaid alpha stars on the map was calculated. Results are summarized in Table 1. The data showed that the percentage of grid squares containing alpha stars dropped from 100% at 1 mo postexposure to 50% at 69 mo postexposure. However, this may be due only to lower plutonium lung burdens at later times postexposure. Therefore the grid field was evaluated from another perspective.

This was done by finding the highest 1-cm² alpha star concentration on each photograph. Table 7 shows the number of stars found in this square, along with the percentage of the total of that section's alpha stars that is

TABLE 7. Distribution of α -Stars in Autoradiographs of Lung Cross-Sections from Dogs Exposed to $^{239}\text{PuO}_2$

Dog No.	913	739	747	817	890	760	761	753
Months Postexposure	1	5	12	21	31	31	69	69
Initial Pulmonary Burden, μCi	4.9	1.5	7.5	3.2	3.1	1.4	1.5	2.4
Final Lung Burden, μCi	4.7	1.4	3.8	2.4	1.1	0.7	0.4	0.4
Total α -Stars in One Section ^(a)	1676	601	1065	930	359	326	497	258
% of Grid Square Containing α -Stars	100	100	84	81	--	56	61	54
Highest Concentration of α -Stars in One Square	31	--	41	22	--	45	82	43
$\frac{\text{Highest Concentration in One Square}}{\text{Total Number of } \alpha\text{-Stars}} \times 100$ ^(b)	1.8	--	3.8	3.5	--	13.7	16.5	16.7
Subpleural %	20	21	51	33	30	41	44	6
Midlung %	20	11	6	16	10	9	11	17
Aggregation %	16	--	35	--	--	30	50	--

(a) Autoradiograph of Right Apical Lobe

(b) The % of Total α -Stars Found in the Centimeter Square of Highest Concentration

contained in that square. Note that no square from the dog killed at 1 mo contains more than 1.8% of the total stars, while both dogs killed at 69 mo have single squares containing over 16% of the entire observed alpha stars. This suggests a focal accumulation of plutonium which tends to increase with time following exposure.

The focal accumulations frequently contained overlapping alpha tracks. These were called "aggregates," and were defined as two or more alpha stars whose tracks overlap. Thus, aggregation percentage is the proportion of the total number of alpha stars that were overlapping. The data on four dogs are summarized in Table 7. The percentage of aggregated alpha stars increases with time postexposure. The overlapping of alpha tracks indicates that a decreasing proportion of lung tissue is absorbing the pulmonary radiation dose. If alpha star distribution were random and uniform, more aggregation should be found in the lung with the most plutonium. This is the opposite of the experimental observation: the dog killed at 1 mo had an aggregation percentage of 16, while that killed at 69 mo, with less alpha activity, had a 50% aggregation percentage.

To determine the site of focal accumulation, a line was drawn through the center of each lung photograph, representing the middle of the lobe. The location of the plutonium was evaluated by directly measuring the position of each alpha star between the

pleural surface and this midline. For each alpha star, the ratio of the distance between midline and pleura was calculated:

$$\frac{\text{Midline to alpha-star distance}}{\text{Midline to pleura distance}} \times 100\%.$$

Histograms made of these ratios were scaled with interval bands at each 20th percentile (Figure 12). These graphs divide the lung into five segments; the plutonium found 80-100% of the distance away from the midline is considered "subpleural." Table 1 lists the subpleural concentrations and those found in the increment closest to the midline. Dogs killed at 1 and 5 mo postexposure exhibit a homogeneous distribution of alpha activity between midline and pleura. Five of the six dogs killed at 12 mo, or later, after exposure have high subpleural ratios and low midline ratios. The degree of subpleural concentration did not increase after 12 mo. However, dog 753 did not fit this trend; even at 69 mo, its subpleural concentration was very low.

These initial quantitative data on the pulmonary distribution of inhaled $^{239}\text{PuO}_2$ in the lung are compatible with previous qualitative descriptions in dogs and morphometric measurements in rats. The sequence of events following inhalation of $^{239}\text{PuO}_2$ includes phagocytosis and then migration, resulting either in clearance or concentration of radioactivity. This seems to be a dynamic process that may influence radiation dose distribution and pathogenesis.

FIGURE 12. Site of Accumulation of $^{239}\text{PuO}_2$ in Dog Lung

• Inhaled Plutonium Nitrate in Dogs

The major objective of this project is to determine dose-effect relationships of inhaled plutonium nitrate in dogs to aid in the prediction of health effects of accidental exposure in man. The critical tissue after inhalation of "soluble" plutonium (such as plutonium nitrate) is generally considered to be the skeleton or liver, on the assumption that such plutonium will be rapidly translocated from the lung to skeleton and liver. In several rodent studies, however, inhalation of "soluble" plutonium has resulted in lung tumors as well as skeletal tumors. Life-span studies are necessary to evaluate the complex interactions between tissues and organ systems directly or indirectly injured by low levels of exposure. Beagle dogs were chosen to correlate relative risks, determined in other studies, with different forms and routes of exposures to plutonium.

DOSE-EFFECT STUDIES WITH INHALED PLUTONIUM NITRATE IN DOGS

Investigators:

G. E. Dagle, W. C. Cannon, A. C. Case, R. M. Madison, J. F. McShane,
D. L. Stevens, S. E. Rowe, H. A. Ragan and R. E. Schirmer

Technical Assistance:

E. T. Edmerson and V. T. Faubert

Beagle dogs given a single inhalation exposure to $^{239}\text{Pu}(\text{NO}_3)_4$, and observed for life-span dose-effect relationship, died from radiation pneumonitis (four of five at the highest dosage level, in 14 to 25 mo postexposure; 1 of 20 at the medium-high dosage level, at 34 mo postexposure). There were also indications in these dogs of radiation osteosis, characterized by peritrabecular fibrosis. One dog, at 39 mo postexposure, has radiographic evidence of an osteosarcoma. Leukopenia, lymphopenia, neutropenia and decreased numbers of circulating monocytes and eosinophils occurred at the two highest dosage levels, as previously reported (Annual Report, 1978). Twelve dogs given a single inhalation exposure to $^{238}\text{Pu}(\text{NO}_3)_4$ showed a more rapid translocation of $^{238}\text{Pu}(\text{NO}_3)_4$ to bone and liver than was observed for $^{239}\text{Pu}(\text{NO}_3)_4$, but at 1 yr postexposure the percentage of the final body burden in bone and liver were similar for the two isotopes.

Six dosage groups (105 dogs) have been exposed to aerosols of $^{239}\text{Pu}(\text{NO}_3)_4$ for life-span observations (Table 8). In addition, 20 dogs were exposed to nitric acid aerosols as vehicle controls, 20 dogs were selected as untreated controls for life-span observations, 25 dogs were exposed to aerosols of $^{239}\text{Pu}(\text{NO}_3)_4$ for periodic sacrifice to study plutonium metabolism and the pathogenesis of developing lesions, and seven dogs were selected as controls for periodic sacrifice.

The dogs were exposed in aerosol chambers using techniques described in previous reports. The appendix (following the entire Annual Report) shows the status of the dogs on these experiments.

A pilot study was conducted, in which 12 dogs were exposed to a single aerosol of $^{238}\text{Pu}(\text{NO}_3)_4$, to study the deposition and translocation up to 1 yr. The aerosol concentration was 14.3 ± 1.2 nCi/l with an

TABLE 8. Protocol for Life-Span Inhalation Exposure of Beagle Dogs to Plutonium Nitrate

Dosage Level Group	Number of Dogs		Initial Alveolar Deposition, ^(a) nCi	AMAD, ^(b) μ m	GSD ^(c)	Aerosol Concentration, nCi/l
	Male	Female				
Control	10	10	0	0	0	0
Vehicle	10	10	0	0	0	0
1	10	10	2 ± 2	0.33	2.07	0.6 ± 0.3
2	10	10	8 ± 4	0.51	1.87	2.2 ± 0.5
3	10	10	56 ± 17	0.65	1.97	18 ± 4
4	10	10	295 ± 67	0.75	1.84	80 ± 33
5	10	10	1709 ± 639	0.87	2.11	306 ± 81
6	3	2	5445 ± 1841	0.85	2.37	534 ± 118

(a) Initial alveolar deposition estimated from thoracic count 2 wk postexposure (mean $\pm$ standard deviation)

(b) Activity Mean Aerodynamic Diameter

(c) Geometric Standard Deviation

AMAD of 0.81μ m, and a GSD of 1.72. The initial alveolar deposition, estimated from thoracic counts 2 wk postexposure, was 66 ± 18 nCi. Groups of three dogs were sacrificed at 3 days, 4 wk, 3 mo, and 1 yr to compare deposition and translocation with that of $^{239}\text{Pu}(\text{NO}_3)_4$ -exposed dogs.

The initial deposition and early clearance of inhaled $^{239}\text{Pu}(\text{NO}_3)_4$ aerosols were discussed in last year's Annual Report. The fraction of $^{239}\text{Pu}(\text{NO}_3)_4$ in the lung decreased to approximately 20% of the final body burden in dogs surviving 1 yr or more (Table 9). There was early translocation to the liver and skeleton, with an average 26% and 49% of final body burden, respectively, present in dogs surviving 1 yr or more. Only minimal amounts were translocated to the thoracic or abdominal lymph nodes. This was in contrast to dogs that inhaled $^{239}\text{PuO}_2$, in which a considerable amount translocated to the thoracic lymph nodes and only minimal amounts translocated to liver or skeleton at these time periods.

The earliest observed biological effect was on the hematopoietic system, with lymphopenia occurring at the two highest dosage levels at 4 wk postexposure to $^{239}\text{Pu}(\text{NO}_3)_4$. The results of these continuing evaluations are shown in Figure 13. Total leukocyte concentrations were reduced significantly in the two highest dose groups, i.e., Group 5 (mean initial alveolar deposition of ~ 1700 nCi), and Group 6 (~ 5500 nCi). Numbers of

leukocytes in Group 4 dogs (~ 300 nCi) tended to be consistently (but not significantly) lower than those from control dogs. The reduction in white cells in Groups 5 and 6 is due to an effect on most leukocyte types (neutrophils, lymphocytes, monocytes and eosinophils). Lymphocyte reductions significantly different from values for controls were present only in Groups 5 and 6. This is in contrast to the effects of both $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$, which significantly depressed lymphocyte concentrations by 21 mo after exposure to initial lung burdens of ~ 80 nCi and greater. The lymphocytopenia at lower dosage levels with plutonium oxides could be related to the more extensive translocation of plutonium oxide to the tracheobronchial lymph nodes.

Four dogs in the highest dose-level group died or were euthanized because of severe radiation pneumonitis at 14 to 25 mo postexposure to $^{239}\text{Pu}(\text{NO}_3)_4$, as described in last year's Annual Report. Since last year's report, one additional dog at the medium-high dose level was euthanized because of severe radiation pneumonitis. Histopathologic examination of these dogs' lungs revealed interstitial fibrosis, alveolar epithelial hyperplasia, increased numbers of alveolar macrophages, occasional small emphysematous cavities and, at times, very small nodules of squamous metaplasia at the termini of respiratory bronchioles. Autoradiographs showed alpha stars concentrated in areas of interstitial fibrosis and

TABLE 9. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$

Dog Number	Time After Exposure, mo	Final Body Burden, μCi	Percent of Final Body Burden					Cause of Death
			Lungs	Thoracic Lymph Nodes(a)	Abdominal Lymph Nodes(b)	Liver	Skeleton	
1359 M	0.1	0.080	90.50	0.15	0.06	2.46	3.20	Sacrifice
1375 F	0.1	0.073	89.61	0.14	0.01	0.97	4.68	Sacrifice
1407 F	0.1	0.092	51.87	0.41	0.13	10.99	18.70	Sacrifice
1389 M	0.5	0.053	24.07	0.38	0.08	41.28	26.21	Sacrifice
1390 M	0.5	0.051	24.62	0.32	0.11	20.05	44.45	Sacrifice
1445 F	0.5	0.057	26.42	0.32	0.11	21.28	44.73	Sacrifice
1329 F	1.0	0.485	70.05	0.16	0.04	8.28	18.79	Sacrifice
1346 M	1.0	0.902	76.81	0.32	0.03	10.45	10.30	Sacrifice
1347 F	1.0	0.699	71.71	0.36	0.08	9.33	14.09	Sacrifice
1336 M	1.0	0.032	71.38	0.22	0.05	5.72	19.73	Sacrifice
1341 F	1.0	0.022	64.43	0.29	0.10	12.92	18.63	Sacrifice
1344 F	1.0	0.052	58.68	0.25	0.04	21.87	16.09	Sacrifice
1335 M	1.0	0.003	19.52	0.07	0.06	6.68	25.04	Sacrifice
1339 F	1.0	0.001	19.08	0.13	0.08	20.92	45.47	Sacrifice
1351 M	1.0	0.002	40.68	1.22	0.09	17.09	28.89	Sacrifice
1522 F	3.0	0.059	54.68	0.57	0.10	11.52	28.24	Sacrifice
1529 F	3.0	0.049	51.68	0.40	0.07	18.48	23.74	Sacrifice
1539 M	3.0	0.072	52.45	0.31	0.05	18.58	25.03	Sacrifice
1564 F	12.0	0.037	18.00	1.27	0.11	33.53	42.63	Sacrifice
1571 F	12.0	0.053	22.37	1.47	0.11	28.76	42.91	Sacrifice
1588 M	12.0	0.053	13.14	0.40	0.12	35.85	46.18	Sacrifice
1424 M	14.3	4.625	33.10	1.43	0.16	26.49	36.88	Radiation Pneumonitis
1517 F	16.3	4.254	17.97	0.89	0.17	27.92	50.68	Radiation Pneumonitis
1510 F	16.6	4.048	22.00	1.15	0.05	20.71	52.00	Radiation Pneumonitis
1420 M	24.6	1.616	16.51	0.86	0.20	7.77	70.06	Radiation Pneumonitis
1471 F	34.4	--	--	--	--	--	--	Radiation Pneumonitis

(a) Includes tracheobronchial, mediastinal and sternal lymph nodes

(b) Includes hepatic, splenic and mesenteric lymph nodes

individual alpha tracks scattered in the parenchyma. The lung changes at these times postexposure were similar in dogs exposed to $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$, but single alpha tracks in the parenchyma were generally seen only in dogs exposed to $^{239}\text{Pu}(\text{NO}_3)_4$ and $^{238}\text{PuO}_2$, not in those exposed to $^{239}\text{PuO}_2$. Lung changes were accompanied by sclerosing tracheobronchial lymphadenitis, but of a lesser degree than that seen after inhalation of either $^{239}\text{PuO}_2$ or $^{238}\text{PuO}_2$. There were indications of radiation osteosis in dogs that died of radiation pneumonitis. This was qualitatively similar to that seen after inhalation of $^{238}\text{PuO}_2$ (but not $^{239}\text{PuO}_2$), and was generally characterized by peritra-

becular fibrosis composed of relatively hypocellular collagen fibers partially surrounding trabeculae in vertebrae, femora, and ribs. One dog, from dose level Group 5, at 39 mo postexposure, has radiographic evidence of an osteosarcoma in a humerus.

Serum enzyme assays have been performed throughout the postexposure period in an attempt to diagnose specific damage to liver and/or bone by plutonium translocated from the lung. Although periodic elevations occurred in mean values for glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, and alkaline phosphatase, there were no dose-related or dose-consistent elevations

FIGURE 13. Mean Leukocyte, Neutrophil and Lymphocyte Values in Dogs After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$.

in these parameters. The periodic excursions in mean values were usually due to one or two dogs that had high values at a particular sampling period, and occurred in all treatment groups, including the controls.

The $^{238}\text{Pu}(\text{NO}_3)_4$ was rapidly translocated from lung parenchyma to other organs (Table 10). Compared with $^{239}\text{Pu}(\text{NO}_3)_4$, $^{238}\text{Pu}(\text{NO}_3)_4$ more rapidly translocated to liver and skeleton but reached a similar plateau at 1 yr postexposure (Figure 14). At 1 yr postexposure there was less $^{238}\text{Pu}(\text{NO}_3)_4$ than $^{239}\text{Pu}(\text{NO}_3)_4$ remaining in the lung. No hematologic or

serum chemistry changes were observed through 12 mo postexposure in these dogs with mean alveolar burdens of ~ 70 nCi $^{238}\text{Pu}(\text{NO}_3)_4$.

A cooperative study with the Radiobiology Laboratory at the University of Utah was initiated this past year. Bones from some of these dogs exposed to plutonium by inhalation will be evaluated using neutron-induced autoradiographic techniques to define more exactly the localization of plutonium and the cells at greatest risk. No results are available from these preliminary studies.

TABLE 10. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{238}\text{Pu}(\text{NO}_3)_4$

Dog Number	Time After Exposure, Mo	Final Body Burden, μCi	Percent of Final Body Burden					Cause of Death
			Lungs	Thoracic Lymph Nodes(a)	Abdominal Lymph Nodes(b)	Liver	Skeleton	
1544 F	0.1	0.126	40.69	0.27	0.15	12.39	22.52	Sacrifice
1549 F	0.1	0.055	49.20	0.37	0.12	12.26	15.07	Sacrifice
1554 M	0.1	0.086	57.70	0.27	0.08	9.49	15.64	Sacrifice
1545 M	1.0	0.091	17.17	0.20	0.08	31.06	43.78	Sacrifice
1552 M	1.0	0.093	18.13	0.21	0.04	40.67	33.99	Sacrifice
1553 F	1.0	0.058	26.46	0.29	0.05	23.19	43.07	Sacrifice
1546 M	3.5	0.152	6.57	0.15	0.13	46.77	41.08	Sacrifice
1551 M	3.5	0.104	12.53	0.23	0.11	32.26	48.47	Sacrifice
1557 F	3.5	0.052	9.54	0.26	0.09	32.31	51.14	Sacrifice
1547 M	12.3	0.099	2.97	0.21	0.27	40.58	52.70	Sacrifice
1550 F	12.3	0.077	3.02	0.21	0.08	42.86	49.88	Sacrifice
1558 F	12.3	0.077	3.89	0.21	0.15	34.86	57.29	Sacrifice

(a) Includes tracheobronchial, mediastinal and sternal lymph nodes

(b) Includes hepatic, splenic and mesenteric lymph nodes

FIGURE 14. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$ or $^{238}\text{Pu}(\text{NO}_3)_4$ with Similar Lung Depositions

INTERACTIVE STORAGE AND RETRIEVAL OF PATHOLOGIST'S OBSERVATIONS

Investigators:

G. M. Trauger, G. E. Dagle, C. R. Watson and J. M. McIntyre

Technical Assistance: J. Meheen

A system was developed for interactive storage and retrieval of pathologic observations. Data are entered on a CRT terminal using modified SNOP (Systematized Nomenclature Of Pathology) codes. They are then stored and processed in a PDP 11/70 computer, and are retrieved on demand, utilizing DATATRIEVE^(a) and a variety of reports convenient for statistical analysis.

We have developed a series of COBOL computer programs to aid in the summary and statistical analysis of histopathologic observations of research animals. By using an interactive system of storage and retrieval we have greatly reduced the time needed for evaluation of histopathologic data.

Observations of each tissue are entered in the Biometrics PDP 11/70 computer by an interactive program which performs elementary error checking. In addition to the typical animal identification and other biological information, numerical SNOP topography and morphology codes are entered. The verbal interpretations of the codes are immediately displayed to ensure proper code selection and entry. The translation dictionary contains SNOP codes frequently used for PNL studies; the pathologist may add other codes as needed. Also, the SNOP coding scheme has been enhanced to allow for severity grading of lesions. A proofreading report may be produced at any time. If errors are detected, the data is edited, using DATATRIEVE, an interactive query

language. DATATRIEVE is also used for ad hoc retrievals for any combination of tissues and/or lesions.

To summarize an experiment, several tabular reports are available, including:

- 1) listings of all lesions for an animal, with detailed background information;
- 2) listing of all animals with lesions in specified locations;
- 3) incidence listing of the number of animals within treatment groups having specific lesions, and the average grade of the lesion;
- 4) listing of all lesions observed for an animal or group of animals.

Future efforts will be directed toward coordinating this information with radio-analysis data for correlation of dose with biological effect. This will be especially important for life-span observations on relatively long-lived animals, e.g., dogs.

(a) DATATRIEVE is a product of Digital Equipment Corp., Maynard, MA

● Inhaled Transuranics in Rodents

The purpose of this project is to examine, in small animals, the fate and effects of inhaled transuranic compounds. This project should provide data required for hazard evaluation and establishment of permissible exposure limits to man for inhaled transuranics, particularly with respect to radiation dose and dose-distribution.

EFFECT OF VITAMIN C ON PULMONARY CARCINOGENESIS FROM INHALED $^{239}\text{PuO}_2$

Investigators:

C. L. Sanders and A. W. Conklin

Technical Assistance: C. White

Vitamin C is being tested as a possible agent to minimize lung cancer induction from inhaled $^{239}\text{PuO}_2$.

The objective of this study is to examine, in the rat, the effect of continuous vitamin C uptake on the development of pulmonary tumors following inhalation of $^{239}\text{PuO}_2$. Therapy with vitamin C may inhibit the tumorigenic action of ^{239}Pu , and may be particularly useful in decreasing cancer risk to large exposed populations.

Seventy-day-old, Wistar, female rats were exposed to an aerosol of high-fired $^{239}\text{PuO}_2$. A total of 101 rats, exposed to PuO_2 in three groups of 33-34 rats, were divided randomly into four experimental groups: 1) 30 rats given only $^{239}\text{PuO}_2$, 2) 31 rats given $^{239}\text{PuO}_2$ + 30 mg vitamin C/day (initially), 3) 31 rats given $^{239}\text{PuO}_2$ + 300 mg vitamin C/day (initially), 4) 35 rats given 300 mg vitamin C/day (initially). Three rats from each ^{239}Pu exposure group were killed 24 hr after exposure and ^{239}Pu lung contents were determined. (Radioanalyses are incomplete.) Initial alveolar depositions

are estimated at 50-100 nCi ^{239}Pu . The AMAD of PuO_2 aerosols was 1.67 μm ; GSD, 1.81. Aerosols were all log-normal particle size distributions.

Vitamin C (Bronson Pharmaceuticals) was 50% sodium ascorbate and 50% ascorbic acid. Mean water intake at start of vitamin C treatment (2 wk after $^{239}\text{PuO}_2$ exposure) was 30 ml/day. Vitamin C was dissolved in drinking water at doses of 1 mg/ml or 10 mg/ml; thus, initial vitamin C uptake averaged 30 mg and 300 mg/day, respectively. Water uptake increased in all groups to about 43 ml/day; vitamin C uptake increased proportionately. No significant difference in water uptake was seen between the treatment groups in these older rats.

Water uptake and body weights were measured monthly. Routine radioanalytical and histopathological procedures will be carried out on dead or moribund rats.

DISTRIBUTION OF INHALED $^{239}\text{PuO}_2$ IN RAT AND HAMSTER LUNG

Investigators:

K. Rhoads, J. A. Mahaffey and C. L. Sanders

Technical Assistance:

D. Moon and J. Jech

Differences were observed in the spatial-temporal distribution of inhaled $^{239}\text{PuO}_2$ in the lung which may contribute to differences in lung tumor response between rats and hamsters.

Previous research has indicated that the distribution of inhaled $^{239}\text{PuO}_2$ is not uniform throughout the lung, and that it changes with time. This study quantitates the distribution of plutonium particles within the lung during the first year after exposure.

Photographic montages of lung cross-sections for rats and hamsters were prepared, and the $^{239}\text{PuO}_2$ particle distribution was analyzed with the aid of morphometric techniques. The lung was divided into 6 major regions by tissue type for this study (Annual Report, 1976).

The percent retention of particles in each lung region is defined as: $100 \times (\text{particles per unit area at } >120 \text{ days after exposure} \div \text{particles per unit area at } <90 \text{ days after exposure})$. A retention value of 100% implies no clearance of particles from a region, whereas a retention of 0 indicates complete clearance.

As shown in Table 11, rats and hamsters had retention values of 32% and 33%, respectively, for the entire lung; retention for the subpleural (SP) region was 47% and 48%; that for parenchymal tissue (P) was

TABLE 11. Retention of Inhaled $^{239}\text{PuO}_2$ Particles in Rat and Hamster Lung

	Retention, % ^(a)		Statistical Analysis ^(b)		
	Rat, n = 24	Hamster, n = 20	Rat vs. 100%	Hamster vs. 100%	Rat vs. Hamster
Subpleural	47 ± 55	48 ± 66	**	**	NS
Parenchymal	23 ± 34	26 ± 40	***	***	NS
Bronchial-Bronchiolar	14 ± 26	0	***		*
Peribronchial-Bronchiolar	228 ± 434	16 ± 45	NS	***	*
Perivascular	406 ± 1122	0	NS		NS
Total Lung	32 ± 43	33 ± 48	***	***	NS

(a) $\text{Retention} = \frac{\text{Particle Concentration at } > 120 \text{ days}}{\text{Particle Concentration at } < 90 \text{ days}} \times 100$

Values are mean ± standard deviation

(b) Statistical Analysis

NS Not Significant

* Retention in rat or hamster is different from 100%, or rat is different from hamster with $p \leq 0.05$

** $p \leq 0.01$

*** $p \leq 0.001$

23% and 26%; and retention in bronchial-bronchiolar tissue (B) was 14% and 0. No particles were found in vascular lumen at any time. Two lung regions in rats had retention values greater than 100%, indicating an increase in particles per unit area. The value for perivascular (PV) was 406% and peribronchial-bronchiolar (PB) was 228%, whereas in hamsters, PV was 0 and PB was 16%; neither PV nor PB in rats was significantly greater than 100%; PB in hamsters was significantly less than 100%. When the two species were compared, rats retained a significantly larger share of particles associated with major airways than hamsters.

A further refinement of the particle distribution data led to definition of the relative particle concentration (RPC = particle concentration in a given region ÷ particle concentration for total lung). When the particle concentration is normalized in this way, a completely uniform particle distribution yields a value of 1 for all components. Therefore, values greater or less than 1 provide a measure of dose in each region according to particle concentration.

The RPC was subjected to linear regression analysis, with the results shown in Tables 12 and 13. The regression coefficient, β_0 , represents RPC at the time of exposure, and the coefficient β_1 indicates the rate of change in RPC over time (Table 12). The β_0 values in rats were greater than 1 in the P

region and less than 1 in B, PB and PV. In hamsters, the initial particle distribution was more uniform, with only the β_0 for PV significantly less than 1. In rats, β_1 showed a significant decrease in RPC over time for P and an increase for PB. None of the other regions in rats or hamsters showed a significant temporal trend. A significant negative correlation was found when the temporal trends in SP and P for rats were compared (Table 13).

The observation that, in rats and dogs, tumors arise preferentially in subpleural regions containing accumulations of $^{239}\text{PuO}_2$ particles prompted a study of particle aggregation. The photomontages utilized in the previous work were divided into segments measuring $1 \times 2 \text{ cm}^2$ (0.22 mm^2 in fixed lung) for the subpleural and internal (P, PB, PV, B, and V combined) regions. The number of particles in each segment was recorded, and a histogram of the results was prepared (Figure 15). Rats had a smaller proportion of empty segments than hamsters in the subpleural region, but they had a greater number of segments containing 2, 3, 4 or more than 10 particles. The internal region showed a species difference only in the 2-particles-per-segment category.

The subpleural and internal particle distributions in rats, as a group, differed from the Poisson distribution ($p < 0.001$), whereas those in hamsters did not. The particles in rat lung showed a greater tendency to aggregate into groups of two or

TABLE 12. Linear Regression Analysis for Relative Particle Concentration in Rat and Hamster Lung Following Inhalation of $^{239}\text{PuO}_2$. Linear regression coefficients are for RPC as a function of time. (a)

Lung Region	Rats (b)			Hamsters (b)		
	$\hat{\beta}_0 \pm S_{\beta_0}$	$\hat{\beta}_1 \pm S_{\beta_1}$	r	$\hat{\beta}_0 \pm S_{\beta_0}$	$\hat{\beta}_1 \pm S_{\beta_1}$	r
Subpleural (SP)	1.17 ± 0.14	0.0014 ± 0.00071	0.38	0.85 ± 0.21	0.0014 ± 0.0014	0.21
Parenchymal (P)	$1.17 \pm 0.066^*$	$-0.00099 \pm 0.00034^{**}$	-0.53	1.06 ± 0.15	-0.0020 ± 0.0010	-0.38
Bronchial-Bronchiolar (B)			-0.025 ^(c)	1.08 ± 0.73	-0.0043 ± 0.0047	-0.18
Peribronchial (PB)	$-0.15 \pm 0.47^*$	$0.00050 \pm 0.0024^*$	0.41	0.50 ± 0.30	-0.0012 ± 0.0020	-0.12
Perivascular (PV)	$0.090 \pm 0.23^{**}$	0.0015 ± 0.0012	0.27	$0.19 \pm 0.13^{***}$	-0.00078 ± 0.00085	-0.19

(a) Values are mean $\pm$ standard error of the mean

(b) Statistical Analyses

* β_0 is different from unity, or β_1 is different from zero with $p \leq 0.05$

** $p \leq 0.01$

*** $p \leq 0.001$

(c) Regression coefficients not reported because low r value indicates a poor fit

more, and the large groups of particles found in subpleural rat lung were absent in hamsters.

Both the RPC and retention data indicate that the initial distribution of $^{239}\text{PuO}_2$ particles in lung is not uniform, and that particle distribution shifts with time from the parenchymal region to the subpleural

and peribronchial. This difference is less pronounced in hamsters than in rats, where it was statistically significant. There were also species differences in the aggregation of particles, with particles occurring in groups more frequently for rats than for hamsters, particularly in the subpleural region.

TABLE 13. Comparison of Temporal Trends for Relative Particle Concentration in Regions of Rat and Hamster Lung Following Inhalation of $^{239}\text{PuO}_2$

Correlation Between	Correlation Coefficient	
	Rats	Hamsters
SP, P	-0.85(a)	0.37
SP, B	0.055	0.15
SP, PB	-0.15	0.051
SP, PV	-0.021	0.059
P, B	-0.24	-0.079
P, PB	-0.21	0.14
P, PV	-0.12	0.10
B, PB	-0.0023	0.096
B, PV	-0.17	-0.059
PB, PV	0.063	-0.081

(a) Correlation coefficient differs from 0 with $p \leq 0.001$.

FIGURE 15. Group Size Distribution of $^{239}\text{PuO}_2$ Particles in Rodent lung

QUANTITATIVE STUDY OF PULMONARY LESIONS AND EPITHELIAL
PROLIFERATION FOLLOWING INHALATION OF $^{239}\text{PuO}_2$ IN RATS

Investigators:

K. Rhoads, R. R. Adee and C. L. Sanders

Technical Assistance:

S. Moe and J. Jech

Morphometric analyses of lung parenchyma exposed to $^{239}\text{PuO}_2$ indicated a volumetric increase in pulmonary fibrosis, epithelial metaplasia, and neoplasia with increasing time after exposure. Metaplastic and neoplastic lesions usually occupied less than 1% of the total lung volume.

Inhaled $^{239}\text{PuO}_2$ induces pathological sequelae characterized by radiation pneumonitis, fibrosis and epithelial metaplasia leading to neoplasia. This study attempted to quantitatively measure the development of these pulmonary lesions. In addition, cell turnover of normal and abnormal pulmonary epithelium was investigated, using ^3H -thymidine and autoradiography.

Thirty-two rats were given a single exposure to $^{239}\text{PuO}_2$ aerosol and killed at 250-530 days after exposure; each rat was also given an intraperitoneal injection of 1.5 nCi ^3H -thymidine about 2 hr prior to necropsy. Lungs fixed with glutaraldehyde-formaldehyde at 20 cm H_2O were embedded in methacrylate, and 0.5- to 1.0-mm-thick sections were cut for morphometric analysis and autoradiography using Ilford K-5 emulsion. The terminal lung burden in all groups averaged 1.5-41 nCi. Lung sections were analyzed by point-counting morphometric methods for relative volume of normal tissue, pneumonitis with or without fibrosis, epithelial metaplasia, and lung tumors.

The volume of normal lung tissue decreased from 100% for controls to about 85% for $^{239}\text{PuO}_2$ -exposed rats at 530 days after exposure (Table 14). Nearly all abnormal lung tissue was characterized as fibrotic, due to radiation damage from ^{239}Pu . Epithelial metaplasia ranged from 0.026% at 257 days to 0.42% at 530 days; metaplasia was not found in control lungs. Epithelial tumors (adenocarcinoma and squamous-cell carcinoma) also increased in volume with increasing time after exposure, up to an average of 1.35% at 530 days. Linear regression analyses indicated a statistically significant decrease in normal lung tissue, and a significant increase in pulmonary fibrosis, epithelial metaplasia, and epithelial tumor volume.

Visual inspection of autoradiograms indicates a much higher labeling of metaplastic and neoplastic cell nuclei with ^3H -thymidine than for normal lung epithelial cells, indicating an increased cell turnover rate for epithelial lesions (Figure 16). Labeling index studies are continuing.

TABLE 14. Relative Volumes of Normal and Pathologic Tissue in Rat Lung Following Inhalation of $^{239}\text{PuO}_2$

Days After Exposure to $^{239}\text{PuO}_2$	Number of Rats	Amount of ^{239}Pu in Lung at Death	% of Lung Volume ^(a)			
			Normal	Fibrosis	Epithelial Metaplasia	Epithelial Tumor
Unexposed Control						
468	2	0	100	0	0	0
530	2	0	100	0	0	0
Exposed						
257	8	32 ± 14	98 ± 1.0	1.8 ± 1.1	0.026 ± 0.010	0
319	8	31 ± 10	97 ± 1.6	2.9 ± 1.6	0.15 ± 0.056	0.069 ± 0.069
375	5	11 ± 2.6	94 ± 2.4	3.3 ± 1.5	0.19 ± 0.094	0.18 ± 0.18
412	5	18 ± 5.6	92 ± 3.7	7.8 ± 3.7	0.094 ± 0.038	0
468	3	41 ± 8.6	96 ± 1.4	3.1 ± 1.2	0.25 ± 0.16	0.36 ± 0.36
530	3	1.5 ± 0.64	84 ± 3.3	12 ± 3.4	0.42 ± 0.24	1.35 ± 1.20

(a) Values are mean ± standard error of the mean

FIGURE 16. Autoradiographs of Epithelial Metaplasias and Neoplasias Following Exposure to a $^{239}\text{PuO}_2$ Aerosol. Rats were given $1.5 \mu\text{Ci } ^3\text{H-thymidine/g}$ body weight 2 hr before autopsy. Exposure time of autoradiographs, 2 wk. Note location of $^3\text{HTdR}$ -labeled cells. All at 220X magnification.

- A. Adenomatosis, 375 days postexposure; terminal lung burden, $9.2 \text{ nCi } ^{239}\text{Pu}$.
- B. Adenocarcinoma, 375 days postexposure; terminal lung burden, $12 \text{ nCi } ^{239}\text{Pu}$.
- C. Squamous cell metaplasia; same lung as A.
- D. Squamous cell carcinoma, 319 days postexposure; terminal lung burden $37 \text{ nCi } ^{239}\text{Pu}$.

TRANSPLANTATION OF HAMSTER LUNG LESIONS INDUCED BY $^{239}\text{PuO}_2$ OR BENZ(a)PYRENE

Investigators:

K. E. McDonald and C. L. Sanders

None (0%) of 1000 recipients of lung lesions from $^{239}\text{PuO}_2$ -exposed hamsters that were transplanted into other hamsters' cheek pouches, developed tumors, whereas 90% of transplants from benz(a)pyrene-induced lung lesions were malignant.

The incidence of lung tumors in Syrian Golden hamsters given intratracheal ^{210}Po has been reported as high as 75%, while the lung tumor incidence observed in hamsters following inhalation of comparable doses of $^{238}\text{PuO}_2$ or $^{239}\text{PuO}_2$ was <5%. One criterion of malignant transformation is transplantability. The purpose of this investigation was to determine whether lesions induced by inhaled $^{239}\text{PuO}_2$ in the hamster lung are truly malignant or merely metaplastic.

One hundred young, female, Syrian Golden hamsters were exposed, nose-only, to an aerosol of $^{239}\text{PuO}_2$; initial alveolar depositions ranged from 12 nCi to 150 nCi ^{239}Pu . At monthly or bimonthly intervals, starting at 5 mo after inhalation of $^{239}\text{PuO}_2$, groups of 10 hamsters were killed. The most severe (from gross appearance) lung lesions were removed, bisected and one portion was transplanted into the cheek pouch of an unexposed recipient hamster. The other portion was embedded in paraffin for histopathological examination (Annual Report, 1978). A control group of 10 hamsters was given serial intratracheal instillations of benz(a)pyrene (BaP) + Fe_2O_3 . Grossly obvious BaP-induced lung tumors were transplanted into the cheek pouches of unexposed hamsters. Lung lesions were tested and examined up to 13 mo following initial exposure. The degree of epithelialization and adenomatosis was determined for the original sample transplanted and for the tissue removed after 3 wk growth within the cheek pouch.

A total of 1000 recipient transplants from 100 donor hamsters following exposure to $^{239}\text{PuO}_2$, and 10 recipient transplants from two lung-tumor-bearing hamsters following BaP instillation, were histopathologically evaluated. The most severe lesions in

but focal regions of adenomatosis; some of these lesions exhibited epithelialization following transplantation (Figures 17 and 18). BaP-induced lung tumors were of the squamous-cell type, and grew up to 1-2 cm in diameter within the recipient transplants. Histologically, transplanted BaP-induced tumors were similar to those seen in donor lungs (Figures 19 and 20).

The degree of epithelialization and adenomatosis was greatest in BaP + Fe_2O_3 hamster lungs, followed by Fe_2O_3 alone, then by $^{239}\text{PuO}_2$; few such lesions were seen in control hamsters (Table 15). None (0%) of the 1000 transplants from 100 donor hamsters exposed to $^{239}\text{PuO}_2$ developed malignant transformations; all of these transplants exhibited some degree of necrosis, calcification and replacement of epithelial tissue with connective tissue at 3 wk. In contrast, 9 of 10 (90%) BaP tumor transplants developed large tumor growths in hamster cheek pouches. None of the Fe_2O_3 or unexposed control lung transplants developed malignant transformations.

An earlier study with inhaled PuO_2 failed to demonstrate the induction of primary lung tumors in more than a few hamsters. However, there was a dose-dependent increase in adenomatosis which might represent a premalignant condition. However, our transplant studies have failed to demonstrate any malignant potential for these lesions. We therefore conclude that, based on the criteria of histopathology and transplantability, inhaled plutonium dioxide induces few primary lung tumors in the hamster at any radiation dose. The hamster is therefore a poor animal model for the study of pulmonary carcinogenesis following inhalation of alpha-emitting radionuclides.

FIGURE 17. Lung Tumor Extending into Pleural Cavity Following Intratracheal Instillation of Benzo (a) pyrene + Fe_2O_3 . 40X

FIGURE 18. Transplanted Lung Tumor (from Figure 17) in Hamster Cheek Pouch. Tumor was about 1 cm in diameter at 3 wk after transplantation. 160X

FIGURE 19. Hamster Lung Exposed to $^{239}\text{PuO}_2$ Showing Bronchiolization and Adenomatous Metaplasia. 160X

FIGURE 20. Transplant from Hamster Lung exposed to $^{239}\text{PuO}_2$ Showing Bronchiolization at 3 wk after Transplantation. This is an example of the most pronounced epithelial proliferation seen in any transplant lung tissues from $^{239}\text{PuO}_2$ -exposed hamsters. 160X

TABLE 15. Degree of Adenomatosis in Donor Lungs and Epithelialization in Hamster Lung Transplants (at 3 wk After Transplantation)

Treatment	Mean ^(a) $\pm$ SD, Epithelial Proliferation	
	Donor Lung (N)	Transplant Lung (N)
Unexposed Controls	0.72 $\pm$ 0.31 (10)	0.71 $\pm$ 0.30 (100)
$^{239}\text{PuO}_2$	1.26 $\pm$ 0.38 (100)	0.81 $\pm$ 0.30 (1000)

(a) Grading system:
 1 - very slight
 2 - slight
 3 - moderate
 4 - marked
 5 - extreme

FATE OF PULMONARY DEPOSITED $^{237,239}\text{PuO}_2$

Investigators:

C. L. Sanders and A. W. Conklin

A ^{237}Pu tag is being tested as a means of rapid analysis for $^{239}\text{PuO}_2$.

Previous studies with inhaled $^{239}\text{PuO}_2$ in rats have demonstrated an increasing incidence of lung tumors with increasing pulmonary dose. Statistical analysis of lung tumor data in rats following inhalation of plutonium compounds predicts a lung tumor incidence of 1.5 rad, two to four times the spontaneous lung tumor incidence (0.14%). Further studies at lower doses are required to determine the accuracy of this prediction.

One problem in performing a life-span study with inhaled $^{239}\text{PuO}_2$ at low doses is the determination of initial ^{239}Pu depositions in individual rats. To accomplish this we propose to tag $^{239}\text{PuO}_2$ particles with ^{237}Pu . This radionuclide, a strong gamma emitter, has a 45-day half-life, which permits whole-body counting. This study was designed to determine whether ^{237}Pu remains with the $^{239}\text{PuO}_2$ particle or is leached from the PuO_2 particle and cleared from the lung at a different rate from that of ^{239}Pu .

Solutions of ^{237}Pu and ^{239}Pu nitrate were mixed and converted to Pu oxalate by treatment with ascorbic acid/hydrazine and oxalic acid solutions. The Pu oxalate preparation was fired for 2 hr at 750°C to convert it into PuO_2 . The PuO_2 particles were suspended in distilled water. Each of 50 female, young adult Wistar rats was given an intratracheal instillation of $^{237,239}\text{PuO}_2$ in 1 ml water. Groups of five rats will be killed at 1, 7, 14, 28, 42, 56, 84, 112 and 140 days after instillation and tissues taken for ^{237}Pu and ^{239}Pu analyses.

Analyses for ^{237}Pu , rapidly accomplished by gamma-ray counting, have been completed only to 28 days after instillation (Table 16); ^{239}Pu analyses are incomplete. Initial data indicate a ^{237}Pu distribution similar to what might be expected for ^{239}Pu .

TABLE 16. Distribution of Intratracheally Instilled $^{237,239}\text{PuO}_2$ in Rat Tissue. Values are $\bar{X} \pm \text{S.E.}$ (n = 5).

Time Postexposure:	1 Day	1 Week	2 Weeks	4 Weeks
Lung	83.7 ± 14.8	88.7 ± 13.9	68.3 ± 20.4	74.2 ± 8.4
Liver	Background	0.4 ± 0.2	0.4 ± 0.1	0.3 ± 0.01
Kidney	Background	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.04
Thoracic Lymph Nodes	1.5 ± 1.4	0.8 ± 0.3	1.2 ± 0.6	2.9 ± 1.2
G.I.	5.3 ± 1.7	0.8 ± 0.1	0.5 ± 0.04	0.4 ± 0.1
Carcass	3.2 ± 1.4	1.1 ± 1.5	0.7 ± 0.5	0.5 ± 0.2
Urine & Feces	--	--	--	$15.9 \pm 8.2^{(a)}$

^(a)Cumulative Excreta Collected for 4 wk.

INFLUENCE OF INHALED CADMIUM OXIDE ON ALVEOLAR CLEARANCE OF PuO_2 PARTICLES

Investigators:

C. L. Sanders and A. W. Conklin

Cadmium oxide inhaled 24 hr or 1 wk previously, reduced the alveolar clearance rate of inhaled $^{239}\text{PuO}_2$ particles.

A previous study demonstrated the impairment of alveolar macrophage function by phagocytized beryllium oxide particles. In that study, the alveolar clearance of $^{239}\text{PuO}_2$ was decreased to 60% of the normal rate when $^{239}\text{PuO}_2$ was inhaled at 1, 30 or 60 days after inhalation of beryllium oxide (Sanders, et al., Arch. Environ. Health 30: 546, 1975). Cadmium, a highly toxic metal, is present in effluents of fossil fuel combustion. Lung-deposited cadmium oxide, (in contrast to highly insoluble beryllium oxide) is very soluble in the lung and accumulates in liver and kidney (Annual Report, 1978). Inhaled cadmium oxide causes pulmonary edema due to membrane damage of Type I alveolar epithelium (Annual Report, 1977). Our objective in this study was to examine the influence of cadmium oxide on the alveolar clearance of a highly insoluble test particle, $^{239}\text{PuO}_2$.

Five groups of five rats each were exposed to $^{239}\text{PuO}_2$ (calcined at 750°C for 2 hr) and/or cadmium oxide. The metal oxides were given to young adult, female, Wistar rats in 1.0 ml 0.9% NaCl by intratracheal instillation; amounts instilled were 20 μg cadmium oxide (MMD, 1-2 μm) and/or 10 nCi $^{239}\text{PuO}_2$ (AMAD, 2 μm). The five treatments were as follows:

- 1) $^{239}\text{PuO}_2$ given alone,
- 2) $^{239}\text{PuO}_2$ given simultaneously with CdO,
- 3) CdO given 24 hr prior to $^{239}\text{PuO}_2$,
- 4) CdO given 24 hr after $^{239}\text{PuO}_2$, and
- 5) CdO given 1 wk prior to $^{239}\text{PuO}_2$.

Rats were placed in individual metabolism cages and excreta were collected for 30 days. The sum of ^{239}Pu in excreta and whole body at 30 days after instillation was defined as the instilled ^{239}Pu dose.

About 60% of instilled ^{239}Pu remained in the lung 30 days after instillation. Previous studies have shown that the majority

of the 40% cleared is phagocytized by alveolar macrophages prior to clearance from the ciliated bronchiolar regions. Instillation of CdO, either 24 hr after $^{239}\text{PuO}_2$ or simultaneously with $^{239}\text{PuO}_2$, did not significantly alter the alveolar clearance of ^{239}Pu . However, instillation of $^{239}\text{PuO}_2$ 24 hr or 1 wk after CdO instillation significantly reduced the alveolar clearance of ^{239}Pu to about half that seen without CdO treatment (Table 17).

TABLE 17. Influence of Cadmium Oxide on the Alveolar Clearance of Intratracheally instilled $^{239}\text{PuO}_2$. Each value ($\pm$ S.D.) is a mean of values for five rats.

Treatment	% of Instilled ^{239}Pu in Lung 30 Days After Instillation
$^{239}\text{Pu}_2$ Only	60 ± 9.1
$^{239}\text{Pu}_2$ CdO Simultaneously	54 ± 6.6
CdO 24 hr After $^{239}\text{PuO}_2$	56 ± 21
CdO 24 hr Before $^{239}\text{PuO}_2$	78 ± 6.3
CdO 1 wk Before $^{239}\text{PuO}_2$	81 ± 4.4

It is theorized that cadmium ions interfered with the phagocytosis of $^{239}\text{PuO}_2$ particles by alveolar macrophages (thus reducing macrophage-related clearance) since simultaneous administration of CdO with PuO_2 did not decrease clearance. Presumably, with simultaneous instillations of both metals, PuO_2 phagocytosis was largely completed before sufficient CdO solubilization occurred to depress phagocytosis.

The study is being repeated with Fischer rats. Similar studies are planned with other toxic metals, such as vanadium and antimony.

CARCINOGENIC EFFECT OF HIGH-FIRED $^{241}\text{AmO}_2$

Investigator: C. L. Sanders

Technical Assistance:

C. White and S. I. Moe

Inhaled $^{241}\text{AmO}_2$ was cleared from the lung with component half-lives of 0.5 days, 6.8 days and several hundred days; histopathology studies are continuing.

Previous studies have considered the metabolism and carcinogenicity of inhaled, high-fired $^{238}\text{PuO}_2$, $^{239}\text{PuO}_2$ and $^{244}\text{CmO}_2$. Americium-241 is produced in nuclear fuels by beta decay of ^{241}Pu . Like $^{244}\text{CmO}_2$, $^{241}\text{AmO}_2$ is much more soluble in tissues than is $^{238}\text{PuO}_2$.

Female Wistar rats were exposed to high-fired (700°C, 4 hr calcination of the oxalate) $^{241}\text{AmO}_2$ (AMAD, $0.92 \pm 0.29 \mu\text{m}$; CMD, $0.02 \pm 0.04 \mu\text{m}$; particle size distribution was not log normal). A total of 283 rats, divided into six dose groups, were held for life-span observation. Initial alveolar depositions (IAD) were approximately 0, 0.1 nCi, 1.0 nCi, 10 nCi, 100 nCi and 1000 nCi.

A preliminary lung clearance curve was fitted to available ^{241}Am data. The ^{241}Am lung clearance curve was somewhat similar to that seen with $^{244}\text{CmO}_2$. A three-compartment model provided the best fit to the data. Percentage of clearance was 54 ± 14 with a half-life of 0.5 ± 0.3 days, 37 ± 13 with a half-life of 6.8 ± 2.9 days; and 8.2 ± 1.9 with a half-life of several hundred days. About 8% of the ^{241}Am IAD was present in bone at 70 days postexposure, 6% in lung and 1% in liver.

About 80% of the life-span rats have been autopsied and examined for pathological lesions (Table 18). No lung, bone or liver tumors have been observed in rats given an

TABLE 18. Number of Tumors in Rats After Inhalation of $^{241}\text{AmO}_2$.

Estimated Initial Alveolar Deposition, nCi ^{241}Am	Number of Rats		Number of Tumors		
	Completed Histology	Exposed	Lung	Liver	Bone
0	18	20 ^(a)	0	0	0
0.1	50	59	0	0	0
1.0	46	59	0	0	0
10	44	57	0	0	0
100	40	58	7	1	0
1000	28	30	8	1	0

(a) An additional 140 common controls are used in the analyses
No tumors have been seen in these rats.

IAD of 0, 0.1 nCi, 1.0 nCi or 10 nCi ^{241}Am . Fifteen lung tumors and two liver tumors have been found in rats with an IAD of 100 or 1000 nCi ^{241}Am . Unexpectedly, no bone tumors have been seen, to date, in any rats given $^{241}\text{AmO}_2$. In contrast, at 374 to 760 days after inhalation, 12 bone tumors were found in rats with similar IADs of $^{244}\text{CmO}_2$. However, histopathology is incomplete for this study.

A short-term study of bone (femur) autoradiography is being carried out. Wistar rats were given inhaled $^{241}\text{AmO}_2$ and bone sections are being examined for alpha activity at intervals of 1 day to 7 wk after inhalation. Autoradiographic exposure time is 6 mo. Mean amounts of ^{241}Am in lung and femur are shown in Table 19.

TABLE 19. Amount of ^{241}Am (nCi) in Lung and Femur Following Inhalation of $^{241}\text{AmO}_2$. Values are means $\pm$ S.D. (n = 3)

Time After Inhalation, Days	Lung	Femur
1	1700 $\pm$ 330	3.9 $\pm$ 3.6
7-8	820 $\pm$ 420	5.2 $\pm$ 2.3
14-15	560 $\pm$ 190	18 $\pm$ 3.3
21-22	300 $\pm$ 110	17 $\pm$ 5.9
32-35	320 $\pm$ 90	21 $\pm$ 3.1
46-49	200 $\pm$ 30	17 $\pm$ 2.6

INHALATION TOXICOLOGY OF $^{241}\text{Am}(\text{NO}_3)_3$

Investigators:

J. E. Ballou, R. A. Gies and A. H. Beasley

Inhaled $^{241}\text{Am}(\text{NO}_3)_3$ was rapidly cleared from the lung (90% in 30 days) and translocated principally to skeleton. Although the estimated radiation dose to lung was twofold greater than the bone dose, the principal treatment-related lesion appeared to be osteosarcoma of the skeleton.

Results of preliminary rangefinding studies and the experimental protocol for a study of the inhalation toxicology of $^{241}\text{Am}(\text{NO}_3)_3$ in rats were reported in the Annual Report for 1977. The present report is an update of the inhalation toxicology study, which is still in progress approximately 700 days after exposure.

Male Wistar rats, 60 days of age, were administered a single, nose-only exposure to $^{241}\text{Am}(\text{NO}_3)_3$ aerosols using a Lovelace nebulizer charged with 0.27N nitric acid solutions of the radionuclide. Ten rats were sacrificed immediately after the 30-min exposure to determine the initial lung burden, and five rats per time point were sacrificed after 7, 30, 60, 100, 150 and 200 days to determine distribution and retention kinetics for dosimetric purposes. Other rats were held for lifespan study of long-term biological effects. Tissues from the long-term rats were analyzed for ^{241}Am content at the time of death, and these data were added to the serial sacrifice results to describe tissue retention throughout the total time course of the study. Long-term animals were necropsied and tissues were examined for histopathological changes at the time of death.

Two groups of rats were exposed to $^{241}\text{Am}(\text{NO}_3)_3$ aerosols as summarized in Table 1. The total amount deposited and the initial lung burden differed by approximately an order of magnitude between the two exposure groups. The early distribution of ^{241}Am and the translocation from the lung did not appear to be influenced by the amount deposited over this range of deposition. The percentage of the total ^{241}Am

deposited that was found in the lung immediately after exposure (25-30%) is compatible with the mass of material deposited and the particle size distribution of these aerosols.

TABLE 20. Initial Deposition and Distribution of Inhaled $^{241}\text{Am}(\text{NO}_3)_3$ Aerosols

	Low-Dose	High-Dose
Total Deposition, nCi	27	381
Initial Lung Burden, nCi	8.2	95.3
Aerosol Characteristics		
AMAD, μm	0.25	0.35
GSD	1.97	2.01
Initial Deposition, % Total Deposition		
Upper Respiratory Tract	9	18
Liver	0.13	0.1
Residual Carcass	1.6	1.2
Lung	30	25
Pelt	60	56

Weight was not influenced significantly by the various ^{241}Am exposures (Figure 21); however, lifespan appeared to be shortened somewhat by the highest ^{241}Am lung burden (Figure 22). There appeared to be no difference in gross mortality between controls and rats in the 8.2-nCi ILB group.

Inhaled $^{241}\text{Am}(\text{NO}_3)_3$ was excreted primarily in the feces during the first week after

FIGURE 21. Weight of Rats Exposed to Inhaled $^{241}\text{Am}(\text{NO}_3)_3$

FIGURE 22. Cumulative Mortality for Rats Exposed to Inhaled $^{241}\text{Am}(\text{NO}_3)_3$

exposure (Figure 23). This is typical of inhaled materials that are cleared via mucociliary processes. The amount of ^{241}Am excreted daily in feces was generally an order of magnitude or more greater than the amount excreted in urine. This may be indicative of the biliary excretion of ^{241}Am reported by others. After 7 days, daily urinary excretion amounted to approximately 1%, and fecal excretion was 6% of ILB.

Tissue retention of inhaled $^{241}\text{Am}(\text{NO}_3)_3$ was determined for only the high-level (95.3 nCi ILB) exposure group, for reasons of economics and because earlier range-finding studies had shown similar tissue retention kinetics in rats with ILB ranging from 52 to 95 nCi (Annual Report, 1977). Retention in major tissues is illustrated in Figure 24.

FIGURE 23. Early Excretion of Inhaled $^{241}\text{Am}(\text{NO}_3)_3$

The major reservoir of translocated ^{241}Am was the skeleton, which retained an amount equivalent to approximately 25% of the ILB. Americium-241 appeared to be retained in skeleton with a long half-life, but the limited data available do not permit an accurate estimation of the magnitude of this retention parameter.

Retention in lung and liver could be described by the sum of three exponential functions with half-lives of 5-10 days, 30-60 days and <1000 days, respectively. After 400 days, about 3% and 4% of an ILB remained in liver and lung, respectively, compared to about 25% in the skeleton.

FIGURE 24. Tissue Retention of Inhaled $^{241}\text{Am}(\text{NO}_3)_3$

Cumulative radiation dose for the major tissues was calculated based on the retention kinetics and amounts of ^{241}Am associated with the various retention components. The estimated dose accumulated over 500 days, and the tumor incidence based on gross observations, are shown in Table 21. It is noteworthy that the major tumorigenic effect of $^{241}\text{Am}(\text{NO}_3)_3$ inhalation appears to be the induction of bone tumors rather than the lung tumors we have seen with other inhaled actinide nitrates. This may be due to the rapid translocation of ^{241}Am from the lung to bone and the relatively large bone dose compared to that sustained by the lung. The lung dose is well within the tumorigenic range; however, approximately 85% of the

dose was delivered within the first 30 days after exposure. This dose rate may be less efficient than the more prolonged radiation insult seen with less rapidly cleared actinides such as plutonium and uranium nitrates. The dose rate to bone appeared to be relatively constant for the 400 days for which we have analytical data.

It is also of interest that two bone tumors have been observed in the control rats that were a part of this study. We have observed similar lesions in control groups and in treated control groups in other studies. This incidence apparently reflects the spontaneous tumors found in male Wistar rats supplied by our vendor.

TABLE 21. Estimated Radiation Dose and Tumor Incidence for Rats Exposed to $^{241}\text{Am}(\text{NO}_3)_3$ Aerosols

Initial Lung Burden, nCi	Number of Rats	500-Day Cumulative Radiation Dose, rad			Observed Tumor Incidence ^(a)		
		Lung	Liver	Skeleton	Kidney	Lung	Skeleton
8.2	58	20	0.7	9	0.8	0/45	1/45
95.3	60	225	8	100	9	3/58	12/58
Control	100	--	--	--	--	0/86	2/86

^(a) Tumor diagnoses were based on radiographs and gross pathology observations. The first number refers to the number of tumors and the second refers to the number of rats examined.

INHALATION TOXICOLOGY OF REPEATED EXPOSURES TO $^{244}\text{CmO}_2$

Investigators:

C. L. Sanders, K. Rhoads and A. W. Conklin

Rats have been exposed to inhaled $^{244}\text{CmO}_2$ at 70 and 280 days of age (IAD, ~ 350 nCi) and at ten 3-week intervals (IAD, ~ 35 nCi per exposure). Autoradiographs are being examined for lung, liver and bone microdosimetry.

Previous studies with inhaled $^{244}\text{CmO}_2$ in Wistar rats have demonstrated its carcinogenicity in lung, liver and bone. The short retention time for $^{244}\text{CmO}_2$ in lung and its rapid translocation to bone result in a temporal dose-distribution pattern quite different from that of plutonium. Repeated exposure to $^{244}\text{CmO}_2$ will significantly alter the radiation dose patterns in lung, liver and bone and may affect its carcinogenicity in these organs. Age at exposure may also be an important factor in transuranic carcinogenesis. This study was designed to test the role of temporal dose-distribution patterns and of age in carcinogenesis from inhaled $^{244}\text{CmO}_2$.

The study was divided among six experimental groups of female Wistar rats:

- 1) 25 unexposed controls;
- 2) 35 sham-exposed controls;
- 3) 35 animals exposed at 70 days of age, initial alveolar deposition (IAD), ~ 350 nCi ^{244}Cm ;
- 4) 70 animals first exposed at 70 days of age and at 3-wk intervals thereafter for 10 consecutive exposures; estimated IAD, 35 nCi ^{244}Cm ;
- 5) 35 animals exposed at 280 days of age with an IAD of ~ 350 nCi ^{244}Cm ;
- 6) 35 animals exposed at 70 days of age; IAD, ~ 1000 nCi ^{244}Cm . These rats were killed at weekly intervals up to 56 days postexposure; lung, liver and bone were taken for radioanalysis of ^{244}Cm content. Tissues were also taken for autoradiographic examination (including femur for bone examination). Exposure times for autoradiograms ranged from a week for lung and liver to 3-6 mo for bone sections.

Groups 1 through 5 are being held for life-span observation of effects, particularly for development of tumors. Sixty-five of 140 ^{244}Cm -exposed rats have died, compared to only 6 of 60 unexposed or sham-exposed control rats. Histopathology studies and radioanalyses are incomplete. Radioanalyses of tissue contents of ^{244}Cm are presented for group 6 in Table 22. About 80% of inhaled ^{244}Cm is cleared from the lung within a few weeks after exposure, being about equally translocated to liver and skeleton. The liver burden declined rapidly, whereas the skeleton burden of ^{244}Cm remained constant (Table 22).

Curium-244 was concentrated on bone surfaces and in bone vasculature following exposure. Activity was buried in bone within several weeks, thus decreasing the radiation dose delivered to osteoblasts. Repeated exposure to $^{244}\text{CmO}_2$ resulted in repeated deposition of ^{244}Cm upon bone surfaces. Autoradiographic and life-span studies are continuing.

TABLE 22. Distribution of Inhaled $^{244}\text{CmO}_2$ in the Rat. Values are means $\pm$ S.D. (n = 4-5)

Time After Inhalation, Days	Amount of ^{244}Cm , nCi		
	Lung	Liver	Skeleton ^(a)
Immediate	1050 $\pm$ 190	17 $\pm$ 19	14 $\pm$ 6.4
7	540 $\pm$ 120	180 $\pm$ 67	120 $\pm$ 24
14	380 $\pm$ 40	100 $\pm$ 45	110 $\pm$ 35
21	400 $\pm$ 90	110 $\pm$ 51	180 $\pm$ 52
28	190 $\pm$ 50	78 $\pm$ 18	180 $\pm$ 53
35	180 $\pm$ 70	42 $\pm$ 15	180 $\pm$ 40
42	180 $\pm$ 90	56 $\pm$ 10	260 $\pm$ 64
49	130 $\pm$ 70	41 $\pm$ 11	230 $\pm$ 42
56	120 $\pm$ 58	31 $\pm$ 13	210 $\pm$ 80

^(a)Femur is examined for each animal, using autoradiography to determine ^{244}Cm microdistribution

A COMPARISON OF METABOLISM AND LATE EFFECTS
IN DOGS AND RATS EXPOSED TO $^{239}\text{PuO}_2$

Investigators:

J. A. Mahaffey, C. L. Sanders, J. F. Park and G. E. Dagle

Lung tumors were observed proportionally earlier in relation to expected life span in dogs than in rats after inhalation of $^{239}\text{PuO}_2$. Dogs appear to translocate a higher percentage of initial lung burden to liver, skeleton and thoracic lymph nodes than rats.

Dogs and rats were given single inhalation exposures to $^{239}\text{PuO}_2$ of similar particle size (Park et al. *Health Phys.* 22: 803-810, 1972; Sanders et al. *Radiat. Res.* 68: 349-360, 1976). Dogs were exposed at about 18 mo, and rats at 70 days of age. Based on a maximum life expectancy (MLE) of 15 yr for dogs and 1000 days for rats, the ages at exposure were 10% and 7%, respectively, of MLE. Rats that had accumulated a dose, at death, of 1100 to 11,000 rad were selected from high-exposure groups of two experiments (Sanders et al., *Radiat. Res.* 68: 349-360, 1976). Only animals surviving the risk of early mortality from radiation pneumonitis (i.e., past 9% of MLE, or 500 days for dogs and 90 days for rats) were included. Mean ($\pm$ standard error) estimates of initial lung burden (ILB), dose at death, and survival are given in Table 23. Mean survival, standardized by MLE, was significantly different for dogs and rats ($p = 0.0024$).

Twenty-seven lung tumors were observed in dogs (77%) and 33 in rats (56%), a marginal significant difference ($p = 0.065$) between the species. However, because of multiple primary lung tumors, only 26 of the rats (44%) had lung tumors, an incidence significantly different from that of dogs ($p = 0.0036$). Cutler-Ederer estimates of lung tumor incidence, adjusted for survival, are given in Table 24, with survival standardized by MLE. Dogs had a significantly higher cumulative incidence of lung tumors than rats between 20-50% of MLE; after 50% of MLE, the differences were not significant. Thus, in proportion to their potential life spans, dogs had more tumors at earlier times than did rats; at later survival times, the differences were not significant.

TABLE 23. Characteristics of Animals Exposed to $^{239}\text{PuO}_2$

	Dogs	Rats
Number of Animals	35	59
Initial Lung Burden		
nCi	1500 $\pm$ 150	250 $\pm$ 16
nCi/g	17 $\pm$ 1.9	130 $\pm$ 8.1
Dose		
rad At Death	4500 $\pm$ 350	5800 $\pm$ 360
rad/Day	2.8 $\pm$ 0.38	14 $\pm$ 1.3
Survival		
Number Days	2100 $\pm$ 150	500 $\pm$ 21
% of MLE (a)	39 $\pm$ 2.7	50 $\pm$ 2.1

(a) Maximum life expectancy

TABLE 24. Cutler-Ederer Estimates (a) of Cumulative Lung Tumor Incidence for Dogs and Rats that had Received Doses to Lung of 1100-11,000 Rad of $^{239}\text{PuO}_2$ at Death

% of Maximum Life Expectancy	Lung Tumor Incidence	
	Dogs	Rats
0-10	0	0
10-20	0	0
20-30	23 $\pm$ 7.7	0
30-40	38 $\pm$ 9.1	20 $\pm$ 2.0
40-50	65 $\pm$ 9.2	11 $\pm$ 4.8
50-60	77 $\pm$ 8.2	46 $\pm$ 8.3
60-70	96 $\pm$ 3.8	79 $\pm$ 8.0
70-80	--	79 $\pm$ 8.0
80-90	--	93 $\pm$ 8.5

(a) $\pm$ Standard Error

Clearance curves were fitted for whole body, lung, thoracic lymph nodes, liver and skeleton, using the model: $\ln Y = b_0 + b_1 t$, where $Y = \% \text{ of ILB}$ and $t = \text{survival in days}$. This equation transforms to $Y = ae^{b_1 t}$ ($a = e^{b_0}$) a single-compartment model. No attempt was made to model data prior to 9% of MLE for any tissue. Two estimates of ILB were employed in dogs, and animals between 1100 and 11,000 rad were selected and modeled for comparison to rats.

Based on both methods of estimating ILB in dogs, the pulmonary and whole body clearance half-times of $^{239}\text{PuO}_2$ in dogs differed

from those of rats. Due to high variation in the data for other tissues, no differences were significant. The observed percentages of ILB in the tissues of dogs and rats at 15-25, 35-45 and 55-65% of MLE are given in Table 25. For dogs, the results based on two estimates of ILB are reported. Significance for the whole body and lung was a function of ILB estimation methodology. For thoracic lymph nodes, liver and skeleton, the differences between dogs and rats were significant regardless of method of ILB estimation. We therefore conclude that the retention and translocation of $^{239}\text{PuO}_2$ to extrapulmonary tissues appears to differ in rats and dogs.

TABLE 25. Percent of Initial Lung Burden Observed in Tissue at 15-25, 35-45 and 55-65% of Maximum Life Expectancy ^(a)

MLE	Number of Animals	Percent of Initial Lung Burden				
		Whole Body	Lung	Thoracic Lymph Nodes	Liver	Skeleton
15-25%						
Dogs (821-1369 days)	8	83 ± 4.6 (42 ± 1.6) ^(b)	46 ± 3.5 (24 ± 2.1)	24 ± 3.3 (12 ± 1.6)	18 ± 12 (16 ± 12)	1.9 ± 0.4 (1.0 ± 0.2)
Rats (150-250 days)	5	38 ± 1.4	28 ± 8.5	4.6 ± 2.3	0.012 ± 0.010	0.15 ± 0.010
35-45%						
Dogs (1916-2464 days)	9	79 ± 0.067 (20 ± 2.1)	24 ± 3.0 (5.8 ± 0.4)	31 ± 2.3 (8.3 ± 1.1)	15 ± 1.1 (4.4 ± 1.0)	4.5 ± 0.7 (1.2 ± 0.2)
Rats (350-450 days)	6	18 ± 2.3	8.9 ± 3.3	2.2 ± 1.2	0.12 ± 0.043	0.17 ± 0.12
55-65%						
Dogs (3011-3559 days)	4	78 ± 0.1 (15 ± 4.3)	10 ± 3.5 (2.2 ± 1.0)	35 ± 5.5 (6.7 ± 2.1)	17 ± 2.0 (3.5 ± 1.3)	7.9 ± 2.2 (1.7 ± 0.9)
Rats (550-650 days)	18	9.4 ± 2.8	2.9 ± 0.4	0.9 ± 0.14	0.050 ± 0.012	0.047 ± 0.008

^(a) ± Standard Error

^(b) Two methods of ILB estimation were used for dogs. The methodology, similar to that used in rats, is reported in parentheses.

● Cigarette Smoke and Plutonium

Cigarette-smoke-induced changes in pulmonary airways of humans and laboratory animals include epithelial squamous metaplasia, with loss of cilia. These changes may impair the mucociliary clearance mechanism, thereby increasing the probability of pulmonary neoplasia induction by inhaled plutonium. The overall objective of this study is to determine whether cigarette smoking increases the probability of plutonium-induced lung cancer. Initial experiments, designed to characterize the effect of chronic cigarette smoke exposure on pulmonary clearance of plutonium aerosols, are described.

EFFECTS OF CIGARETTE SMOKE EXPOSURE ON PULMONARY CLEARANCE OF $^{239}\text{PuO}_2$ IN RATS

Investigators:

R. E. Filipy, J. L. Pappin, D. L. Stevens and S. G. Irby

Groups of rats were exposed or sham exposed to cigarette smoke for 7 mo, at which time they were exposed to an aerosol of $^{239}\text{PuO}_2$. Rats were then subjected to whole-body counting (17-keV X-rays) periodically, beginning at day 4 after plutonium exposure, and smoke exposures or sham exposures were resumed on day 7. Clearance of plutonium from the lungs of cigarette-smoke-exposed rats was significantly slower than that from the sham-exposed rats' lungs. The difference between the two groups became significant 7 days after the resumption of cigarette-smoke exposures.

Since a large portion of the population smokes cigarettes, a synergistic effect between inhaled plutonium and cigarette smoking might influence estimates of the health risk of plutonium and other trans-uranics released to the environment. Cigarette-smoke-induced changes observed in pulmonary airways of smokers include epithelial squamous metaplasia with loss of cilia. These changes may impair the mucociliary clearance mechanism, thereby influencing the pulmonary distribution and retention of inhaled particulate material.

During the initial phase of the experiment, four groups of Fischer rats were exposed to smoke from 10 University of Kentucky (1R3) research cigarettes per day, or were sham exposed, for 7 mo. Following initial smoke exposure or sham exposure, one-half the rats were given a single inhalation exposure to produce an average initial lung burden of 50 nCi $^{239}\text{PuO}_2$. The exposures received by each group of rats are listed in Table 26.

TABLE 26. Exposure Histories of Rats in an Experiment to Determine the Effect of Cigarette Smoke on Pulmonary Clearance of Inhaled $^{239}\text{PuO}_2$

Group	Number of Rats	Exposure Regimen
1	35	Cigarette Smoke Only
2	35	Cigarette Smoke + $^{239}\text{PuO}_2$ (~ 50 nCi)
3	35	Sham Smoke + $^{239}\text{PuO}_2$ (~ 50 nCi)
4	35	Sham Smoke (controls)

An additional group of smoke-exposed animals was sacrificed immediately following exposure to one cigarette (7 puffs), and measurements were made to determine carboxy-hemoglobin (COHb) concentration in the blood, a value indicative of the amount of smoke inhaled. The results demonstrate a significantly ($p < 0.01$) elevated blood COHb concentration in the smoke-exposed rats. Mean concentrations ($\pm$ standard deviations) were $3.8 \pm 0.2\%$ ($n = 5$) and $7.5 \pm 1.3\%$

(n = 10) for the sham-exposed and smoke-exposed groups, respectively.

Five rats from each group were killed shortly after plutonium exposure; 10 rats from each group were killed 1 day post-exposure; 10 more animals from each group were killed on the 7th day after exposure. Rats were not exposed or sham exposed to smoke during the first week after plutonium exposure until contamination of fur, feces and urine were at levels low enough to permit routine handling. Smoke exposure or sham exposure of the remaining 10 rats in each group was resumed on day 7 after plutonium exposure, and continued until the final sacrifice on day 42.

Whole-body counting (17-keV X-rays) was done on the plutonium-exposed rats of groups 2 and 3 on day 4 and 6 after exposure. These counts were made prior to resuming smoke exposure, and are presumed to correlate with the initial lung burden for each rat. Smoke exposure resumed on day 7, and whole-body counting continued periodically through day 42, a total of nine counts (Figure 25). Whole-body counts indicate significantly ($p < 0.003$) slower lung clearance rates in rats chronically exposed to cigarette smoke before and after a single inhalation exposure to a $^{239}\text{PuO}_2$ aerosol than in sham-exposed rats.

The excised and fixed lungs of animals serially sacrificed after plutonium exposure were subjected to the counting procedure. Although no attempt was made to remove the tracheobronchial lymph nodes and the heart before counting, the activity was considered to represent the lung burden at the time of sacrifice. No significant difference was observed between lung counts of smoke-exposed and sham-exposed rats sacrificed on either day 0, day 1 or day 7 after plutonium exposure. Lungs of smoke-exposed rats contained significantly higher ($p < 0.0005$) concentrations of ^{239}Pu 42 days after exposure than did the lungs of sham-exposed rats. Lung counts also correlate well with whole-body counts at each time of sacrifice (Figure 26). A somewhat lower whole-body counting efficiency at day 41 than at day 6 is suggested by the data. This could be a result of plutonium accumulation in the tracheobronchial lymph nodes of the rats sacrificed at the later time. However, the possibility of external surface contamination in the day 7 rats cannot be definitely ruled out.

Further analysis of pulmonary tissue is underway, using a newly developed autoradiographic method for detection of radionuclides on the epithelial surfaces of pulmonary airways (Annual Report, 1978). This method is expected to generate information on the

FIGURE 25. Comparison of Whole-Body Counts in Sham-Exposed and Smoke-Exposed Rats After Inhalation of a $^{239}\text{PuO}_2$ Aerosol.

FIGURE 26. Comparison of Whole-Body Counts with Counts of Excised, Fixed Lungs of the Same Animals.

distribution as well as on the quantity of $^{239}\text{PuO}_2$ particles deposited in pulmonary airways. The remaining lung tissue and tracheobronchial lymph nodes will undergo radiochemical analysis to determine the thoracic radioactivity in each rat.

A replicate of this experiment is currently being performed. Two new exposure groups were added: One group is exposed to cigarette smoke before, but not after, plutonium

exposure; the other group is exposed to smoke after (but not before) plutonium exposure. This protocol is expected to help clarify whether cigarette-smoke exposure before or after inhalation of the plutonium aerosol was most influential in inhibiting the pulmonary clearance mechanism. The distribution of the plutonium body burden at each sacrifice time will be characterized, and the surface contamination potential will also be investigated.

• Toxicology of Plutonium-Sodium

Scenarios for liquid metal fast breeder reactor (LMFBR) accidents predict the release of mixed sodium-LMFBR fuel aerosols into the environment. It has been demonstrated that the physicochemical and biological properties of these aerosols, which contain sodium as well as plutonium and uranium oxides, are different from those of aerosols generated from LMFBR fuel alone. This project seeks to devise methodologies for continuous generation of fuel and sodium aerosols under various conditions, and to determine the physicochemical aerosol properties that significantly alter biological behavior. Deposition and translocation studies in rodents and dogs following exposure to these aerosols by inhalation or gavage will provide data to evaluate and interpret the potential biological effects.

BIOLOGICAL BEHAVIOR OF MIXED LMFBR-FUEL-SODIUM AEROSOLS IN RODENTS

Investigators:

D. D. Mahlum, J. O. Hess, P. L. Hackett and J. K. Briant

Clearance of ^{239}Pu from lung and other tissues was determined after nose-only exposure of rats to mixed aerosols of sodium-LMFBR fuel or to LMFBR fuels only. The rates of clearance from lung and from total body were both higher after exposure to the mixed sodium-fuel than after exposure to fuel-only aerosols.

We previously reported (Annual Report, 1978) that both pulmonary deposition and clearance of mixed aerosols of LMFBR fuel and sodium are different from those of the fuel-only aerosols. We have now examined pulmonary and total body clearance to 365 days after nose-only exposure of rats to a fuel-only aerosol or a sodium-fuel aerosol (sodium:fuel ratio = 159).

Groups of female Wistar rats were exposed to each of the above aerosols for 30 min. Animals were killed at 0, 7, 182 and 365 days after exposure and tissues taken for ^{239}Pu analysis. Aerosol characteristics and exposure parameters are shown in Table 27.

The deposition and retention of ^{239}Pu in the lung are shown in Figure 27. These data,

TABLE 27. Characteristics of LMFBR-Fuel Aerosols With and Without Sodium

^{239}Pu Conc., nCi/l	Sodium Conc., $\mu\text{g}/\text{X}$	Ratio of Sodium: Fuel	AMAD/GSD ^(a)
37	--	0	0.81/1.52
6.4	98	159	3.83/1.43

(a) Activity Median Aerodynamic Diameter/Geometric Standard Deviation

FIGURE 27. Deposition and Retention of ^{239}Pu in Lung After Inhalation Exposure to LMFBF-Fuel or Sodium-Fuel Aerosols

expressed as percent of the initial body burden, show that the deposition of the mixed sodium-fuel aerosol is lower than for the fuel-only aerosol. The higher rate of loss from the lung of the mixed aerosol can be seen when calculated in this manner, but is less apparent than if the absolute levels are considered. On an absolute basis, 60, 10 and 6% of the initial lung burden was retained in the lung at 7, 182 and 365 days, respectively, after exposure of rats to the fuel-only aerosol. Values of 38, 3 and 4%

were obtained for the same time intervals for those exposed to the sodium-fuel aerosol. The total body burden was about 112% of that inhaled (calculated on the basis of 4.5 μl inhaled) for animals exposed to the mixed aerosol, but only 54% for those exposed to the fuel-only aerosol. These results probably reflect the marked difference in particle size between the two aerosols (0.81 for fuel-only and 3.83 μm for the sodium-fuel aerosol).

The distribution of ^{239}Pu among various tissues immediately after exposure (Figure 28) is different for rats exposed to the fuel-only aerosol than for those exposed to the sodium-fuel aerosol. The lung and the skin contain a significantly greater fraction, and the GI tract significantly less of the body burden in animals exposed to the fuel-only aerosol.

Liver (Figure 29) and femur (not shown) values are relatively higher for animals exposed to the mixed aerosol than for those exposed to fuel-only aerosol. These data suggest that the ^{239}Pu in the mixed aerosol was more soluble than that in the fuel alone.

FIGURE 28. Distribution of ^{239}Pu Among Tissues Immediately After Exposure of Rats to LMFBR-Fuel or Sodium-Fuel Aerosols

FIGURE 29. Distribution of ²³⁹Pu Among Tissues of Rats at Several Times After Inhalation Exposure to LMFBF-Fuel or Sodium-Fuel Aerosols

DEPOSITION AND CROSS-PLACENTAL TRANSFER OF LMFBR FUEL-SODIUM AEROSOLS IN RATS

Investigators:

P. L. Hackett, J. O. Hess, D. D. Mahlum and J. K. Briant

Technical Assistance:

B. L. Egbert and S. C. Johnson

Pregnant rats in late gestation were exposed to aerosols of LMFBR fuel or sodium fuel to determine the deposition and distribution of ^{239}Pu within the fetoplacental unit: 0.008% of the initial body burden of the dam appeared in the fetus immediately after exposure, in both treatment groups; however, a larger fraction of the burden was apparently present in the sodium-fuel-exposed fetuses after 48 hr.

Pregnant Wistar rats were exposed for 1 hr, nose-only, to an LMFBR fuel aerosol or a sodium fuel aerosol at 19 days gestation. Groups of six rats were killed at 0 or 48 hr postexposure for ^{239}Pu analyses of maternal and fetal tissues.

Although the ^{239}Pu concentrations of the two aerosols differed by a factor of 10, the total ^{239}Pu activity measured in fuel-exposed rats was only three times higher than the amount deposited in the sodium-fuel group (Table 28). In the fuel aerosol (AMAD, $0.78\ \mu\text{m}$), 88% of the particles were less than $1.2\ \mu\text{m}$ in size; while in the mixed

aerosol (AMAD, $3.5\ \mu\text{m}$), only 2% of the particles were smaller than $1.2\ \mu\text{m}$. A large fraction of the total deposited fuel aerosol was observed on the pelt, particularly on the head skin; therefore, the initial body burden (IBB), which excludes pelt activity, was used as a basis for comparing biological disposition of the two aerosols.

The gastrointestinal tracts of the sodium-fuel rats contained the major fraction of the IBB (Table 29). The increased gastrointestinal deposition and decreased lung burdens observed following exposure to mixed

TABLE 28. Properties and Deposition of LMFBR Fuel and Sodium Fuel Aerosols

Aerosol Properties	Fuel	Sodium-Fuel
Sodium-Fuel Ratio	0:1	145:1
Fuel Concentration (nCi/ℓ)	39	3.7
AMAD ^(a)	0.77	3.53
GSD ^(b)	1.51	1.64
Total Deposition (nCi)	92.6 ± 5.24	27.5 ± 1.55
Pelt (nCi)	38.8 ± 1.50	2.96 ± 0.31
Initial Body Burden (nCi)	53.7 ± 4.95	24.6 ± 1.59
Lung Deposition (nCi)	18.8 ± 2.28	0.52 ± 0.10

^(a) Activity Median Aerodynamic Diameter
^(b) Geometric Standard Deviation

TABLE 29. Distribution of ^{239}Pu in Rats After Exposure to LMFBR Fuel or Sodium-Fuel Aerosols (% of Initial Body Burden)

	Immediately After Exposure		Two Days Postexposure	
	Fuel	Sodium-Fuel	Fuel	Sodium-Fuel
Lung	35.9 ± 5.29	2.17 ± 0.43	41.7 ± 5.36	1.57 ± 0.28
Gastrointestinal Tract	58.1 ± 5.83	93.0 ± 0.94	5.41 ± 0.94	1.63 ± 0.43
Carcass ^(a)	3.87 ± 0.94	2.09 ± 0.26	0.33 ± 0.07	0.53 ± 0.04
Blood ^(b)	0.018 ± 0.005	0.098 ± 0.045	0.014 ± 0.005	0.095 ± 0.036
Pregnant Uterus ^(c)	0.12 ± 0.04	0.27 ± 0.08	0.034 ± 0.009	0.13 ± 0.02

(a) Carcass includes major organs and excludes respiratory and gastrointestinal tracts.

(b) Calculated from activity/ml and a total blood volume of 6% of the body weight.

(c) Average litter size of 11 ± 2

aerosols have been attributed to the larger particle size of the sodium-fuel aerosols (Annual Report, 1978). Carcass values, which include major organ activity but exclude respiratory and gastrointestinal-tract deposition, were calculated to estimate absorption and retention and were found to be similar in the two exposure groups at both time intervals. The percentages of the IBB in the blood and pregnant uterus of the sodium-fuel-exposed rats were apparently higher, but due to individual variability, were not significantly different from those of the fuel-exposed group.

The fraction of the IBB within individual fetoplacental units (FPUs) was very low (Table 30). Fetal values for both aerosol groups were equal immediately after exposure, but a somewhat larger percentage of the dose

was retained at 2 days postexposure in the fetuses of the mixed-aerosol-exposed rats. The apparent clearance of ^{239}Pu activity from the fetus after 2 days seemed unusual during this period of fetal skeletal growth and calcification. The higher membrane values at 2 days postexposure for the mixed-aerosol-exposed FPUs were also unexpected.

These interesting aspects of the biological behavior of sodium-fuel aerosols in the FPU, i.e. fetal clearance and membrane accumulation of ^{239}Pu , will be clarified using more sensitive analytical methods. Aerosol properties will also be altered by lowering sodium-fuel ratios to produce smaller-sized particles. Exposure to these aerosols with lower AMADs should increase the lung burden, thus promoting ^{239}Pu absorption.

TABLE 30. Disposition of ^{239}Pu Activity in Components of the Fetoplacental Unit

Aerosol	Days After Exposure	Percent Of Initial Body Burden (X 100)		
		Placenta	Membranes	Fetus
Fuel	0	0.13 ± 0.05	0.10 ± 0.05	0.80 ± 0.35
	2	0.07 ± 0.01	0.09 ± 0.03	0.17 ± 0.04
Sodium-Fuel	0	0.38 ± 0.07	0.17 ± 0.06	0.86 ± 0.31
	2	0.20 ± 0.04	0.43 ± 0.04	0.33 ± 0.10

EFFECT OF EXPOSURE SEQUENCE ON THE DISTRIBUTION OF
LMFBR-FUEL-SODIUM AEROSOLS IN RATS

Investigators:

D. D. Mahlum, J. O. Hess, P. L. Hackett and J. K. Briant

Female, Wistar rats were exposed for 30 min to sodium aerosols containing approximately 370 $\mu\text{g}/\ell$ of sodium. Together with nonexposed rats, they were then exposed, nose-only, for 30 min to aerosols of LMFBR fuel containing approximately 36 nCi/liter. Animals from each group were killed immediately after exposure to the fuel aerosol, or 7 days later. Lungs and other tissues were removed and analyzed for plutonium content. Both the animals exposed previously to sodium aerosol, and those not exposed, showed similar patterns of deposition and retention of ^{239}Pu in tissues at both time periods. Thus, prior exposure of animals to relatively high levels of sodium did not affect the deposition and retention of ^{239}Pu .

In the case of a hypothetical core disruptive accident (HCDA) of a liquid metal fast-breeder reactor, sodium vapors could be released and inhaled by personnel working in the reactor building or by the general population. Exposure to released fuel aerosols could occur subsequently. We performed an experiment to determine whether prior exposure to sodium aerosols would influence the deposition and retention of LMFBR fuel aerosols.

Female Wistar rats were exposed, whole body, to aerosols containing approximately 370 μg of sodium per liter. Upon completion of this exposure, this group and a group of nonexposed animals were exposed, nose-only,

to an aerosol of LMFBR fuel (36 nCi/ ℓ). Half the animals from each group were killed immediately after exposure to the fuel aerosol, and the other half of each group were killed 1 wk later. Tissues were removed and analyzed for ^{239}Pu content.

Table 31 compares the distribution of ^{239}Pu among several tissues at both times of sacrifice. There were minor variations between groups at the two times of sacrifice; but overall, there was little difference in tissue levels between groups. It thus appears that prior exposure of animals to aerosols of sodium did not influence either the deposition of ^{239}Pu or its clearance during the week subsequent to exposure.

TABLE 31. Effect of Prior Exposure to Sodium Aerosols on the Deposition and Retention of LMFBR Aerosols

Time Post Exposure, days :	nCi/Tissue			
	0		7	
Tissue	Controls	Sodium	Controls	Sodium
Blood	0.0021 ± 0.0023	0.0009 ± 0.0003	0.0022 ± 0.0020	0.0034 ± 0.0025
Lung	20.2 ± 3.6	17.35 ± 3.05	15.9 ± 4.1	16.77 ± 1.91
Trachea	0.26 ± 0.09	0.504 ± 0.16	0.05 ± 0.07	0.122 ± 0.17
Esophagus	0.11 ± 0.02	0.458 ± 0.35	0.04 ± 0.04	0.017 ± 0.02
Liver	0.03 ± 0.02	0.014 ± 0.007	0.012 ± 0.010	0.022 ± 0.02
G.I. Tract	37.14 ± 11.88	38.39 ± 6.70	0.1609 ± 0.0298	0.279 ± 0.0709
Carcass	1.075 ± 0.24	1.41 ± 0.78	0.064 ± 0.044	0.085 ± 0.046
Head Skin	10.69 ± 4.94	14.76 ± 2.55	0.102 ± 0.069	0.192 ± 0.089
Nasal Bones	0.883 ± 1.57	0.510 ± 0.30	0.007 ± 0.009	0.062 ± 0.14
Total	75.85 ± 11.54	78.94 ± 8.88	16.54 ± 4.21	17.77 ± 2.08

DEPOSITION OF INHALED LMFBR-FUEL-SODIUM AEROSOLS IN BEAGLE DOGS

Investigators:

P. L. Hackett, D. D. Mahlum, J. K. Briant, D. L. Catt,
L. R. Peters and A. J. Clary

Technical Assistance:

G. J. Powers, J. O. Hess, J. R. Rossignol, J. P. Herring,
C. L. Baldwin, T. C. Kinnas, R. L. Music, and B. L. Egbert

Initial alveolar deposition of LMFBR-fuel aerosols in beagle dogs amounted to 30% of the inhaled activity, but only 5% of the total inhaled activity was deposited in dogs exposed to sodium-fuel aerosols. Aerosol deposition in the gastrointestinal tract amounted to 4% of the initial body burden of fuel-aerosol exposed dogs and 24% of the burden of animals receiving sodium-fuel aerosols. Preliminary analytical data for the dog exposures appear to agree with rodent data for deposition and distribution patterns of aerosols of similar sodium:fuel ratios.

Previous studies in this laboratory (Annual Reports 1977 and 1978) comparing the deposition and distribution of inhaled LMFBR-fuel and sodium-fuel in rodents demonstrated that pulmonary deposition was lower and ^{239}Pu activity in the gastrointestinal tract was higher following exposure to the sodium-fuel aerosol. The observed difference in particle partition was attributed to the larger particle size of the sodium-fuel aerosols. Studies with beagle dogs were initiated to determine if the observed differences in the biological behavior of the two types of aerosols in rodents occurred in a species with a much larger major-airway volume, cross-sectional area, and pulmonary vascular area.

Mature female beagles were exposed for 30 min to fuel or sodium-fuel aerosols by the single-dog, nose-only technique. The eight dogs of group A were sacrificed at 0, 3, 7 and 28 days after exposure, and 13 dogs each in groups B and C were killed at 1, 7 and 28 days, and ultimately, 270 days post-exposure. A tranquilizer was administered to animals of groups B and C to attempt to equalize respiratory characteristics among treatments. Alveolar deposition was estimated by external thorax counting at 3, 4, 7, 14 and 28 days postexposure. Serial blood samples were obtained during the first 48 hr; urine and feces were collected (daily for the first week and subsequently pooled

by week for the first month) from all animals; tissues were obtained at necropsy for ^{239}Pu analyses.

Table 32 shows the properties of the fuel and sodium-fuel aerosols utilized in these exposures. Although ^{239}Pu activities and sodium concentrations of sodium-fuel aerosols for exposure groups B and C varied considerably, the mass ratios and AMADs of the inhaled aerosols were similar. Solubilities of sodium-fuel aerosols for groups A and C were more nearly alike. Except for ^{239}Pu activity levels, fuel aerosol properties were more uniform for all three exposures.

The coefficients of variation calculated for respiration parameters of the dogs during the 30-min exposure period (Table 33) ranged between 16.2 and 35.0, suggesting that use of the tranquilizer produced no apparent decrease in respiratory variability. The respiratory rate of the group C dogs exposed to the sodium-fuel aerosol (the highest sodium concentration) was elevated. Further comparisons of the data should reveal whether this higher value was characteristic of this group of animals, or was due to the sodium concentration.

Total ^{239}Pu activities were calculated from the total volume inhaled during exposure and the ^{239}Pu concentration of the aerosol, and initial alveolar deposition

TABLE 32. LMFBR-Fuel and Sodium-Fuel Aerosol Characteristics

Aerosol	Exposure	Na: Fuel Ratio	Activity nCi ²³⁹ Pu/ℓ	Sodium Conc. µg Na/ℓ	% Solubility (2 hr - H ₂ O)	Inhaled Aerosol		Exhaled Aerosol	
						AMAD µm	GSD	AMAD µm	GSD
Na-Fuel	A	54:1 ^(a)	15 ± 2.6	79	8.3	2.8	1.5	3.8	1.8
	B	29:1	18 ± 1.2	66	10	2.1	1.6	2.9	2.3
	C	26:1	53 ± 7.0	170	40	2.2	1.5	3.6	2.0
Fuel	A	0:1	22 ± 1.4	0	< 0.1	0.85	1.5	0.84	1.5
	B	0:1	41 ± 5.5	0	< 0.1	0.75	1.5	1.00	1.5
	C	0:1	77 ± 4.4	0	< 0.1	0.78	1.5	0.87	1.5

(a) Solubility and AMDA indicate ratio may be lower

TABLE 33. Respiration Parameters for Beagle Dogs During Exposure ($\bar{X} \pm S.D.$)

Exposure	Aerosol	Respiratory Rate Breaths/min	Minute Volume ℓ/min	Tidal Volume ml/Breath
A	Na-Fuel	11 ± 1.8 (a)	1.4 ± 0.48	130 ± 37
	Fuel	15 ± 2.5 (a,b)	1.9 ± 0.38	120 ± 21
B*	Na-Fuel	15 ± 5.1 (a,b)	1.8 ± 0.62	130 ± 48
	Fuel	13 ± 1.9 (a)	2.6 ± 0.34	210 ± 44
C*	Na-Fuel	23 ± 5.2 (b)	2.6 ± 0.26	110 ± 34
	Fuel	13 ± 2.6 (a)	2.6 ± 0.64	180 ± 41

(a,b) Values with different letters are significantly different ($P < 0.05$)

* Tranquilized during exposure

values were estimated from external thorax counts (Figure 30). The percentage of inhaled aerosols deposited in the sodium-fuel-exposed dogs in all groups was less than 20% of amount deposited in the fuel-aerosol groups. The magnitude of this decrease in deposition was similar to the difference observed in rodents exposed to aerosols of 0:1 or 24:1 sodium-fuel ratios (Annual Report, 1978).

Initial body burdens calculated from ²³⁹Pu analyses of group A dogs are shown in Figure 31. Group averages for lung burdens

compare favorably with the estimated alveolar deposition (Figure 30). A comparison of ²³⁹Pu distribution in dogs from each treatment group sacrificed immediately after exposure showed lower lung levels and higher values for ²³⁹Pu on the skin, in the turbinates, and in the gastrointestinal tracts of dogs exposed to the sodium-fuel aerosol compared to fuel-exposed animals. The major portion of the gastrointestinal ²³⁹Pu activity appeared in the feces by the third day after exposure. Fractions of the initial body burden found in the gastrointestinal tract of rodents exposed to aerosols of comparable

FIGURE 30. Fuel and Sodium Fuel Aerosol Deposition in Beagle Dogs

FIGURE 31. Tissue Distribution in Beagle Dogs Immediately After Inhalation of Sodium - Fuel or Fuel Aerosols

sodium-fuel ratios were much higher (53% after fuel exposure and 90% after the sodium-fuel aerosol); however, lung values of fuel-exposed rats were lower, as were skin and nasal-bone levels of the sodium-fuel rodents.

These initial data, which compare deposition and distribution of inhaled fuel and sodium-fuel aerosols, appear to agree with observations from the previous rat inhalation studies. Absorption and clearance estimations await completion of plutonium analyses.

DISSOLUTION OF LMFBF FUEL-SODIUM AEROSOLS

Investigators:

J. K. Briant, M. D. Allen, and O. R. Moss

Technical Assistance: E. J. Rossignol

Aerosols of either LMFBF fuel or PuO_2 are essentially insoluble in water or biological fluids. If either of these aerosols is exposed to sodium metal vapor, the resulting aerosol is much more soluble in aqueous solutions. Preferential dissolution of uranium from mixed sodium-fuel aerosols makes the plutonium more readily soluble than for sodium- PuO_2 aerosol. Ultrafiltration of the dissolution test solvent revealed the plutonium to be associated with particles between 2 nm and 10 nm in diameter, in both cases.

LMFBF fuel aerosol (or PuO_2 aerosol) was generated by laser vaporization, subsequently exposed to sodium vapor in an inert atmosphere, and finally exposed to air. The solubility of aerosol samples increased with increasing sodium:fuel (or sodium: PuO_2) mass ratios. Characteristics of the aerosol and the flow-past dissolution system for testing solubility of the aerosol have been described in previous Annual Reports (1977,1978).

Filter samples of sodium-fuel and sodium- PuO_2 aerosol were tested for dissolution rate in distilled water for 120 min. For sodium-actinide mass ratios on the order of 10, sodium-fuel aerosols were several hundred times more soluble than fuel aerosol alone. At equivalent sodium-actinide mass ratios, sodium-fuel aerosol was about 10 times more soluble than sodium- PuO_2 aerosol. Figure 32 illustrates this influence of sodium metal, as well as the influence of the uranium present in the sodium-fuel aerosol. The uranyl tricarbonate compound formed is very soluble in aqueous solution and dissolves preferentially, thus exposing a higher percentage of the plutonium compound in fuel aerosol than in the sodium- PuO_2 aerosol. The availability of carbonate therefore has a significant role in dissolution of the uranium lattice in the aerosol and, consequently, in dissolution of the plutonium. It increases the dissolution rate of sodium- PuO_2 aerosol to a much lesser, but significant, degree.

Another factor influencing the dissolution rate was the age of the sample. All samples shown in Figure 32 had been aged for several months, except the sodium- PuO_2

FIGURE 32. Dissolution of LMFBF-Fuel and of PuO_2 , With and Without Exposure of the Aerosols to Sodium Vapor. (Note log scale.)

sample, at a 9:1 ratio, which was only 2 days old when the dissolution test was run. Sodium-fuel samples also exhibited increased solubility with age: a 50% increase during the first week for samples with a 27:1 mass ratio. Between 23°C and 35°C, temperature of the solvent had little effect on the dissolution rate.

Ultrafiltration of solvent from dissolution tests, in stirred cells, showed greater than 90% of the dissolved plutonium to be associated with particles between 2 nm and 10 nm in diameter. This was true for both sodium-fuel and sodium-PuO₂ samples with distilled water as the solvent.

Dissolution in simulated lung fluid proceeded much more slowly. As reported last year, there was about a 20-fold decrease in dissolution, when compared to water as the solvent. This has been attributed to a complex interaction with the phosphate in the simulated lung fluid. The mechanism of this interaction is not yet clear.

The pronounced effect of sodium on plutonium solubility in LMFBR fuel aerosol dissolution is clear. We are investigating the mechanism of this enhanced solubility by comparing it with that for sodium-PuO₂ and sodium-UO₂ aerosols. Spectrophotometric analysis of aerosol samples will hopefully provide further information about the chemical species involved.

SPECTROPHOTOMETRIC DETERMINATION OF URANIUM AND PLUTONIUM IN
LMFBR FUEL-SODIUM AEROSOLS

Investigators:

L. G. Morgan,^(a) J. L. Ryan,^(a) M. D. Allen and J. K. Briant

Spectrophotometric investigations of sodium-(Pu,U)O₂ and sodium-PuO₂ aerosols were conducted with aerosols collected both in dry air on filters or in impinger solutions. These studies confirm previous observations (Annual Report, 1978) that the uranium present in the LMFBR fuel-sodium aerosol was rapidly converted to a soluble uranyl carbonate complex ion species in the presence of air and moisture. In addition, our results demonstrate that previous speculations concerning the valence of plutonium in the aerosol must be reexamined.

Spectrophotometric studies of LMFBR fuel-sodium aerosols were conducted to identify the valence state of the actinides in aerosols and in soluble species derived from suspension of the aerosols in various media. Techniques involving direct spectrophotometric determinations of the valence state of the actinides (uranium and/or plutonium) in the aerosol have been developed and used successfully in previous studies.

Aerosols were collected on a suitable filter in a dry air stream and immediately placed in a desiccator until they were mixed with Kel-F® (3M Co., St. Paul, MN) grease under a blanket of argon to preclude exposure to moisture. The Kel-F containing the aerosol was placed between glass plates, whose edges were then taped and sealed. The thickness of this sample, known as a "mull," may be adjusted to give optimal spectrophotometric results.

Although this spectrophotometric technique had been used successfully to identify valence states of various actinide compounds, the results from the sodium-PuO₂ aerosol mull were equivocal. Although some weak structure was observed in the visible region of the spectrum, it was not identical to any known valence-state spectrum of plutonium. A uniformly increasing apparent absorbance, with decreasing wavelength, in this sample and in a similar sample of sodium-(Pu,U)O₂, indicated that severe scattering of the radiation had occurred.

These sodium-PuO₂ and sodium-(Pu,U)O₂ aerosol samples and their respective mulls, which were collected on the filters in dry air were light gray in color; after prolonged exposure to laboratory air (moisture) the color of the mulls changed to a greenish yellow-brown. There was no change in the spectrophotometric result following this color change. The spectrophotometric results and the appearance of the aerosol samples on the filters were consistent with the possibility that metallic sodium remained in these samples even after prolonged exposure to dry air. Therefore, a filter-collected aerosol sample of sodium-PuO₂ that had been in a dessicator of dry air for 8 days was quickly added to a test tube containing liquid ammonia. The intense blue color which immediately developed indicated the presence of metallic sodium in the aerosol sample. The aerosol material was thoroughly immersed in liquid ammonia and loosely capped, so that the ammonia could slowly distill off, meanwhile minimizing infiltration of air and moisture. The following day, the material remaining in the test tube was leached with 6M HClO₄, which will not alter the valence of the plutonium. A spectrum of the supernate obtained from this leaching test indicated the presence of Pu(VI) in addition to Pu(IV). Since sodium dissolved in liquid ammonia is a powerful reducing agent, an oxidizing environment would not be expected at any time during the treatment of this sample. Therefore, this may be the first evidence that Pu(VI) exists in the aerosol.

^(a) Chemical Technology Department

The evidence for the presence of metallic sodium in aerosol samples collected in dry air gave rise to concern that erroneous valence state information might also be obtained for aerosol samples collected in impinger solutions, since the point of collection for impinger solutions was identical to that of the filter-collected samples. To determine if this concern was valid, spectrophotometrically pure solutions of Pu(VI) in HClO_4 and Pu(IV) in HCl were prepared and exposed to sodium aerosol at the same point of collection with the same aerosol generation parameters as filter-collected samples and previous impinger samples. Spectrophotometric results, which were obtained as rapidly as possible (approximately 30 min), indicated that a portion of the plutonium in both solutions had been reduced to lower valence states. A mixture of Pu(III), Pu(IV), and Pu(VI) was found in the impinger solution originally containing only Pu(VI), and a mixture of Pu(III) and Pu(IV) was observed in the solution originally containing only Pu(IV). These results indicate that previous spectrophotometric results reported for plutonium valence states in impinger-collected samples probably do not represent the valence state of plutonium in the aerosol itself, and that the technique of impinger solution collection is

not suitable for determination of valence states in aerosol samples.

However, impinger-collected samples can be used successfully for determination of the valence states of the actinides in solution, and have been used to demonstrate that the uranium in sodium- $(\text{Pu},\text{U})\text{O}_2$ aerosols was rapidly converted in the presence of air and moisture to a soluble uranyl carbonate complex ion species in the following experiment. A sodium- $(\text{Pu},\text{U})\text{O}_2$ aerosol was generated and collected in distilled water by the impinger technique. The resulting solution, which had a pH of 11.77 (compared to a pH of 11.78 for a sodium carbonate solution) was centrifuged and the supernate was examined spectrophotometrically. The spectrum of the supernate was identical to that of a solution containing uranyl ion in excess carbonate.

Further studies are in progress to determine the valence state of both uranium and plutonium in sodium- $(\text{Pu},\text{U})\text{O}_2$ aerosols when both air and moisture are excluded. The determination of the initial valence states of these metals in the aerosol is essential for the prediction of their chemical behavior once they are exposed to a variety of environmental conditions.

MEASUREMENTS OF DYNAMIC SHAPE FACTORS OF LMFBR AGGREGATE AEROSOLS

Investigators:

M. D. Allen, O. R. Moss and J. K. Briant

Dynamic shape factors for branched, chain-like aggregates of LMFBR mixed-oxide fuels have been measured with a LAPS spiral-duct centrifuge. The aerosol was generated by repeatedly pulsing a focused laser beam onto the surface of a typical LMFBR fuel pellet. The measured values of the dynamic shape factor, corrected for slip, vary between $\kappa = 3.60$ at $D_{ae} = 0.5 \mu\text{m}$, and $\kappa = 2.23$ at $D_{ae} = 1.5 \mu\text{m}$.

Reactor-fuel materials could be vaporized by the heat produced in a criticality accident. The vapors would condense into small, spherical particles which would then coagulate to form branched, chain-like aggregates. An understanding of the aerodynamic behavior of these aggregates is important in evaluating the hazards due to certain defined reactor accidents.

The aerodynamic diameter, D_{ae} , of aggregates can be calculated using the following semi-empirical equation:

$$D_{ae} = \sqrt{\frac{\rho C(D_e)}{\rho_0 \kappa C(D_{ae})}} n^{1/3} D_g \exp[1.5 \ln^2 \sigma_g], \quad (1)$$

where

ρ = density of the material
($\rho \approx 10.96 \text{ g/cm}^3$ for UO_2),

ρ_0 = unit density (=1),

D_e = volume equivalent diameter,

$C(D)$ = Cunningham-Knudsen-Weber slip correction factor,

n = number of primary particles per aggregate,

D_g = geometric mean diameter of the primary particles, and

σ_g = geometric standard deviation of the primary particle size distribution.

The empirical term, which is called the dynamic shape factor κ , relates the drag on a nonspherical particle to the drag on a

spherical particle of the same volume.

Dynamic shape factors for aggregates generated by high-temperature vaporization of LMFBR fuel have been measured for five aerodynamic sizes.

Aggregates were generated by pulsing a focused CO_2 laser beam on the surface of a rotating LMFBR fuel pellet. Each laser pulse produced an incandescent vapor plasma, which rapidly condensed and coagulated to form aggregates. The aerosol passed continuously through a 25-l stainless steel chamber, from which Lovelace Aerosol Particle Separator (LAPS) spiral-duct centrifuge samples were taken.

To determine the dynamic shape factor κ , carbon-coated copper grids were taped to the centrifuge foil at distances corresponding to $D_{ae} = 0.5, 0.6, 0.8, 1.0$, and $1.5 \mu\text{m}$, respectively, so that the average mass per aggregate ($\bar{m}$) could be determined. The total mass per cm^2 (M) at a certain aerodynamic size was determined by counting the Pu-alpha activity deposited on a spiral centrifuge foil. The number of aggregates per cm^2 (N) was determined by counting the number of aggregates in an area on electron photomicrographs. The average mass per aggregate, ($\bar{m}$), then, is M divided by N . The average number of primary particles per aggregate ($\bar{n}$) can be calculated from the equation:

$$\bar{m} = \frac{\pi}{6} \rho \bar{n} D_g^3 \exp[4.5 \ln^2 \sigma_g]. \quad (2)$$

The values of D_g and σ_g were determined by Zeiss analyses of transmission electron photomicrographs to be $0.025 \mu\text{m}$ and 1.46 , respectively.

Figure 33 shows typical LMFBR aggregates of four different aerodynamic sizes and

FIGURE 33. Typical LMFBR Mixed-Oxide Fuel Aggregates Generated by Laser Vaporization

TABLE 34. Measured Values for the Determination of the Dynamic Shape Factor for $(\text{Pu}_{0.14}\text{U}_{0.86})\text{O}_{2.22}$ Aggregates

D_{ae} (μm)	M (g/cm^2)	N (No. Of Aggregates/ cm^2)	$\bar{m}$ ($\text{g}/\text{aggregate}$)	$\bar{n}$ No. of Primary Particles/ Aggregate	D_e (μm)	κ
0.5	2.95×10^{-8}	3.09×10^5	9.55×10^{-14}	560	0.255	3.60
0.6	8.19×10^{-8}	4.95×10^5	1.65×10^{-13}	966	0.306	3.48
0.8	2.57×10^{-7}	7.77×10^5	3.31×10^{-13}	1938	0.386	3.04
1.0	3.01×10^{-7}	5.16×10^5	5.83×10^{-13}	3413	0.466	2.78
1.5	9.17×10^{-7}	6.25×10^5	1.47×10^{-12}	8606	0.635	2.23

spherical polystyrene latex particles of the same respective aerodynamic sizes. Values of D_{ae} , M , N , $\bar{m}$, $\bar{n}$, and D_e are listed in Table 34. Equation (1) was used to calculate values for κ at each aerodynamic diameter. Measured dynamic shape factors ranged between

$\kappa = 3.60$ at $D_{ae} = 0.5 \mu\text{m}$, and $\kappa = 2.23$ at $D_{ae} = 1.5 \mu\text{m}$. These values are in good agreement with values measured at Battelle, Columbus Laboratories using a modified Millikan oil-drop apparatus.

• Toxicology of Sodium and Lithium

This project will obtain data for establishing emergency standards for sodium and lithium release accidents involving heat transfer systems such as those designed for use in LMFBs or, in the case of lithium, magnetic fusion reactors. It will utilize an existing aerosol generation and exposure system in exposing young adult and adult rats to different concentrations of sodium and lithium combustion products. The ED₅₀ and LD₅₀ will be determined for the predominant combustion products. Target organs, location of lesions and pathogenesis of lesions will be studied. Additional experiments will be designed on the basis of the data obtained from these studies.

TOXIC EFFECTS OF SODIUM CARBONATE AEROSOLS IN RODENTS

Investigators:

R. H. Busch and K. E. McDonald

Studies were conducted to establish LC₅₀ concentrations of Na₂CO₃ aerosols in rats, mice, hamsters and guinea pigs. The order of decreasing species sensitivity appears to be guinea pigs, mice, rats and hamsters, with an LC₅₀ of 750, 1900, 1600 and >8130 µg/ℓ concentrations, respectively, for 2-hr exposures.

The objectives of studies conducted during FY 1979 were: (1) to determine Na₂CO₃ aerosol concentrations which would result in acute toxicity to rats, mice, guinea pigs and hamsters as calculated by LC₅₀, so that this data would be available for consideration in setting standards for accidental exposure of human subjects; and (2) to determine mechanisms involved in acute deaths.

Groups of male and female animals of four species were exposed to aerosols consisting primarily of sodium carbonate in order to establish relative toxicity, as well as relative species and sex sensitivities.

Generation of pure Na₂CO₃ aerosols at higher (e.g., 2600-µg/ℓ), dose levels has proven to be difficult, apparently because of difficulty in reaching optimal conversion rates of NaOH to Na₂CO₃. Factors known to affect this conversion are: 1) partial pressures of CO₂ and H₂O vapor, 2) temperature, and 3) particle size and distribution.

When composition is constant, toxic effects of Na₂CO₃ appear to be concentration-related.

Guinea pigs appear to be the most sensitive animal tested, with an LC₅₀ of approximately 750 µg/ℓ when exposures are conducted over 2-hr periods. Rats and mice appear to be significantly more resistant, having LC₅₀s of 2600 µg/ℓ (rats) and 1900 µg/ℓ (mice). Golden Syrian hamsters have relatively high levels of resistance. It was not possible to obtain mortality data sufficient to calculate the LC₅₀ for this species even though exposure levels up to 8130 µg/ℓ for 2 hr were tested. Males appear to be slightly more sensitive in all species tested.

Exposure-related deaths appear at two time intervals relative to exposure: during or immediately following exposure, and 2 to 10 days following exposure. Mechanisms responsible for early death are laryngospasm, pharyngeal and laryngeal edema (acute), hemorrhagic lung, and severe gastric tympany.

There were a variety of causes for the later deaths; the most significant was bacteremia from normally noninvasive flora of the respiratory system. Since the causes for the later deaths are complex, and might be prevented by administration of antibiotics,

those data have not been used in calculations of LC_{50} s. This complication may be mechanistically similar to respiratory symptoms in NaOH vat workers, who have been reported to be antibiotic-responsive.

● Inhalation Hazards to Uranium Miners

This project determines the specific biological effects of exposure to known levels of uranium mine air contaminants, using both large and small experimental animals to model human respiratory disease. Lung cancer and deaths by degenerative lung disease have reached epidemic proportions among uranium miners, but the cause-effect relationships for these diseases are based on inadequate epidemiological data. This project identifies agents or combinations of agents (both chemical and radiological), and their exposure levels, that produce respiratory tract lesions, including respiratory epithelial carcinoma, pneumoconiosis, and emphysema.

INFLUENCE OF RADON-DAUGHTER EXPOSURE RATE AND URANIUM ORE DUST CONCENTRATION ON OCCURRENCE OF LUNG TUMORS

Investigators:

F. T. Cross, R. F. Palmer and R. H. Busch

Technical Assistance:

C. R. Petty, W. Skinner, J. C. Gaven and M. J. Pipes

Groups of male SPF Wistar rats were exposed concurrently to several levels of radon daughters and uranium ore dust to study the effect of these variables on pulmonary disease states. Clinical pathology data at 1 yr postexposure indicate no significant differences among exposed animals when compared with controls. Preliminary histopathologic data suggest a trend toward increasing lung tumor risk as the exposure rate is decreased (constant total dose), but the differences are not statistically significant at the 0.05 level. A similar trend occurs with decrease in ore dust concentration (except for the 2560-WLM exposure group), but these differences are also not significant at the 0.05 level. The tumor risk is significantly (0.05 level) increased as the exposure level increases from approximately 320 and 640 WLM to 2560 WLM at the high ore dust concentration (15 mg/m³).

These studies were initiated in 1977 to test the roles of radon-daughter exposure rate and uranium ore dust concentrations on the production and progression of lung lesions. Human radon-daughter epidemiology suggests that radiation delivered at very low exposure rates is the most efficient in the production of lung cancer. Corroboration in animal studies would indicate that present-day uranium miners, who are exposed at low dose rates, are at greater risk per unit dose than those early miners whose experience was used to derive current limits of exposure.

Groups of 32 or 48 male SPF Wistar rats were exposed for 30 hr/wk to several levels of radon daughters and uranium ore dust, according to the protocols in Tables 35 and 36, and were placed on a periodic sacrifice schedule for observation of developing lung lesions. The ore dust concentrations shown in Table 36 were chosen to maintain low fractions of unattached radon daughters (thus keeping the lung deposition sites constant), while allowing sufficient change in concentration to study the pathogenic role of the dust per se.

TABLE 35. Protocol for Study of Radon-Daughter Exposure Rate Versus Total Exposure in Rats

Exposure Regimen	Cumulative Exposure, WLM
1000 WL Radon Daughters 15 mg/m ³ Uranium Ore Dust	320, 640, 2560
500 WL Radon Daughters 15 mg/m ³ Uranium Ore Dust	640, 2560
250 WL Radon Daughters 15 mg/m ³ Uranium Ore Dust	640
Controls	Background

TABLE 36. Protocol of Uranium Ore Dust Exposures in Rats

Exposure Regimen	Cumulative Exposure, WLM
1000 WL Radon Daughters 15 mg/m ³ Uranium Ore Dust	320, 640, 2560
1000 WL Radon Daughters 3 mg/m ³ Uranium Ore Dust	320, 640, 2560
Controls	Background

Data from approximately 75% of the animals are included in Table 37; some animals are still living, and histopathologic examinations of all dead animals have not been completed. The data indicate a trend toward increased lung tumor risk as the exposure rate is decreased, but this trend is not significant (0.05 level). The data on lung tumor occurrence are statistically significant (0.05 level) as the exposure level increases from approximately 320 and 640 WLM to 2560 WLM at the 15 mg/m³ ore dust concentration. The data also indicate a trend

toward increased lung tumor risk with decrease in ore dust concentration at the lower exposure levels, but the trend is reversed at the high (2560 WLM) level. None of these data are presently significant at the 0.05 level. Whether there is a difference in deposition site at these two dust concentrations is yet to be evaluated. We have conducted experiments which measured radon daughter burden in sections of the respiratory tract as a function of ore dust concentration and radon daughter disequilibrium; these data have not yet been analyzed.

TABLE 37. Current Summary of Primary Epithelial Tumors of the Lung

Exposure Regimen ^(a)			% of Examined Animals with Epithelial Tumors	% Animals Not Examined
Exposure Rate, WL	Total Exposure, WLM	Ore Dust Conc., mg/m ³		
1000	320	15	3.8	45
250	640	15	9.4	0
500	640	15	12	19
1000	640	15	2.6	17
500	2560	15	44	44
1000	2560	15	25	35
1000	320	3	8.8	26
1000	640	3	12	15
1000	2560	3	5.3	39
Controls			0	30

^(a)Nominal Values

PULMONARY FUNCTION EVALUATIONS OF DOGS EXPOSED TO URANIUM ORE DUST

Investigators:

S. M. Loscutoff, R. L. Buschbom, R. F. Palmer and F. T. Cross

Technical Assistance:

J. L. Pappin and W. Skinner

Pulmonary function evaluations were conducted on dogs exposed to carnotite uranium ore dust. Significant changes were detected in the slope of the single-breath N₂ washout curve, suggesting an uneven distribution of ventilation.

Pulmonary function evaluations were conducted on dogs exposed to 14.0 ± 1.9 mg/m³ ($\bar{X} \pm SD$) carnotite uranium ore dust for 4 hr/day, 5 days/wk. Age, body weight and exposure duration are listed in Table 38.

TABLE 38. Age, Exposure Duration and Body Weight in Dogs Exposed to Uranium Ore Dust

		Age, Mo	Length of Exposure, Mo	Body Wt., kg
Control (Old)	$\bar{X}$	56.7	21.8	14.8
	SE	0.3	1.6	0.5
	N	9	9	9
Exposed (Old)	$\bar{X}$	55.9	24.9	13.9
	SE	1.1	0.0	0.5
	N	16	16	16
Control (Young)	$\bar{X}$	36.3	5.6	13.8
	SE	0.1	0.0	0.8
	N	2	2	2
Exposed (Young)	$\bar{X}$	33.1	7.8	13.4
	SE	0.3	0.0	1.1
	N	4	4	4

Pulmonary function measurements included arterial blood gases and respiration rates on awake dogs; and closing volume maneuvers, quasi-static and dynamic compliance, pulmonary resistance, and lung volume on pentothal-anesthetized dogs. Statistical evaluations were based on a two-way analysis of variance, comparing age and treatment effects.

Results of pulmonary function tests are shown in Tables 39-42. Because of differences in group size, effects of age or aerosol exposure at times reflected only differences in one group. For example, a significant effect of aerosol exposure was measured in values of hemoglobin concentration (Table 39). This effect was due to the high hemoglobin values measured in the old control animals

compared with all other groups. Since the same trend was not seen in the young animals, and since values for the young control and young exposed groups were similar to those of the old exposed group, this measurement should be repeated to verify that a reduced hemoglobin concentration is an effect of aerosol exposure. Respiration rate was also significantly higher in the young compared with the old animals (Table 39). This effect is due to a very high respiration rate in one of the young control dogs, and is not a significant physiological effect of aging.

Quasi-static and dynamic pulmonary compliance were significantly lower in young compared with old dogs (Table 40). However, when the data were examined, only the young dogs exposed to ore dust had low compliance values. Because the 16 old exposed dogs showed no change in compliance, even though the four young dogs had substantially lower values for compliance, no effect of aerosol exposure was found. If these young exposed dogs continue to have low compliance when tested further, a significant effect of aerosol exposure might be suggested. Measurements of lung volumes (Table 41) showed no significant effects of age or aerosol exposure.

The slope of the alveolar N₂ plateau (M3), (M3), and the slope of the single-breath N₂ washout curve from closing volume to residual volume (M4), showed significant effects of aerosol exposure (Table 42). Increased slopes during single-breath N₂ washout maneuvers indicate an uneven distribution of ventilation. The results of these measurements indicate that after either 6 mo or 2 yr exposure to uranium ore dust there is a significant decrease in the uniformity of ventilation in the lung. In examining the

TABLE 39. Arterial Blood Gases in Dogs Exposed to Uranium Ore Dust

		O ₂ Hb, %	Hb, g/100 ml	COHb, %	PO ₂ , mm Hg	PCO ₂ , mm Hg	pH	Respiration Rate(a)
Control (Old)	$\bar{X}$	93.2	16.7	2.9	85.8	41.5	7.378	22
	SE	1.3	0.4	0.1	4.5	1.2	0.010	2
	N	9	9	9	9	9	9	9
Exposed (Old)	$\bar{X}$	93.2	15.5	2.9	91.1	41.8	7.363	25
	SE	0.8	0.3	0.1	3.2	1.0	0.009	2
	N	15	15	15	15	15	15	15
Control (Young)	$\bar{X}$	95.7	15.6	2.9	100.3	36.4	7.407	45
	SE	0.2	0.2	0.4	0.2	0.2	0.005	9
	N	2	2	2	2	2	2	2
Exposed (Young)	$\bar{X}$	95.0	15.9	2.4	98.0	39.3	7.354	24
	SE	0.2	0.4	0.1	4.2	2.0	0.014	3
	N	4	4	4	4	4	4	4

Significant Effects
P < 0.05 2-way ANOVA

-- Aerosol -- -- -- -- Age

(a) Breaths per minute

TABLE 40. Pulmonary Mechanics in Dogs Exposed to Uranium Ore Dust

		Resistance (cm H ₂ O/ l /sec)	Dynamic Compliance (ml/cm H ₂ O)	Quasi-Static Compliance (ml/cm H ₂ O)
Control (Old)	$\bar{X}$	2.78	122	130
	SE	0.14	11	5
	N	9	9	9
Exposed (Old)	$\bar{X}$	2.82	120	128
	SE	0.08	7	7
	N	16	16	16
Control (Young)	$\bar{X}$	2.57	113	129
	SE	0.12	1	12
	N	2	2	2
Exposed (Young)	$\bar{X}$	2.99	84	92
	SE	0.12	6	7
	N	4	4	4

Significant Effects
P < 0.05 2-Way ANOVA

-- Age Age

TABLE 41. Lung Volume in Dogs Exposed to Uranium Ore Dust

		Residual Volume RV ml	RV/BW, ml/kg	Functional Residual Capacity, FRC ml	FRC/BW, ml/kg	Vital Capacity, VC ml	VC/BW, ml/kg	Total Lung Capacity, TLC ml	TLC/BW, ml/kg
Control (Old)	$\bar{X}$	310	20.9	524	35.1	1123	75.8	1433	96.7
	SE	15	1.6	39	1.7	46	2.0	59	2.3
	N	9	9	9	9	9	9	9	9
Exposed (Old)	$\bar{X}$	346	25.6	554	40.4	1141	82.3	1487	108
	SE	25	2.3	24	2.3	41	2.3	52	4.2
	N	16	16	16	16	16	16	16	16
Control (Young)	$\bar{X}$	406	29.5	621	45.1	1195	86.3	1601	116
	SE	31	0.6	46	0.8	208	10.5	240	11.1
	N	2	2	2	2	2	2	2	2
Exposed (Young)	$\bar{X}$	301	22.0	489	36.0	964	72.0	1265	94.0
	SE	50	2.0	72	2.6	82	1.1	130	2.4
	N	4	4	4	4	4	4	4	4

No Significant Effects of Age or Aerosol Exposure

TABLE 42. Results of Single Breath N₂ Washout Maneuvers in Dogs Exposed to Uranium Ore Dust

		M3, % N ₂ /ℓ	M4, % N ₂ /ℓ	Closing Volume, ml	Closing Volume/ Vital Capacity, %	Closing Capacity, ml	Closing Capacity/ Total Lung, Capacity, %
Control (Old)	$\bar{X}$	0.61	4.34	91.2	8.3	401	28.1
	SE	0.04	0.63	10.8	1.1	17	1.0
	N	9	9	9	9	9	9
Exposed (Old)	$\bar{X}$	0.86	5.73	73.7	6.5	420	27.8
	SE	0.05	0.35	5.3	0.4	25	1.2
	N	16	16	16	16	16	16
Control (Young)	$\bar{X}$	0.56	3.88	85.1	7.1	491	31.0
	SE	0.08	0.84	15.2	0.0	46	1.8
	N	2	2	2	2	2	2
Exposed (Young)	$\bar{X}$	0.95	5.82	65.4	6.6	366	28.3
	SE	0.14	1.17	13.8	1.0	64	2.3
	N	4	4	4	4	4	4
Significant Effects P < 0.05 2-way ANOVA		Aerosol	Aerosol	--	--	--	--

magnitude of the changes, dogs exposed for only 6 mo appear to be affected as much as dogs exposed for 2 yr. If the change is as great after 6 mo as after 2 yr of exposure, when (after beginning exposure) does the

distribution of ventilation begin to change; and how long does it continue to change before reaching a new, relatively stable level?

PULMONARY FUNCTION EVALUATIONS OF DOGS EXPOSED TO CIGARETTE SMOKE

Investigators:

S. M. Loscutoff, R. L. Buschbom, R. F. Palmer and F. T. Cross

Technical Assistance:

J. L. Pappin and C. R. Petty

Pulmonary function evaluations were conducted on dogs exposed to cigarette smoke from 10 cigarettes per day. The most consistent effects of exposure were decreases in lung volumes.

Pulmonary function evaluations were conducted on dogs exposed to cigarette smoke from 10 cigarettes/day (University of Kentucky, 1R1 research cigarettes) for 7 days/wk. Age, body weight and exposure duration are listed in Table 43. Three of the dogs also received a total radon daughter exposure of about 4000 working level months (WLM) during 1973 and 1974. During this same period, they were simultaneously exposed to 15 mg/m³ uranium ore dust. One of these dogs has subsequently been exposed to cigarette smoke, along with the other smoke-exposed dogs. Although values for the three radon daughter-exposed dogs are listed in the tables, these

dogs were not included in the statistical analyses because of the small sample size and because no age-matched controls were tested.

Pulmonary function measurements included arterial blood gases and respiration rates on awake dogs, and closing volume maneuvers, quasi-static and dynamic compliance, pulmonary resistance, and lung volumes on pentothal-anesthetized dogs. Statistical evaluations were based on a two-way analysis of variance, comparing age and treatment effects. Results of pulmonary function tests are shown in Tables 44-47.

TABLE 43. Age, Exposure Duration and Body Weight in Dogs Exposed to Cigarette Smoke and Radon Daughters

		Age, Mo	Length of Exposure, Mo	Body Wt, kg
Control (Old)	$\bar{X}$	136	107	13.6
	SE	2	3	0.6
	N	5	5	5
Smoke (Old)	$\bar{X}$	141	114	12.3
	SE	3	2	0.6
	N	11	11	11
Control (Young)	$\bar{X}$	33.6	18.4	9.6
	SE	0	0	1.2
	N	2	2	2
Smoke (Young)	$\bar{X}$	34.1	15.3	12.5
	SE	0.5	3.1	0.6
	N	4	4	4
Radon	$\bar{X}$	101.2	73.3	11.8
	SE	0	0	0.2
	N	2	2	2
Radon + Smoke	$\bar{X}$	101.2	73.8	12.3
	SE	--	--	--
	N	1	1	1

TABLE 44. Arterial Blood Gases in Dogs Exposed to Cigarette Smoke and Radon Daughters

		Resistance, cm H ₂ O/ℓ /sec	Dynamic Compliance, ml/cm H ₂ O	Quasi-Static Compliance, ml/cm H ₂ O
Control (Old)	$\bar{X}$	2.89	86	121
	SE	0.16	6	15
	N	5	5	5
Smoke (Old)	$\bar{X}$	3.19	56	98
	SE	0.16	6	7
	N	11	11	11
Control (Young)	$\bar{X}$	2.47	141	148
	SE	0.15	63	54
	N	2	2	2
Smoke (Young)	$\bar{X}$	2.87	114	123
	SE	0.09	20	18
	N	4	4	4
Radon	$\bar{X}$	3.37	56	102
	SE	0.46	8	6
	N	2	2	2
Radon + Smoke	$\bar{X}$	2.65	67	102
	SE	--	--	--
	N	1	1	1
Significant Effect P < 0.05 2-way ANOVA		--	Age	

TABLE 45. Pulmonary Mechanics in Dogs Exposed to Cigarette Smoke and Radon Daughters

		O ₂ Hb, %	Hb, g/100 ml	COHb, %	PO ₂ , mm Hg	PCO ₂ , mm Hg	pH	Respiration Rate (a)
Control (Old)	$\bar{X}$	95.3	15.1	3.0	96.4	38.0	7.408	36
	SE	0.6	1.0	0.3	3.0	1.4	0.009	5
	N	5	5	5	5	5	5	5
Smoke (Old)	$\bar{X}$	94.1	14.3	2.9	91.6	44.2	7.375	26
	SE	0.4	0.3	0.1	2.3	1.2	0.006	2
	N	11	11	11	11	11	11	11
Control (Young)	$\bar{X}$	94.8	15.1	2.1	97.2	35.8	7.391	28
	SE	0.2	1.0	0.1	0.1	1.2	0.010	--
	N	2	2	2	2	2	2	1
Smoke (Young)	$\bar{X}$	94.2	16.6	2.2	96.9	41.8	7.367	21
	SE	0.2	0.1	0.2	3.4	1.3	0.013	1
	N	4	4	4	4	4	4	4
Radon	$\bar{X}$	92.6	15.7	3.0	84.4	44.3	7.361	21
	SE	1.6	1.1	0.0	8.7	1.7	0.021	5
	N	7	2	2	2	2	2	2
Radon + Smoke	$\bar{X}$	93.4	13.7	2.8	80	47.9	7.343	17
	SE	--	--	--	--	--	--	--
	N	1	1	1	1	1	1	1
Significant Effect < 0.05 2-way ANOVA		--	Age	Age	--	Smoke	Smoke	Smoke

(a) Breaths per minute

Measurements of arterial blood gases (Table 44) showed effects of both age and smoke exposure. Hemoglobin concentration was significantly lower in old compared with young dogs, while percent carboxyhemoglobin was higher in old compared with young dogs. Both these effects probably reflect an increase in hemolytic processes in old dogs, reducing the concentration of hemoglobin and (since carbon monoxide is formed during hemolysis) increasing the level of carboxyhemoglobin. Arterial partial pressure of carbon dioxide (PaCO_2), pH, and respiration were all significantly affected by smoke exposure. The PaCO_2 was significantly higher in smoke-exposed compared with control dogs and, because CO_2 acts like a weak acid in blood, the pH was significantly lower in smoke-exposed compared with control animals. Respiration rate was significantly slower in smoke-exposed compared with control dogs. These differences in respiration rate, PaCO_2 and pH, although they appear to be smoke-related, may be caused by greater anxiety in the control dogs, thus increasing respiration rate and causing them to lose CO_2 . In other studies with dogs (and also in humans), as the lung becomes diseased, arterial oxygen (rather than CO_2) is usually the first parameter to be affected. Carbon dioxide is a much more soluble gas than O_2 , and is therefore often normal in early pulmonary impairment, while O_2 is decreased. In these dogs, O_2 levels were not different in smoke-exposed and control animals, suggesting that gas exchange was not impaired. The low CO_2 values in control animals were probably caused by greater anxiety in these animals due to handling, since these dogs are no longer being sham-exposed and are not used to handling.

Dynamic pulmonary compliance (Table 45) was significantly lower in old compared with young dogs but was not significantly affected by smoke exposure. Pulmonary resistance and quasi-static pulmonary compliance showed no effect of age or smoke exposure.

Lung volumes and lung volumes divided by body weight (Table 46) were significantly affected by age and smoke exposure. Residual volume, divided by body weight, was significantly lower in old compared with young animals. Other lung volumes affected by both age and smoke exposure were functional residual capacity (FRC), FRC divided by body weight, vital capacity divided by body weight, and total lung capacity divided by body weight. In all cases, lung volumes were lower in old than young animals, and lung volumes, except for residual volume, were lower in smoke-exposed compared with control animals.

Single-breath N_2 washouts (Table 47) showed significant age effects but no significant effects of smoke exposure. Closing volume, closing volume divided by vital capacity, and closing capacity (closing volume plus residual volume) were all significantly lower in old compared with young animals. These results suggest that, whatever factors determine the lung volume at which airways begin to close, these factors occur at lower lung volumes in old compared with young animals. Under these exposure conditions, cigarette smoke does not appear to affect the distribution of ventilation assessed from changes in the slope of the N_2 washout curve or airway closure as assessed from measurements of closing volume.

TABLE 46.¹ Lung Volume in Dogs Exposed to Cigarette Smoke and Radon Daughters

		Residual Volume RV, ml	RV/BW, ml/kg	Functional Residual Capacity FRC, ml	FRC/BW, ml/kg	Vital Capacity VC, ml	VC/BW ml/kg	Total Lung Capacity TLC, ml	TLC/BW ml/kg
Control (Old)	$\bar{X}$	343	25.1	491	36.1	1112	81.0	1455	106
	SE	33	2.0	23	1.0	120	5.9	131	5.1
	N	5	5	5	5	5	5	5	5
Smoke (Old)	$\bar{X}$	286	23.3	391	32.0	934	75.7	1219	99.0
	SE	21	1.4	28	2.1	57	2.6	72	3.3
	N	11	11	11	11	11	11	11	11
Control (Young)	$\bar{X}$	356	37.5	644	66.7	1274	131	1630	169
	SE	11	3.5	108	2.9	278	12.6	289	9.0
	N	2	2	2	2	2	2	2	2
Smoke (Young)	$\bar{X}$	365	29.2	556	44.3	1158	92.3	1524	121
	SE	26	1.5	38	1.0	104	6.3	129	7.6
	N	4	4	4	4	4	4	4	4
Radon	$\bar{X}$	289	24.6	327	27.8	740	62.8	1028	87.4
	SE	33	2.3	37	2.5	30	1.2	63	3.6
	N	2	2	2	2	2	2	2	2
Radon + Smoke	$\bar{X}$	320	26.4	476	39.3	881	72.8	1201	99.3
	SE	--	--	--	--	--	--	--	--
	N	1	1	1	1	1	1	1	1
Significant Effect <0.05 2-way ANOV,		--	Age	Age Smoke	Age Smoke	--	Age Smoke	--	Age Smoke

TABLE 47. Results of Single Breath N₂ Washout Maneuvers in Dogs Exposed to Cigarette Smoke and Radon Daughters

		M3, % N ₂ /ℓ	M4, % N ₂ /ℓ	Closing Volume, ml	Closing Volume/ Vital Capacity, %	Closing Capacity, ml	Closing Capacity/ Total Lung Capacity, %
Control (Old)	$\bar{X}$	0.85	8.49	66	6.2	410	28.6
	SE	0.18	1.20	4	0.6	36	2.8
	N	5	5	5	5	5	5
Smoke (Old)	$\bar{X}$	1.05	11.63	51	5.6	337	27.6
	SE	0.09	0.77	4	0.4	23	1.1
	N	11	11	11	11	11	11
Control (Young)	$\bar{X}$	0.67	4.12	94	7.4	450	28.2
	SE	0.08	1.30	19	0.1	30	3.2
	N	2	2	2	2	2	2
Smoke (Young)	$\bar{X}$	0.76	3.83	106	9.7	471	31.4
	SE	0.04	0.20	16	2.1	12	2.0
	N	4	4	4	4	4	4
Radon	$\bar{X}$	1.95	9.27	48	6.4	336	32.6
	SE	0.04	1.02	4	0.2	37	1.4
	N	2	2	2	2	2	2
Radon + Smoke	$\bar{X}$	1.07	8.20	64	7.2	384	31.9
	SE	--	--	--	--	--	--
	N	1	1	1	1	1	1
Significant Effect <0.05 2-way ANOVA		--	Age	Age	Age	Age	--

• Toxicology of Krypton-85

The purpose of this project is to obtain biological data to supplement earlier evaluations of the hazards of ^{85}Kr exposure. The studies include both short-term and chronic exposures of rats, dogs and sheep to determine tissue distribution and retention kinetics for metabolic modeling purposes. Dose-effect studies in rats exposed chronically for their life span are included to identify tissues at risk and determine tumorigenic potency.

EFFECT OF CHRONIC ^{85}Kr EXPOSURE

Investigators:

D. H. Willard, H. S. DeFord and J. E. Ballou

Groups of rats exposed continuously for 15 mo to ^{85}Kr atmospheres have received measured dose rates of 2750, 370 and 38 rad/yr. With more than 75% of the rats still alive, there has been no dose-related effect on weight gain but a probably significant effect on survival.

Krypton-85, a radioactive noble gas, is released to the environment during nuclear reactor operation and fuel reprocessing. The studies reported here concern the biological effects of lifetime exposure of rats to ^{85}Kr atmospheres ranging from 10^2 to 10^4 times the maximum permissible air concentration, $3 \times 10^{-7} \mu\text{Ci/ml}$. A detailed experimental protocol was presented in the Annual Report for 1978.

Adult male and female Wistar rats, 50 of each sex per dose group, are exposed to ^{85}Kr atmospheres of 3×10^{-5} , 3×10^{-4} and $3 \times 10^{-3} \mu\text{Ci/ml}$; a control group is exposed under identical conditions to room air. Exposures continue 24 hr per day, 7 days per week with scheduled 4-hr interruptions at monthly intervals to weigh rats, transfer them to clean racks and cages, and clean exposure chambers. Animals are fed daily through glove ports in the exposure chamber, and dead and moribund animals are removed through a pass-through for necropsy and radiographic examination. Krypton-85 is supplied to the high-level chamber, under pressure, from a compressed-gas cylinder; diluent air is added by means of a jet pump designed to maintain a flow rate of 500 l/min

through the chamber. The activity level of the high-level chamber is monitored with a Triton radioactive-gas monitor equipped with a chart recorder. The ^{85}Kr for lower-level chambers is drawn from the exhaust stream of the next-higher-level chamber, and combined with appropriate diluent air to make up the exposure atmospheres.

Weight records and mortality percentages are available for the 15 mo during which the study has been in progress. No significant differences in weight have been observed among the different exposure groups (Figure 34). The erratic course of the average weight curves is due to the death of sick animals and the resulting change in weight averages due to the loss of the emaciated subjects.

Dose-related differences in mortality rates (Figure 35) between the higher and lower exposure groups are already apparent. Approximately 25% of the rats in the two highest-level exposure groups have died, compared to only 10% of the low-level and control animals. Most of the mortality among female rats has resulted from mammary tumors; 39 females have died vs. 28 males. Deaths occurred after 6-8 mo exposure in the

FIGURE 34. Weight Gain of Rats Exposed Chronically to ⁸⁵Kr Atmospheres

two high-dose groups, while the earliest deaths in the low-level and control groups occurred after 10-11 mo.

Rats in the highest-dose group, and controls, were screened for leukemia after 1 yr of continuous exposure. Blood samples with white cell counts in excess of 15,000 cells/mm³ were subjected to additional differential cell counts. None of the differential counts were considered to be indicative of leukemia. Additional tests for leukemia will be made at 6-mo intervals. Blood analyses included total white cell count, red blood cell count, hemoglobin,

hematocrit, mean cell volume and mean cell hemoglobin concentration.

The radiation dose to rats housed in the exposure chambers is measured by thermoluminescent dosimeters (TLDs) suspended between cages in simulated animal locations. The TLDs are replaced each month. The average, integrated, yearly dose (based on TLDs for rats exposed to the high, medium or low dose of ⁸⁵Kr) is 2750, 370 and 38 rad/yr, respectively. The radiation dose estimated from the TLD readings is slightly higher (10%) than the calculated dose reported in last year's Annual Report.

FIGURE 35. Cumulative Mortality of Rats Exposed Chronically to ^{85}Kr Atmospheres

TOTAL BODY EXPOSURE OF NEWBORN RATS IN ^{85}Kr ATMOSPHERES

Investigators:

H. S. DeFord and J. E. Ballou

Newborn rats exposed to 2300-4800 rad total body radiation from ^{85}Kr developed radiation dermatitis, and showed stunted growth and abnormal development of ears, eyelids and paws. A dose of 1000 rad was tolerated without grossly observable effect.

Irradiation of the skin is considered to be the primary hazard associated with ^{85}Kr exposure, since the gas is poorly absorbed and rapidly excreted via respiration, and the principal beta emission is absorbed in surface skin layers. Earlier reports by Mahlum (Annual Report, 1969) have shown the skin of newborn rats to be more susceptible to beta radiation effects than the skin of adults. It is of interest to investigate the effect of ^{85}Kr on skin of newborns since the gas is ubiquitous in the environment and all segments of the human population, including infants, are exposed to ^{85}Kr radiation.

Newborn male and female Wistar rats, less than 48 hr of age, were exposed continuously for 6 hr to ^{85}Kr atmospheres. The exposure chamber consisted of a series of connected, 8-in., glass bell jars. The chambers were mounted on a cage rack that was housed in a ventilated enclosure, vented to the building exhaust. The infant rats were suspended separately within the bell jars in groups of five, each held in a 1/2-in.-mesh, galvanized-metal screen holder. The ^{85}Kr atmosphere was recirculated through the exposure system by a pump. A physiological breathing atmosphere was maintained with CO_2 and moisture absorbents; oxygen was replaced on demand as CO_2 absorption reduced the pressure within the closed system.

Preliminary rangefinding studies with this exposure system indicated that an atmospheric concentration of about $23 \mu\text{Ci/ml}$ would produce a skin surface dose of about 4300 rad in 6 hr, as measured by thermoluminescent dosimeters. Newborn rats exposed to this dose regimen survived the radiation insult but sustained permanent damage to the ears and paws, and permanent thinning of the hair coat.

The present study includes three dose groups, exposed to 1000, 2300, or 4800 rad accumulated over a 6-hr continuous exposure period, and a control group exposed to room air under identical conditions.

Exposed animals were grossly indistinguishable from controls until 5 days after the radiation exposure. Severe effects then became apparent in the groups receiving 2300 and 4800 rad. Two animals in the 4800-rad group apparently died and were cannibalized by the dam. Grossly observed effects included stunted growth, poorly developed toes, ears and eyelids, and desquamating epithelium with pinpoint lesions and reduced hair growth. The effects were more severe in the 4800-rad group, but damage was significant in both groups. Rats exposed to 1000 rad were not affected and were indistinguishable from controls. (Figure 36) illustrates the effects observed 12 days after exposure.

Immediately after exposure, histopathologic examination of skin sections showed no effects that could be attributed to ^{85}Kr radiation. After 6-7 days there was evidence of radiation dermatitis in the two highest dose groups, including neutrophils and fibrosis in the subepithelial dermis and slight edema in the epidermis. The changes were most severe in animals exposed to 4800 rad. No changes were observed in controls or in animals exposed to 1000 rad.

We will continue observation of the progressive damage/repair process of the radiation-induced lesions throughout the life span of these animals. Since the study has been in progress for only about 60 days, there is little to report on dose-related weight changes, mortality or tumor incidence, which are expected to be important late effects of this radiation insult.

FIGURE 36. Gross Effect of ^{85}Kr Radiation 12 Days after Exposure

• Toxicity of Thorium Cycle Nuclides

The purpose of this project is to investigate the biological hazards associated with uranium-thorium breeder fuels and fuel recycle process solutions. Initial studies will emphasize the metabolism and long-term biological effects of inhaled ^{233}U - ^{232}U nitrate and oxide fuel materials and ^{231}Pa , a major long-lived radioactive waste product.

DISPOSITION AND LATE EFFECTS OF INHALED $^{232}\text{UO}_2(\text{NO}_3)_2$ AND $^{233}\text{UO}_2(\text{NO}_3)_2$ IN RATS

Investigators:

J. E. Ballou, R. A. Gies and A. C. Case^(a)

^{232}U and ^{233}U appeared to be metabolized similarly in rats except for an unexplained greater lung retention of ^{232}U in one dose group. Retention in skeleton was similar for the two isotopes. Preliminary results indicate that ^{232}U and ^{233}U are about equally effective in inducing lung tumors.

The metabolism and life-shortening effects of inhaled uranium isotopes of high specific activity (^{233}U and ^{232}U) have been reported previously (Annual Report, 1978). This report summarizes recent progress in these studies.

The experimental protocol is described briefly in Table 48. Male Wistar rats, 60 days of age, were exposed for 30 min, nose-only, to aerosols of uranyl nitrate, generated from 0.27N nitric acid solutions, using a Lovelace nebulizer. All animals held for study of long-term effects have died; tissues are being examined for histopathologic changes. Animals sacrificed periodically after exposure have been analyzed for ^{232}U and ^{233}U content to permit comparison of metabolism, and to estimate early radiation dose to tissues with significant deposition.

Pulmonary retention of inhaled $^{233}\text{UO}_2(\text{NO}_3)_2$ is shown for the three exposure groups in Figure 37. Early clearance kinetics varied during the first week after exposure because of differences in aerosol particle size

TABLE 48. Experimental Protocol

Initial Lung Burden (nCi $\pm$ SD)		Number of Rats	
		Periodic Sacrifice	Late Effects Study
$^{233}\text{UO}_2(\text{NO}_3)_2$ (ng)			
0.63 $\pm$ 0.2	66.2	35	65
10 $\pm$ 2	1050	35	65
36 $\pm$ 14	3759	35	65
$^{232}\text{UO}_2(\text{NO}_3)_2$			
0.74 $\pm$ 0.2	0.037	35	65
5.85 $\pm$ 3	0.29	35	65
53 $\pm$ 14	2.5	35	65
Controls			
Treated (0.27 N HNO_3)			62
Nontreated			45

distribution (the largest particle size was associated with the greatest initial lung burden, ILB), and differences in the mass of uranium deposited. Retention half-lives after the first week were comparable for all dose groups, ranging from 65 to 85 days.

^(a) Physical Sciences Department

FIGURE 37. Retention of Inhaled $^{233}\text{UO}_2(\text{NO}_3)_2$ in the Lung

FIGURE 38. Retention of Inhaled $^{232}\text{UO}_2(\text{NO}_3)_2$ in the Lung

Animals exposed to the three dose levels of inhaled $^{232}\text{UO}_2(\text{NO}_3)_2$ showed similar clearance from the lung (Figure 38) except for the group with the largest ILB (53 nCi). Clearance from the latter group was best described by a single exponential function with a half-life of 45 days. No explanation is available for this apparent difference in rate of clearance. Particle size (0.34–0.43 μm) was very similar for the three dose groups, and the mass of uranium is probably of negligible consequence, considering the high specific activity (0.046 g/Ci) of this isotope.

Pulmonary retention of the highest dose levels of ^{232}U and ^{233}U are compared in Figure 39. The points represent averages of five rats; standard deviations are indicated. Differences in lung clearance for the two uranium isotopes appear to be statistically significant for approximately the first 150 days after inhalation exposure.

Subsequent clearance, after 150 days, is fairly comparable for the two isotopes. The difference in early pulmonary clearance kinetics results in a relatively greater radiation dose to the lung from ^{232}U . A major part of the dose is accumulated during the first 2 mo postexposure, before the lung burden is appreciably reduced.

FIGURE 39. Retention of Inhaled Uranyl Nitrate in the Lung

The major reservoir of translocated uranium was the skeleton. Deposition and retention of ^{233}U in skeleton are shown in Figure 40. There appeared to be no significant difference in early deposition or later retention of ^{233}U for the different dose groups. An amount equivalent to between 10 and 20% of an ILB was deposited after 7 days; from 6 to 10% was retained after 200 days. Retention half-lives ranged from 100 to 350 days.

Completed results for animals exposed to ^{232}U are not yet available, except for those of the highest dose level. These data are shown in Figure 41, and are compared with the highest dose level of ^{233}U . Also shown are preliminary results, based on relatively few analyses, which indicate a preferential uptake and retention of ^{232}U by the skeleton. This finding was not confirmed by subsequent results, as indicated by the solid curve for ^{232}U shown (with standard deviations) in

FIGURE 40. Retention of Inhaled $^{233}\text{UO}_2(\text{NO}_3)_2$ in the Skeleton

Figure 41. There appeared to be no significant difference in early deposition or retention of ^{232}U and ^{233}U for the first 200 days postexposure. Data for ^{232}U retention at later time points are not yet available.

Only preliminary results are available for lung tumor incidence in groups exposed to the highest dose levels of ^{232}U and ^{233}U . Animals exposed to ^{233}U accumulated 130 rad in 500 days, and had a lung tumor incidence of 22%. Rats exposed to ^{232}U sustained a radiation dose of 350 rad over 500 days, and had a tumor incidence of 67%. The dose calculation for ^{232}U is tentative, and will require further adjustment since it does not include the dose from ^{232}U decay products. Studies are now in progress to determine the parent-daughter relationships in major tissues to better define the dose contribution from the ^{232}U daughter products.

FIGURE 41. Retention of Inhaled Uranyl Nitrate in the Skeleton

COMPARATIVE TOXICOLOGY OF INHALED ACTINIDE NITRATES IN RATS

Investigators:

J. E. Ballou, R. A. Gies and A. H. Beasley

A preliminary comparison of data being obtained on the metabolism, cumulative radiation dose and tumorigenesis of actinide nitrates in rats suggests there is no unusual toxicity associated with the inhalation of high-specific-activity uranium isotopes (^{233}U and ^{232}U) compared to the toxicity of other inhaled actinide nitrates.

Investigation of the comparative toxicity of inhaled actinide nitrate aerosols has identified a number of factors which may influence the initial disposition and ultimate metabolism and toxicity of seemingly similar radioisotopes. Among these factors are particle size distribution of the aerosol, mass of material deposited and subtle differences in chemical/physical form of the aerosolized materials. In order to monitor these parameters, the aerosol exposure protocols have included carefully scheduled preparation of radionuclide solutions to assure comparable chemical/physical species, characterization of particle size, chamber atmosphere and generator solutions, along with determination of early deposition patterns in the animals. It is obvious that not all potential problems (e.g., differences in mass due to large differences in specific activity) can be resolved when such materials are compared. However, these differences can be identified and their implications considered in the interpretation of results.

In this article, data have been selected from several similar studies with inhaled actinide nitrates which used male Wistar rats exposed, nose-only, for 30 min to aerosols generated from 0.27N nitric acid solutions of ^{238}Pu , ^{239}Pu , ^{241}Am , ^{253}Es , ^{233}U or ^{232}U . Experimental groups were selected in which the total amount of radioactivity initially deposited was fairly similar. The long-term biological effects were also expected to be similar in quality and magnitude for radionuclides of comparable toxicity. The initial disposition of radioactivity, as determined immediately after exposure in groups of 10 rats for each radionuclide, is shown in Table 49a. The total initial deposition of ^{253}Es was unusually large because of the short physical

half-life of 20.5 days (effective half-life in lung, 10 days) and the requirement for a large initial lung burden to deliver a radiation dose that was in the tumorigenic range.

The effect of particle size on deposition in the upper respiratory tract (URT) is clearly seen by the increased deposition of the larger particle size distribution (see for example, ^{233}U and ^{239}Pu). Translocation to tissues (liver and carcass residue) is apparently not related to particle size but is associated with the individual radionuclide. The relatively large translocation of ^{239}Pu to the carcass residue is probably an artifact due to contamination, since values for one animal were unusually high in the group sacrificed immediately after exposure.

It is of interest that deposition in the lung was notably less with the uranium isotopes (7 to 9% of the total amount deposited) than with the transuranic elements (24 to 28% of that deposited). Such differences in pulmonary deposition are typically due to variations in particle size distribution, but this was not the case with the uranium isotopes. Aerosols containing particles of 2.12 μm AMAD (^{233}U) and 0.36 μm (^{232}U) deposited similar percentages in the lung. That is to say, the amount recovered in the lung after the 30-min exposure was similar for ^{232}U and ^{233}U but relatively less than the amount recovered after exposure to the transuranic elements. It is possible that clearance from the lung and translocation to excretory pathways during the 30-min exposure period could account for the relatively low initial lung burden of the uranium isotopes. This possibility is supported by the cumulative excretion curves shown in Figures 42 and 43.

TABLE 49a. Initial Disposition of Inhaled Actinide Nitrates in Rats

	²³³ U	²³² U	²³⁹ Pu	²³⁸ Pu	²⁴¹ Am	²⁵³ Es
Total Deposited (nCi)	510	608	318	247	381	1523
Aerosol Characteristics						
AMAD (μm) (a)	2.12	0.36	1.04	0.44	0.35	0.49
GSD (b)	2.19	2.24	1.73	1.65	2.02	1.70
Distribution (% Deposited)						
URT (c)	62	20	30	18	18	25
Lung	7	9	24	28	25	28
Liver	0.2	0.7	0.01	0.05	0.1	0.13
Carcass Residue	3	3.3	8	1.6	1.2	2.1
Pelt	28	67	37	52	56	45

(a) Activity Median Aerodynamic Diameter

(b) Geometric Standard Deviation

(c) Upper respiratory tract; includes head, trachea, esophagus, stomach, and jejunum

FIGURE 42. Cumulative Urinary Excretion of Inhaled Actinide Nitrates

Urinary excretion of inhaled uranium was 5 to 10 times greater on the first day after exposure than that of comparable transuranic elements (Figure 42). The early fecal excretion of uranium was also greater than that observed for the transuranic elements (Figure 43). Since fecal clearance might also include uranium excreted via bile, the possibility of rapid clearance of uranium from the lung during the exposure period cannot be ruled out at this time. Studies are in progress to better define the early disposition of inhaled uranium and gain some understanding of the early translocation processes.

Uranium-233 was rapidly cleared from the lung, similar to ²⁴¹Am, during the first few weeks after exposure. Later clearance (after 100 days) appeared to parallel that of ²³⁹Pu (Figure 44). Completed analyses are not available for ²³²U, but partial results indicate that retention of the two isotopes will be the same. The clearance of ²⁵³Es closely followed that of ²⁴¹Am for the first 100 days after exposure. No further data were obtained because of the short half-life of ²⁵³Es.

FIGURE 43. Cumulative Fecal Excretion of Inhaled Actinide Nitrates

Retention in the skeleton, the main reservoir of translocated actinides, is shown in Figure 45. Americium-241 and ²³⁸Pu are characterized by preferential translocation to the skeleton and tenacious retention of the radioelements over a long period of time. Relatively less ²³⁹Pu was initially translocated to bone, and it was lost more rapidly than was either ²⁴¹Am or ²³⁸Pu. Uranium-233 retention (and that of ²³²U, from all indications) is unique in that it is best described by two exponential functions with half-lives of about 100 days and 2000 days, respectively. Uranium retention appeared to follow that of ²³⁹Pu more closely than the other actinides.

Actinide retention in the liver was over an order of magnitude less than that in the skeleton (Figure 46). The curves for ²⁴¹Am and ²³⁸Pu appeared to correspond fairly well, while those for ²³³U and ²³⁹Pu were set apart at a value about 1/10 to 1/2 the ²⁴¹Am-²³⁸Pu level. The kidney, traditionally the critical organ for uranium toxicity, contained about the same amount of ²³³U as the liver. Since the kidney is considerably smaller than the liver, the radiation dose to kidney would be about fivefold larger because of the mass difference.

The radiation dose accumulated over the initial 500 days in the major tissues of animals in the various exposure groups is shown in Table 49b. Also shown is the tumor

incidence observed in lung and skeleton at the time of death. It appears from these results that inhaled ²⁵³Es and ²⁴¹Am were relatively inefficient at delivering a radiation dose to the lung, and were correspondingly inefficient in inducing lung tumors. This is presumably due to the greater dose rate, compared to the more prolonged radiation insult from the other radionuclides. Bone tumors were produced about equally by all the actinides. It should be noted that the radiation dose estimated for ²³²U included only that dose associated with the decay of the parent, and did not take into account the radiation dose from daughter products. Since daughter decay could result in a significant increase in the dose estimate for the lung, it probably explains the apparent high incidence for lung tumors in rats exposed to ²³²U. The incidence of bone tumors has not yet been confirmed by histopathologic examination, but it is expected to be no higher than has been observed with the plutonium isotopes.

Although this comparison is of a preliminary nature, and has not included complete results from all the studies cited, the results suggest that there is no unusual toxicity associated with the radionuclides we have selected. The studies with ²⁴¹Am and uranium are still in progress; early results suggest a trend similar to that observed with the plutonium isotopes.

FIGURE 44. Retention of Inhaled Actinide Nitrates in Rat Lung

TABLE 49b. Cumulative Radiation Dose and Lung and Bone Tumor Incidence for Rats Exposed to Actinide Nitrate Aerosols

Radionuclide	ILB, nCi	Number of Rats	500-Day Cumulative Radiation Dose, rad			Tumor Incidence,%	
			Lung	Skeleton	Liver	Lung	Skeleton
²³⁹ Pu	78	20	610	17	1	45	5
²³⁸ Pu	70	52	425	76	3	46	10
²³³ U	36	63	134	12	0.4	22	NA ^(a)
²³² U	53	60	350	35	0.6	67	NA ^(a)
²⁴¹ Am	95	60	225	100	8	5 ^(b)	20 ^(b)
²⁵² Es	520	60	390	68	4.5	27	7

^(a) NA = Not available at this time

^(b) Tumor incidence based on gross observation at necropsy and examination of radiographs

FIGURE 45. Retention of Inhaled Actinide Nitrates in Rat Skeleton

FIGURE 46. Retention of Inhaled Actinide Nitrates in Rat Liver

URANIUM REMOVAL THERAPY

Investigators:

R. A. Gies and J. E. Ballou

Therapeutic treatment regimens used in this study, including DTPA, NaHCO_3 , sodium salicylate and combinations of these agents, were notably ineffective in uranium removal.

Methods for uranium decorporation are of interest because of the potential for personnel exposure to the highly radioactive isotopes, ^{233}U and ^{232}U , which are produced in the thorium breeder process. Exposure to ^{233}U or ^{232}U represents a radiological hazard unlike the chemical toxicity usually associated with the isotopes of lower specific activity, ^{238}U and ^{235}U , used in more conventional reactors.

Forty young adult, female, Wistar rats, average weight 200 g, were injected intravenously via the great saphenous vein with 0.25 ml of a subacutely toxic dose of ^{233}U citrate. Thirty-five of these rats (five per group) were administered therapeutic agents by intraperitoneal injection 1 hr after ^{233}U administration. Five rats served as nontreated controls. Ten additional rats (five per group) were injected intravenously with a mixture of DTPA and ^{233}U , or with a mixture of DTPA plus sodium salicylate and ^{233}U . The ^{233}U dose in the latter two groups was 1/50 the amount injected as ^{233}U citrate. Details of the experimental protocol are given in Table 50.

The ^{233}U citrate injectant was prepared immediately before administration, using a 2N nitric acid solution of ^{233}U with added sodium citrate (1%) and sufficient sodium hydroxide to neutralize the solution. Injectants containing mixtures of ^{233}U and DTPA, or DTPA plus sodium salicylate, were prepared several days before use and were stored in a refrigerator.

After injection, the rats were housed in metabolism cages, where urine and feces were collected daily for 4 days. The animals were then sacrificed. The pelt, feet and GI tract were carefully removed to avoid contamination of the remaining carcass, and were discarded. Tissues analyzed for ^{233}U content included liver, kidneys, skeleton, and residual soft tissue. The injection site was excised and analyzed

separately to verify injection integrity. Tissue and excreta samples were dry-ashed at 400-500°C, solubilized in 2N HNO_3 , and analyzed by liquid scintillation counting, using the method of Keough and Powers (1970).

The agents used were selected because of their demonstrated effectiveness in uranium removal, as reported in the literature. Sodium salicylate in combination with DTPA was used because of the recent report of its effectiveness in heavy metal removal (Schubert and Derr, *Nature*, 1978). Two groups were injected with premixed combinations of chelating agent and ^{233}U to demonstrate the in vivo stability of the uranium-DTPA complex, and to illustrate the potential maximum effectiveness of removal under optimal conditions of therapy.

The results of tissue and excreta analyses are shown in Table 50. The percentage of the injected ^{233}U retained in the kidneys is dramatically lower in the two groups administered the chelated ^{233}U , and deposition in the skeleton is also relatively lower compared to that in rats injected with ^{233}U citrate, followed by removal therapy. Both urinary and fecal excretion of ^{233}U was enhanced in rats administered chelated ^{233}U , implying that uranium may be effectively removed from the body through excretory processes, providing a U-DTPA chelate is formed. When the therapy agents were administered 1 hr after ^{233}U injection, they were relatively ineffective; apparently because the therapeutic agents could not successfully compete for uranium that was previously bound to normal biological ligands in the circulatory system and body tissues.

The metabolic effects of the various treatment agents are indicated in Table 51. Only minimal effects on weight and excretion rate were noted in the two groups administered the lower ^{233}U dose combined with

TABLE 50. Summary of Results of Treatment for ^{233}U Removal

Treatment ^(b)	Values are averages of five rats, expressed as percent of injected dose ^(a)							
	Liver	Kidney	Skeleton	Soft Tissue	Total Rat ^(c)	Urine	Feces	Recovery
Nontreated Control	0.27	15	28	3.5	47	37	7.6	91
DTPA (0.1 mM)	0.20	9.8	22	5.4	37	41	6.1	85
Sodium Salicylate (0.4 mM)	0.36	14	26	1.7	42	43	5.8	91
Sodium Bicarbonate (0.9%)	0.26	14	29	1.9	45	27	13	85
Sodium Chloride (0.9%)	0.29	17	26	2.3	46	36	7.7	89
DTPA (0.1 mM) + Sodium Salicylate (0.4 mM)	0.32	9.7	32	2.1	44	30	9.3	83
DTPA (0.1 mM) + Sodium Bicarbonate (0.9%)	0.25	11	26	5.8	43	43	5.6	92
DTPA (0.1 mM) + Sodium Chloride (0.9%)	0.40	15	29	2.5	47	36	6.3	89
^{233}U - DTPA (0.06 mM)	0.21	0.098	19	3.7	23	61	22	106
^{233}U - DTPA (0.06 mM) + Sodium Salicylate (0.24 mM)	0.17	0.034	19	5.2	24	70	11	105

(a) The first eight groups of rats were injected intravenously with ^{233}U ($0.86 \pm 0.02 \mu\text{Ci}$) in 1% sodium citrate solution. The last two groups received ^{233}U ($0.016 \pm 0.002 \mu\text{Ci}$) in 1 % sodium citrate solution combined with the chelating agents.

(b) The first seven groups (excluding controls) were administered 1 ml of the agent by intraperitoneal injection, 1 hour after ^{233}U IV injection; the animals were sacrificed after 4 days.

(c) The pelt, feet, gastrointestinal tract, and injection site were excluded.

TABLE 51. Metabolic Effects of ^{233}U Citrate Incorporation. Values are the average of five rats ($\pm$ SD) observed 4 days after ^{233}U administration. ^(a)

Treatment ^(b)	Weight Loss, % Original Wt	Excretion Rate	
		Urine, ml/rat/day	Feces, g/rat/day ^(c)
^{233}U Non-treated	16 ± 3	7 ± 10	0.73 ± 0.69
^{233}U + DTPA	17 ± 7	10 ± 8	0.92 ± 1.88
^{233}U + Sodium Salicylate	11 ± 6	22 ± 14	1.71 ± 1.54
^{233}U + Sodium Bicarbonate	22 ± 6	10 ± 12	0.51 ± 1.87
^{233}U + Sodium Chloride	20 ± 7	10 ± 12	0.51 ± 0.91
^{233}U + DTPA + Sodium Salicylate	7 ± 2	23 ± 16	1.90 ± 1.11
^{233}U + DTPA + Sodium Bicarbonate	20 ± 4	9 ± 8	0.62 ± 1.76
^{233}U + DTPA + Sodium Chloride	13 ± 8	11 ± 11	0.75 ± 0.82
^{233}U - DTPA combined	$+2 \pm 3$	9 ± 5	3.13 ± 0.68
^{233}U - DTPA + Sodium Salicylate combined	0 ± 2	11 ± 6	3.23 ± 1.32

(a) The first eight groups received $0.86 \pm 0.02 \mu\text{Ci}$ ^{233}U citrate by intravenous injection. The last two groups received $0.016 \pm 0.002 \mu\text{Ci}$ ^{233}U citrate combined with the indicated chelating agent.

(b) The first seven groups (excluding controls) received 1 ml of the agent by intraperitoneal injection, 1 hour after ^{233}U citrate administration.

(c) Feces were dried overnight at 62°C .

chelating agents. This is thought to be due to the lower toxicity of the dose of uranium administered, and to the more efficient excretion of the chelated metal. The volume of urine excreted was somewhat higher in two of the groups treated with sodium salicylate. This is in agreement with the diuretic effect expected with relatively large doses of sodium salicylate. Increased uranium excretion was not, however, consistently associated with the increased urine volume. This suggests that induction of diuresis was not a major factor in the removal of uranium by the kidneys. It is of further interest that the highest per-

centage of the ^{233}U dose excreted in urine (although not the greatest amount of ^{233}U excreted) was associated with treatment groups having relatively normal urinary excretion rates.

Weight loss was a consistent finding in all animals administered the subacutely toxic dose of ^{233}U citrate. The results indicate that none of the treatment regimens initiated 1 hr after ^{233}U citrate injection were effective in reducing uranium retention; nor did any treatment appear to alter the course of uranium toxicity, as evidenced by weight loss.

PRELIMINARY INVESTIGATION OF THE ACUTE TOXICITY OF URANIUM ISOTOPES

Investigators:

R. A. Gies and J. E. Ballou

The acute toxicity of ^{233}U in the rat is due primarily to heavy-metal toxicity rather than to radiation effects.

The effect of specific activity on uranium toxicity is of interest because of the variety of uranium isotopes used and produced in the nuclear industry. This report presents results of a range-finding study in rats injected with ^{238}U or ^{233}U (representing differences of $\sim 2 \times 10^4$ in specific activity) to determine dose levels for more extensive studies comparing acute effects of ^{238}U , ^{233}U , and ^{232}U plus decay products.

Twenty-four 500-g, adult female Wistar rats were injected intraperitoneally with ^{238}U citrate or ^{233}U citrate at one of three dose levels (Table 52), and survival was followed for 30 days. Uranium citrate injection solutions were prepared from 2N

nitric acid solutions of uranyl nitrate by adding sodium citrate crystals and adjusting to pH 7 with 2N sodium hydroxide; the final solutions contained 1% citrate. At death (or sacrifice, after 30 days) rats were necropsied and examined for gross evidence of uranium toxicity. Kidneys were sectioned for histopathologic examination.

Animal survival time and weight changes are shown in Table 52. Two rats that received ^{238}U survived, and were sacrificed at the end of the 30-day observation period. The remaining animals in the study died within 18 days after injection. Weight loss was observed in all treatment groups.

TABLE 52. Summary of Results for Rats Injected Intraperitoneally with ^{238}U or ^{233}U Citrate

(Values are averages $\pm$ SD for four rats unless otherwise noted)

Radioisotope	mgU/kg	$\mu\text{Ci/kg}$	Days Survival	% Weight Loss
^{238}U	5.0	--	$7 \pm 1^{(a)}$	11.8 ± 6.6
	4.4	--	8 ± 4	14.0 ± 9.0
	3.2	--	$6 \pm 1^{(a)}$	8.2 ± 3.1
^{233}U	4.1	39.4	6 ± 1	6.4 ± 1.5
	3.6	34.2	6 ± 1	5.2 ± 0.6
	2.8	26.6	10 ± 6	9.4 ± 11.2

^(a)Values for three rats; 1 rat survived more than 30 days and was sacrificed.

The results indicate that uranium is acutely lethal in the 3-5 mg/kg range used in this preliminary study. Lower dose levels will be used in future studies to unmask possible effects of radiation. It is felt that the acute deaths observed in the present rangefinding study were due principally to heavy-metal poisoning. This argument is supported by gross pathological observations in the kidneys of all groups (medullary hemorrhage, necrosis, cortical anemia) and by the fact that, in similar studies with a fourfold-higher radiation dose from ^{238}Pu , death did not occur before 34 days (Annual Report, 1962). In rats administered ^{238}Pu , death was attributed to bone-marrow depletion resulting from the higher radiation dose sustained by the skeleton. Since the skeleton is also a

major deposition site of incorporated uranium, it is conceivable that bone marrow depletion could be the cause of death after incorporation of similar amounts of high-specific-activity uranium isotopes such as ^{232}U . Also, ^{232}U is associated with a highly radioactive series of decay products that tend to magnify the radiation toxicity of this isotope. Since thorium-breeder-produced ^{233}U may contain varying amounts of ^{232}U and its decay products, it is important to investigate the mode of toxicity and possible synergistic action of mixtures of uranium isotopes. Studies designed for this purpose are currently being initiated, using combinations of ^{238}U , ^{233}U , and ^{232}U plus its decay products.

• Fetal and Juvenile Radiotoxicity

A number of studies conducted under this project have demonstrated that many of the biological parameters used to calculate permissible levels of exposure of adults to radioactive materials are inappropriate for the rapidly growing infant or child or for the pregnant female. These include age-related differences in radionuclide deposition, distribution, and retention and associated differences in microdosimetry, as well as the greater intrinsic radiosensitivity of the immature organism. These findings emphasize the need for more detailed information on the metabolism and toxicity of radionuclides in the prenatal and juvenile mammal. The continuing objective of this project is to obtain such information, which is needed to establish appropriate exposure limits for radionuclides of greatest potential hazard to these age groups.

LATE EFFECTS OF ^{239}Pu ADMINISTERED AT REPRESENTATIVE STAGES OF GESTATION

Investigators:

F. D. Andrew, M. R. Sikov, G. E. Dagle and D. B. Carr

Technical Assistance:

R. Music and L. F. Montgomery

Prenatal exposure to ^{239}Pu depressed offspring growth and survival rates in a relatively dose-related fashion. Histopathological evaluations of the tumors and other lesions removed at necropsy are currently underway.

Previous studies in this laboratory provided extensive data indicating that there are age-related differences in long-term responses of rodents to plutonium (^{239}Pu) injection. Since these earlier studies did not include lifespan evaluations following exposure during early gestation, such studies were initiated (Annual Report, 1976).

Pregnant Wistar rats were injected intravenously at 9, 15, or 19 days of gestation (dg) with 0, 0.3, 3, or 30 $\mu\text{Ci/kg}$ of ^{239}Pu citrate preparation. Fifty-six offspring from each of the 12 age-dose groups, about evenly divided between sexes, were selected as a colony for this long-term study. The dams (at least seven per age-dose group) were also retained for estimating relative risk. Growth rates and survival times were monitored for 30 months after injection of the dams.

As reported previously (Annual Report, 1978), male offspring exposed to the highest dose (30 $\mu\text{Ci/kg}$) had significantly lower body weights than their respective controls (Figure 47A). This has now been shown to be true for the female offspring as well. Additional analyses are required to evaluate possible effects on growth and survival from the interaction of different stages of gestation with dose (Figure 47B).

Cumulative mortality among the offspring exposed to the highest dose (30 $\mu\text{Ci/kg}$) was higher than that of the controls during most of the 30-mo observation period (Figure 48). Median survival times, however, were not greatly shortened and show little effect of sex and the stage of gestation when exposed. This finding differs from the dose-related decrease in median survival times reported for the dams (Annual Report, 1978), but this probably reflects the fact that only a

FIGURE 47. Late Effects of ^{239}Pu Administration at Representative Stages of Gestation

FIGURE 48. Longevity of Offspring

small portion of the dose administered intravenously to the dam reached the offspring in utero. Cumulative radiation doses to the fetuses and neonates during the perinatal period were similar for all age groups. Further dosimetry measurements are required for the subsequent maturation period.

The rats were monitored throughout their lifespan without intervention, except for surgical removal of mammary tumors as soon as they were detected. Moribund animals (sacrificed prior to death) and survivors

at 30 mo of age were necropsied. Tissues showing gross pathology are being studied microscopically. No maternal tissues had obvious dose-related increases in incidence of lesions (Table 53); this may be due, in part, to the significantly shortened lifespan of the middle and high-dose maternal groups. Further analysis of the offspring tissues may reveal dose-related differences. From 52 to 96% of the adult pregnant rats were examined for evidence of pathology at necropsy; approximately 64 to 96% of these animals had tumors, the majority of which were benign.

TABLE 53. Maternal Histopathology

Group Size		Pregnant				Nonpregnant			
		Dose (μCi/kg)				Dose (μCi/kg)			
		0	0.3	3.0	30.0	0	0.3	3.0	30.0
Number Dosed		30	26	32	27	7	6	3	6
Number Examined		27	25	26	14	7	5	1	3
Tumor Incidence									
Number with Tumors		26	22	23	9	7	4	1	1
% with Benign Tumors (a)		85	76	62	29	86	80	100	0
% with Malignant Tumors (a)		19	56	46	50	29	0	100	33
% with Benign and Malignant Tumors (a)		11	36	19	14	14	0	100	0
Primary Tissues Affected (%)									
Breast	benign	13	17	16	3	4	4	1	1
	malignant	1	7	3	5	1	0	0	1
Pituitary	benign	15	--	--	--	2	--	--	--
	malignant	--	--	--	--	--	--	--	--
Bone	benign	--	--	--	--	--	--	--	--
	malignant	2	4	5	1	1	--	2	--

(a)Based on number of rats necropsied

LATE EFFECTS OF ^{239}Pu INJECTION IN ADULT,
WEANLING, NEWBORN, AND FETAL RATS

Investigators:

M. R. Sikov, D. D. Mahlum, G. M. Zwicker,
J. O. Hess and D. B. Carr

Technical Assistance:

M. J. Kujawa and P. L. Savignac

Exposure to Pu decreased the latency of mammary tumor induction in all age groups; incidence varied with the age at injection, and with the dose. There were age-related differences in the effect of plutonium exposure on pituitary tumor incidence, as well as an association between the presence of both mammary and pituitary tumors. Prenatal exposure also increased the incidence of adrenal and pancreatic neoplasms in males.

We previously described (Annual Report, 1972) the protocol for a study to compare long-term effects in rats injected at various ages with ^{239}Pu citrate. The doses ($\mu\text{Ci/kg}$) were 0.3, 1, and 3 for adults and weanlings, 3, 10 and 30 for neonates, and 6, 20 and 60 for prenatal exposure via maternal injection, and are referred to as low, medium, and high, respectively. Some litters from the high prenatal group were fostered to control mothers (EC) and control litters were nursed by exposed dams (CE). Preliminary reports of the overall results were presented earlier (Annual Report, 1973, 1974). The anatomic distribution and incidence of bone tumors (Annual Report, 1977), and comparisons of survival and terminal organ weights (Annual Report, 1978) have also been described. Incidence values have been obtained for the more prevalent neoplastic lesions and associations between co-existent tumor sites, which might be expected a priori on pathophysiologic bases, have been examined. Although overall incidences are influenced by differing survivorship, they show clear relationships with dose and age at injection.

The percentage of control females with tumors of the mammary gland ranged from 4-18% (average, 12%) by 550 days of age (Table 54). By 900 days of age, the average incidence had increased to 47%, with a range of 40-56%. At the earlier time, incidence was elevated in the medium and high adult, high weanling, high prenatal groups, and in the group exposed prenatally

and cross-fostered to control dams (EC). The differences were less pronounced by 900 days of age: only the medium adult, low and high weanling, and medium and high prenatal groups had incidences that were significantly above control levels. The incidences for the high adult and EC group were no longer different from those of the controls at this age. The percentage of tumors that were malignant was increased at higher doses in the newborn and prenatal groups, unchanged in the weanling group, and tended to decrease with increasing dose in the animals exposed as adults. The percentage of females with pituitary tumors decreased with increasing dose in adult and newborn groups; remained unchanged in weanlings; and showed a slight increase in those exposed prenatally (Table 54). A similar pattern was seen when the percentage of animals that showed both mammary and pituitary tumors, or the percentage of females with mammary tumors that also showed pituitary tumors was considered.

Although the percentage of rats with pheochromocytomas was not high in either sex, it was somewhat greater in males than in females (Table 55). The incidences were clearly elevated in males prenatally exposed to plutonium at all three levels and in those from exposed dams fostered to control mothers. A lesser elevation was seen in the weanling and newborn groups, and adults were unaffected. The incidence of pancreatic tumors (adenomas and carcinomas combined) was markedly higher in males than in

TABLE 54. Effect of ^{239}Pu on Incidence of Mammary and Pituitary Tumors in Female Rats

Group	Percent Of Rats With Mammary Tumor At Indicated Age		Percent Of Mammary Tumors Histologically Malignant (a)	Percent With Pituitary Tumors At Necropsy	Conditional Percentage (b)
	Age, 550 Days	Age, 900 Days			
Adult					
Control	4	48	17	56	75
Low	11	56	36	21	31
Medium	50	71	18	8	12
High	40	52	8	12	23
Weanling					
Control	14	40	14	43	14
Low	14	69	25	45	40
Medium	24	48	14	32	54
High	39	67	29	33	46
Newborn					
Control	18	56	23	33	36
Low	13	48	36	52	64
Medium	15	46	17	15	17
High	26	26	75	5	0
Prenatal					
Control	5	40	25	50	75
Low	18	48	25	64	69
Medium	22	65	53	57	60
High	36	71	55	72	76
EC (c)	24	48	25	42	58
CE (d)	7	46	17	62	83

(a) Calculated on basis of all tumors obtained by surgery or at necropsy

(b) Percent of rats with mammary tumor which also had pituitary tumor

(c) Prenatally exposed pups cross-fostered to control dams

(d) Pups from control dams cross-fostered to exposed dams

TABLE 55. Effect of ^{239}Pu on the Percent of Male Rats with Soft Tissue Tumors

	Pituitary Chromophobe Adenoma	Adrenal Pheochromocytoma	Pancreas All Tumors (a)
Adult			
Control	4	4	4
Low	21	0	0
Medium	4	0	0
High	0	0	0
Weanling			
Control	14	3	8
Low	15	5	8
Medium	9	7	11
High	2	2	9
Newborn			
Control	9	0	5
Low	12	8	16
Medium	4	4	0
High	0	0	0
Prenatal			
Control	17	0	10
Low	17	9	31
Medium	22	11	19
High	29	17	25
EC (b)	14	14	17
CE (c)	26	5	11

(a) Primarily islet cell adenoma, but combined for all tumor types

(b) Prenatally exposed pups cross-fostered to control dams

(c) Pups from control dams cross-fostered to exposed dams

females. The incidence of these tumors showed a general elevation in the exposed animals of the prenatal groups and in those cross-fostered from exposed mothers to control dams. The incidence in the other age groups was essentially unaffected. Although the incidence of chromaphobe adenomas of the pituitary was substantially less in males than in females, age and dose relationships were similar for the two sexes. There was an association between the presence of either pancreatic or adrenal tumors and tumors of the pituitary gland.

We are presently attempting to establish mechanisms for the interactions among age at injection, radiation dose and the incidence of tumors in the various endocrine organs. A number of the degenerative lesions are readily explained in terms of the greater damage produced in adult animals due to higher radiation doses delivered over a longer time. Some of the proliferative lesions are of a compensatory nature and incidences may be explained on the basis of age-related differences in destruction, fibrosis, and in the ability of tissues to compensate.

INTERACTIONS OF ANIMAL AGE AND PARTICLE SIZE
WITH DEPOSITION AND RETENTION OF INHALED ⁵¹Cr-LABELED MICROSPHERES

Investigators:

P. L. Hackett, M. R. Sikov, W. E. Skiens,
W. C. Cannon, J. O. Hess and D. Hall

Technical Assistance:

E. F. Blanton, A. J. Clary, K. A. Poston,
J. P. Herring, R. L. Music and B. L. Egbert

Newborn, juvenile, weanling and adult rats were exposed to aerosols of ⁵¹Cr-labeled microspheres with AMADs of 0.91, 1.4, 2.3, 3.4 and 4.4 μ m. Alveolar deposition was negligible in newborn rats for all particle sizes, and decreased to less than 6% at an AMAD of 2.3 μ m in juveniles and weanlings, and an AMAD of 3.4 μ m in adults.

Previous studies of the deposition and translocation of inhaled plutonium in newborn and adult rats revealed that animal age and particle size markedly influenced aerosol deposition, but not retention (Annual Report, 1976). Furthermore, while adult lung contained a wide range of particle sizes following exposure to an aerosol of a single AMAD distribution, newborn lung contained only the smaller particles of the size spectrum. Therefore, studies utilizing aerosols with more-specific, better-defined size ranges were initiated to determine the influence of the progressive development of the respiratory tree on deposition characteristics.

Polystyrene, polyvinyltoluene, or styrene/vinyltoluene microspheres with diameters of 0.5, 1.1, 2.0, 2.8 and 4.3 μ m (Table 56) were labeled with ⁵¹Cr by emulsion polymerization of chromium acetylacetonate dissolved in a styrene monomer. Aqueous suspensions of the ⁵¹Cr microspheres were nebulized and used to expose rats for 60 min. Groups of 50 newborn (less than 24 hr old), juvenile (13 days old), weanling (21 days old), and adult females were exposed to the 0.5-, 1.1- and 2.0- μ m microspheres; only adult rats were exposed to 2.8- and 4.3- μ m particles. Six rats from each age group were killed immediately after exposure, at 2 and 6 hr, and at 1, 3, 7, 21 and 56 days after exposure.

TABLE 56. Aerosol Properties

Aerosol Properties	Microsphere size, μ m				
	0.481 $\pm$ 0.002	1.091 $\pm$ 0.008	2.020 $\pm$ 0.014	2.770 $\pm$ 0.14	4.330 $\pm$ 0.54
nCi/l	47.8 $\pm$ 10.1	32.3 $\pm$ 6.25	18.7 $\pm$ 6.36	16.1	39.9
AMAD, μ m	0.91	1.42	2.34	3.40	4.36
GSD	1.78	1.49	1.77	2.14	2.11
MMD, μ m	0.70	1.42	2.34	3.40	4.36

Tissue samples were analyzed for the 0.325-MeV gamma emission of ^{51}Cr . Filter paper and cascade impactor samples were taken during exposure to determine aerosol concentrations and particle size distributions. To compensate for aerosol concentration differences among exposures (Table 1), all radioanalytical values were normalized for an exposure to an aerosol concentration of 40 nCi/l.

Calculated values for alveolar deposition for each age group (Table 57) show the effects of age and microsphere size on alveolar deposition. Total deposition values, based on radioanalytical results for material balance (Figure 49), suggest that the aerosol concentrations may have been underestimated for the larger particle sizes. We have used these tentative values for adjustment of aerosol concentrations to 40 nCi/l in order to facilitate age comparisons; more-precise values are being determined. Total deposited activities were significantly different for all ages and microsphere sizes, except for juvenile and weanling animals exposed to 2.3- μm particles. Deposition in adults increased with increasing AMAD; values in newborns and juveniles were constant; and deposition in weanlings showed a significant peak with microspheres of 1.1 μm . Partition of this deposited activity among the lung, gastrointestinal tract, and the head (which includes the nasopharynx and mouth) is shown

in Figure 50. Lung values for all ages decreased following exposure to increasing microsphere size. Correspondingly, at these larger microsphere sizes, the activity in the gastrointestinal tract increased in adult and weanling rats, but showed an apparent (but not significant) decrease in the younger age groups. Percentages of ^{51}Cr activity in the head were extremely variable, so that temporal changes within each age group were not significant. However, a larger fraction of the deposited particles appeared in the heads of newborn and juvenile rats.

Lung deposition was decreased to less than 5% of the total body burden when adults were exposed to microspheres greater than 3 μm in size, or when weanling and juvenile rats were exposed to microspheres larger than 2 μm . Since lung deposition in newborn animals was extremely low, even at an AMAD of 0.9 μm , studies utilizing 0.2- μm microspheres have been initiated.

Preliminary evaluations of retention data for the 0.9-, 1.4- and 2.3- μm microspheres indicated that the adults retained 25-35% of the deposited particles, while only 10-15% of the 1.4- and 2.3- μm particles remained in weanling and juvenile lungs after 8 wk.

TABLE 57. Respiratory and Alveolar Deposition Values for Rats of Different Ages ($\bar{X} \pm \text{S.E.}$)

	Adult	Weanling	Juvenile	Newborn
Body Weight, g	273 $\pm$ 3.6	43.7 $\pm$ 1.8	31.8 $\pm$ 0.9	7.09 $\pm$ 0.20
Minute Volume, cc/min ^(a)	144 $\pm$ 5.5	35.7 $\pm$ 3.4	28.1 $\pm$ 1.9	9.12 $\pm$ 0.63
Inhaled Activity, nCi ^(b)	346 $\pm$ 13.2	85.7 $\pm$ 8.2	67.4 $\pm$ 4.6	21.9 $\pm$ 2.98
Alveolar Deposition, %				
AMAD: 0.9 μm	17.6 $\pm$ 2.9	12.6 $\pm$ 4.8	7.59 $\pm$ 2.5	0.14 $\pm$ 0.07
1.4	11.7 $\pm$ 1.5	20.2 $\pm$ 2.8	8.34 $\pm$ 2.6	0.75 $\pm$ 0.68
2.3	11.9 $\pm$ 3.3	0.39 $\pm$ 0.1	2.76 $\pm$ 1.5	0.76 $\pm$ 0.56
3.4	4.48 $\pm$ 1.0			
4.4	2.75 $\pm$ 0.3			

(a) Minute volume = $2.10 (\text{Body weight})^{0.75}$

(b) Inhaled activity = minute volume x 60-minute exposure x 40 nCi/l aerosol concentration

FIGURE 49. Total Deposited Activity in Rats After Exposure to ^{51}Cr -Labeled Microspheres of Different AMADs. (Mean values are adjusted to an aerosol concentration of 40 nCi/ℓ.)

FIGURE 50. Distribution of Deposited ^{51}Cr -Labeled Microspheres in Adult, Weanling, Juvenile and Newborn Rats.

• Modifying Radionuclide Effects

The metabolism and effects of radionuclides may be influenced by a number of dietary, physiological, and environmental factors. Since persons who might be exposed to radionuclides do not live under carefully controlled and isolated conditions, we are studying some factors which have been identified as potentially important determinants of radionuclide behavior. This year, we are reporting studies on the effect of pregnancy, lactation and repeated breeding on the response of the rat to ^{239}Pu .

EFFECTS OF PREGNANCY AND LACTATION ON THE TOXICITY OF ^{239}Pu

Investigators:

D. D. Mahlum, J. O. Hess and M. R. Sikov

Nulliparous, pregnant or lactating Wistar rats were injected intravenously with 0, 0.5 or 2.0 μCi of ^{239}Pu per kilogram of body weight. Half of each group were repeatedly bred while the others were held for observation without further treatment. Treatment with either dose of ^{239}Pu decreased the median time to appearance of mammary tumors. Repeated breeding tended to increase the mean time to appearance. Longevity was decreased in a dose-related fashion by Pu administration. Repeated breeding did not influence survival in a major way.

Three groups of rats (nulliparous, pregnant or lactating), representing three discrete physiological states, were injected with 0, 0.5 or 2.0 $\mu\text{Ci/kg}$ ^{239}Pu citrate. Half the animals in each group were held for observation without further treatment. Thirty days after injection, the other half of each group was bred and allowed to deliver. The young were weighed and destroyed at 7 days postpartum. Dams were rebred until each had experienced five pregnancies.

Each physiological group showed a dose-related decrease in survival times (Figures 51-53). Although there were a few differences between those repeatedly bred and those held without further treatment, they were not major. Pregnancy and lactation appeared to offer some protection

against the life-shortening effect of the 2.0- $\mu\text{Ci/kg}$ dose.

Rates of mammary tumor appearance are shown in Figures 54-56 for groups injected with ^{239}Pu while nulliparous, pregnant, or lactating. In each group, ^{239}Pu administration increased the rate of appearance in a dose-related manner. Repeated breeding tended to delay tumor appearance in all groups, at least until breeding was completed.

Although further analysis is required, these data suggest that longevity is decreased and that rates of mammary tumor appearance are increased by the administration of ^{239}Pu . Physiologic state at time of administration, or repeated pregnancy, may modify the response to ^{239}Pu .

FIGURE 51. Survival After Administration of ^{239}Pu to Virgin Rats

FIGURE 52. Survival After Administration of ^{239}Pu to Pregnant Rats

FIGURE 53. Survival After Administration of ^{239}Pu to Lactating Rats

FIGURE 54. Influence of ^{239}Pu on Mammary Tumor Incidence in Rats Injected as Virgins

FIGURE 55. Influence of ^{239}Pu on Mammary Tumor Incidence in Rats Injected at 19 Days of Gestation

FIGURE 56. Influence of ^{239}Pu on Mammary Tumor Incidence in Rats Injected During Lactation

• Gut-Related Studies of Radionuclide Toxicity

This project is concerned with the behavior of radioactive materials of importance in the nuclear industry that may be ingested as a consequence of a reactor accident, from unavoidable occupational exposure, or after release to the environment and incorporation into the food chain. Current emphasis is directed toward evaluating hazards from actinides that pass through the gastrointestinal (GI) tract after ingestion or as a result of clearance from the lung, and the effect of animal age or species, or the influence of chemico-physical state of the radionuclide on GI tract function.

TRANSPLACENTAL ABSORPTION OF ^{238}Pu IN RATS AND GUINEA PIGS

Investigator: M. F. Sullivan

Technical Assistance: L. S. Gorham

Pregnant rats and guinea pigs were injected intravenously with ^{238}Pu citrate to determine if the potential for in utero accumulation of ^{238}Pu by these two species is related to the stage of development at which immunity is gained. Although guinea pigs retained more ^{238}Pu after birth than rats, the difference was not significant.

Both newborn rats and guinea pigs absorb 100 times more plutonium from their gastrointestinal tract than adults (Annual Report, 1977, 1978). Newborn rats accumulate 10 to 20 times more of the actinide in the mucosa of the small intestine than is absorbed. It is retained for several days in the mucosa before maturation results in its excretion. Presumably, this increased permeability of the bowel occurs during the time that immunoglobulins from colostrum are absorbed by the neonate. Since rats gain immunity both pre- and postnatally, while guinea pigs and humans attain it only prenatally, it seemed possible that fetuses of these species that absorb those macromolecules prenatally might also absorb more plutonium from their dams than species that absorb antibodies postnatally.

To test that possibility, pregnant rats and guinea pigs were each injected intravenously with 10 μCi of ^{238}Pu citrate. The ^{238}Pu retention in offspring born at various intervals after injection of the dams is shown in Table 58. Results for rats suggest

that the amount of ^{238}Pu absorbed by each fetus is dose-related, since the number born 3 days after injection was about half the number born at 2 days, and the retained dose per animal was twice as high.

Guinea pigs delivered 3 days after ^{238}Pu injection of dams were stillborn. Absorption was not much different from those born at 5 days after injection. Both litters contained more ^{238}Pu than those killed 10 or 13 days after injection.

There was no substantial difference between the in utero absorption of plutonium by guinea pigs and rats. Therefore, it appears that species differences in development of immunity are not related to accumulation of plutonium in utero. This suggests that if humans obtain immunity prenatally, as does the guinea pig, the hazard of obtaining plutonium from their mother is not appreciably more than it is for those species that absorb macromolecules from the GI tract postnatally.

TABLE 58. Retention of ^{238}Pu by Neonatal Rats and Guinea Pigs that Absorbed Plutonium in Utero from Dams Injected Intravenously with ^{238}Pu Citrate $\pm$ SEM.

Rats						Guinea Pig					
Interval between ^{238}Pu Injection and Birth, days	Age at Sacrifice, days	No. of Animals	Percent of Maternally Injected ^{238}Pu (b)			Interval Between ^{238}Pu Injection and Birth, days	Age at Sacrifice, days	No. of Animals	Percent of Maternally Injected ^{238}Pu (b)		
			Liver	Carcass	Total				Liver	Carcass	Total
2	7	17	0.06 $\pm$ 0.003	0.15 $\pm$ 0.01	0.21 $\pm$ 0.01	3	3	3	0.01 $\pm$ 0.01	0.62 $\pm$ 0.03	0.63 $\pm$ 0.03
3	7	8	0.12 $\pm$ 0.03	0.36 $\pm$ 0.01	0.38 $\pm$ 0.02	5	7	2	0.08 $\pm$ 0.01	0.76 $\pm$ 0.12	0.84 $\pm$ 0.13
4(a)	11	14	0.05 $\pm$ 0.01	0.15 $\pm$ 0.01	0.20 $\pm$ 0.01	10	7	4	0.03 $\pm$ 0.004	0.12 $\pm$ 0.07	0.15 $\pm$ 0.08
4	11	12	0.09 $\pm$ 0.01	0.26 $\pm$ 0.01	0.35 $\pm$ 0.02	13	7	4	0.05 $\pm$ 0.01	0.30 $\pm$ 0.04	0.35 $\pm$ 0.05

(a) Retention values for three neonates from each of the litters born 4 days after ^{238}Pu injection of the dam and killed 12 hours later did not differ from those of animals that lived for 11 days; values of all newborns were therefore pooled
(b) Percent of ^{238}Pu dose in each neonate

SPECIES VARIABILITY IN THE GASTROINTESTINAL ABSORPTION
AND DISTRIBUTION OF SOLUBLE OR INSOLUBLE COMPOUNDS OF PLUTONIUM

Investigators:

M. F. Sullivan, L. S. Gorham and E. F. Blanton

Absorption of plutonium was measured after administration to neonatal rats, dogs and swine by gavage or inhalation. The amount absorbed was not appreciably different for any of the three species, but absorption of the soluble nitrate form was 1000 times higher than that of the insoluble oxide. The higher quantities of ^{238}Pu absorbed and retained in the GI tract of swine suggested that solubilization of the oxide in the lung may have caused the increase. Studies with the more insoluble $^{239}\text{PuO}_2$ also resulted in increased retention.

We have observed that neonatal swine absorb more plutonium from the gastrointestinal (GI) tract than either newborn rats or guinea pigs (Annual Report, 1977 and 1978). Furthermore, a large fraction of plutonium is stored in the small bowels of young rats and swine for several days before it is excreted. Since a major fraction of inhaled plutonium is cleared through the GI tract, that route of entry may contribute substantially to the quantity absorbed after inhalation. To study the importance of the GI route in plutonium absorption of either "soluble" ^{238}Pu nitrate or "insoluble" ^{238}Pu and ^{239}Pu oxides, litters of swine were administered aerosols or suspensions of plutonium by inhalation. In addition, absorption was measured in neonatal dogs, because this species has been used to obtain much of our information on the effects of inhaled plutonium.

To obtain information on the absorption of gavaged plutonium for comparison with the data from inhalation-exposed animals, nitric acid solutions (pH 2) of ^{238}Pu nitrate, or water suspensions of the oxides, were administered by stomach tube to rats (0.1 ml), and to dogs or swine (1 ml). Depending on the litter size, animals were killed at 1, 2 and 3 wk after treatment. Neonatal animals were exposed by inhalation to aerosols of PuO_2 in water, or $\text{Pu}(\text{NO}_3)_4$ in 3 to 5N nitric acid. The exposures were nose-only, under restraint in plastic containers for 0.5 to 1 hr, depending on the plutonium concentration of the aerosol. At the conclusion of the exposure, two or three animals were killed, skinned and analyzed for plutonium.

Data obtained after ^{238}Pu nitrate gavage of rats, dogs and swine (some of which were killed a week after treatment) are shown in Figure 57. Absorption was between 5 and 10% of the dose for all three species. The major difference among species was the smaller quantity of ^{238}Pu retained in the gut of dogs as compared to either rats or swine. One week after treatment the gut content had not been excreted. Therefore, the retained dose represents gastrointestinally bound plutonium, accounting for about half the gavaged dose. A similar but less substantial absorption resulted after gavage with a ^{238}Pu oxide suspension (Figure 58). The decreased absorption was presumably due to the lower solubility of the oxide.

A comparison of plutonium retention after gavage with that resulting after inhalation of ^{238}Pu nitrate is shown by the data in Figure 59. Animals killed immediately after inhalation had half the retained dose in the stomach or in the small intestine. Consequently, a substantial amount of the ^{238}Pu retained in the skeleton and liver must have resulted from absorption via the GI tract. Retention in the mucosa of the gut or in gut content amounted to half the administered dose, regardless of the route of entry. The neonatal dog absorbed more ^{238}Pu after inhalation than swine (as shown by skeletal content) but very little was retained in the gut.

The difference between absorption after gavage or after inhalation was more evident after administration of the insoluble oxide

(Figure 60), although the amount absorbed was much less. Retention in the skeleton and liver by both swine and dogs was about 10 times higher after inhalation than after gavage. Retention in the gut wall of swine was also higher after inhalation. This suggested that some solubilization of the ^{238}Pu may have occurred during clearance from the lung. To test for that possibility, ^{238}Pu oxide was administered both by gavage and by inhalation. A comparison of distribution in the carcass after each method of administration is shown in Figure 5. Absorption of ^{238}Pu oxide was less than half of that for ^{238}Pu oxide (0.015 vs 0.07%). In

addition, inhalation caused an increase of 0.27% in bone and liver content of plutonium. The amount of ^{238}Pu retained by the intestine after inhalation also increased by a factor of five, from 2.3 to 10.1%. Further experiments are planned to determine if solubility changes occur in the lung that lead to increased absorption from the GI tract after clearance from the lung.

These studies demonstrate that, in the neonatal animal, absorption from the GI tract provides a major pathway for the entry of inhaled plutonium into the body.

FIGURE 57. Distribution of Plutonium in Neonatal Rats, Dogs and Swine Gavaged with ^{238}Pu Nitrate.

FIGURE 58. Distribution of Plutonium in Neonatal Rats, Dogs and Swine Gavaged with ^{238}Pu Oxide.

FIGURE 59. Distribution of Pu in Neonatal Swine and Dogs Administered ²³⁸Pu Nitrate by Gavage or by Inhalation

FIGURE 60. Distribution of Pu in Neonatal Swine and Dogs Administered ^{238}Pu Oxide by Gavage or by Inhalation

FIGURE 61. Distribution of ^{239}Pu in Neonatal Swine Administered ^{239}Pu Oxide by Gavage or by Inhalation

EFFECT OF X-IRRADIATION OR CHELATE ADMINISTRATION
ON TRANSPORT OF PLUTONIUM ACROSS RAT INTESTINE

Investigators:

M. F. Sullivan and L. S. Gorham

Technical Assistance: B. L. Southward

Experiments carried out after abdominal X-irradiation indicated increased permeability of the gastrointestinal tract to ^{238}Pu at doses of 800 rad or above. Measurement of movement of ^{238}Pu from the isolated duodenum also indicated increased ^{238}Pu transport after 800-1500 rad. By the same technique, we were able to show that the increased urinary excretion of ^{239}Pu measured after gavage with ^{239}Pu -DTPA by Baxter and Sullivan (Health Phys. 22: 785, 1972) was indeed due to increased transport across the GI tract. Bile, however, had no measurable effect on ^{238}Pu transport.

Although Foreman (Radiat. Res. 7: 167, 1957) reported 20 years ago that whole-body exposure of rats to doses between 250 and 2000 rad had no effect on ^{239}Pu absorption from the GI tract, we have obtained contradictory results after abdominal exposures, using two different procedures to measure transport. We also reported that DTPA stimulated ^{239}Pu absorption from the GI tract (Baxter and Sullivan, Health Phys. 22: 785, 1972) but were unable to measure gut transport by the procedure used, and could only infer increased absorption from an observed increase in urinary ^{239}Pu excretion. Results from the present studies were obtained by a procedure that specifically measures intestinal transport.

Rats were exposed to partial body X-irradiation under nembutal anesthesia. Only the body region distal to the xiphoid process was irradiated. Three days after exposure the rats were gavaged with 7.5 μCi of ^{238}Pu in a nitric acid solution (pH 2), and were killed at 5 days for tissue analysis.

Animals used to study ^{238}Pu transport from the duodenum were anesthetized with nembutal 3 days after X-ray. A 15- to 18-cm segment of the small bowel, immediately distal to the pylorus, was isolated with ligatures and injected with 7.5 μCi of ^{238}Pu nitrate. The animals were killed 4 hr later for analysis. Animals used to study the effect of DTPA were treated by the same procedure. The ^{238}Pu nitrate, pH 4, was mixed with a 10^4 molar excess of a solution

of calcium DTPA, pH 4, immediately before injection into the duodenal segments. The ^{238}Pu for the experiment with bile was mixed with bile or saline so that 8 μCi could be injected in 1 ml. The ^{238}Pu concentration in the skeleton in these studies was estimated by multiplying the femur content by 23.

Data on ^{238}Pu absorption obtained after exposure of the abdomen of a rat to various doses of X-irradiation are shown in Figure 62. They indicate that no change occurred until a dose of 800 rad was reached; at the two doses, the data were quite variable. Severe diarrhea occurred at 1000 rad. Since changes in gastric emptying that are dose-dependent may also influence measurements by this procedure, we measured transport directly by a procedure (unpublished) that we have used to show that the duodenum is the region where plutonium transport occurs to the greatest extent. The results are shown in Figure 63. These data are also quite variable, but they support the results obtained after gavage, i.e., increased absorption occurred after exposure of the GI tract to high X-irradiation doses. These results suggest that irradiation of the gut, such as might occur from an ingested beta-emitting source, or from an external source that irradiates the abdomen, may lead to increased actinide absorption.

Results obtained after injection of ^{238}Pu nitrate or ^{238}Pu nitrate and DTPA into duodenal segments are shown in Figure 64. They show a higher absorption of ^{238}Pu nitrate

FIGURE 62. $^{238}\text{Pu}(\text{NO}_3)_4$ Absorption ($\pm$ S.E.M.) by Rats Exposed to Lower-Body X-Irradiation (No. of Rats in Parentheses)

than ^{238}Pu citrate, probably due to contamination of the carcass during the injection procedure, or to leakage of the intestinal segment during the 4-hr incubation period. Nevertheless, the procedure demonstrates that DTPA promoted transport of ^{238}Pu across the duodenum. We saw no such effect of DTPA in the stomach. Bile apparently did not promote Pu transport at the concentration used.

These results demonstrate that an in vivo isolated gut segment technique may be used to demonstrate the effect of chemical agents or injury on plutonium transport across the GI tract. It may be especially useful when retention in the liver or skeleton is low because of rapid excretion, as for Pu-DTPA, or when gut function is abnormal.

FIGURE 63. Distribution of ^{238}Pu in Rats Exposed to Abdominal X-Irradiation. Animals received an intraduodenal injection of ^{238}Pu Nitrate (pH 2) 3 days after exposure and were killed 4 hr later.

FIGURE 64. Absorption ($\pm$ S.E.M.) of ^{238}Pu from Intestines of Rats After Injection of Various Solutions into Ligated Duodenal Segments

TRANSPORT OF PLUTONIUM ACROSS ISOLATED SEGMENTS OF THE INTESTINE OF NEONATAL SWINE

Investigators:

M. F. Sullivan and L. S. Gorham

Absorption of ^{239}Pu was measured in neonatal swine gavaged with either $^{239}\text{Pu}(\text{IV})$ or $^{239}\text{Pu}(\text{VI})$ nitrate 1 day after birth. When they were killed, 1 or 3 wk later, retention of the $\text{Pu}(\text{VI})$ was about 50% greater than retention of $\text{Pu}(\text{IV})$. Studies with isolated intestinal segments indicated no appreciable difference in ^{238}Pu transport by the duodenum, midgut or terminal ileum, and a substantial decrease in absorption between 2 and 19 days after birth.

We have demonstrated the high rate of ^{238}Pu and ^{233}U absorption shown by the neonatal swine (Annual Reports, 1977 and 1978), and the effect of oxidation state on the absorption of ^{239}Pu by adult animals. Elsewhere in this report, we showed that hexavalent ^{239}Pu is absorbed in higher quantities from the lung after inhalation (Annual Report, 1979) than is $^{239}\text{Pu}(\text{IV})$. In an effort to learn more about the mechanism of transport in swine, $^{239}\text{Pu}(\text{IV})$ and $^{239}\text{Pu}(\text{VI})$ were administered by gavage to day-old littermates. Animals from two other litters were used to determine the rate of ^{238}Pu absorption from different segments of the small intestine.

Plutonium-239 nitrate, cleaned a year earlier to remove ^{241}Am , was oxidized to $\text{Pu}(\text{VI})$ with ozone. A spectrographic analysis made a few weeks before this study showed it to be 100% $\text{Pu}(\text{VI})$. A small amount of reduction probably occurred during that interval, but 90% should have been in the $^{239}\text{Pu}(\text{VI})$ state at the time of gavage. Two animals treated with 15 μCi of $^{239}\text{Pu}(\text{IV})$ and two treated with $^{239}\text{Pu}(\text{VI})$ were killed at both 1 and 3 wk after gavage. The results (Figure 65) indicate that about twice as much

^{239}Pu was in the skeleton of animals treated with $^{239}\text{Pu}(\text{VI})$ as in those treated with $^{239}\text{Pu}(\text{IV})$ either 1 or 3 wk after gavage. There was a smaller difference in the total amount retained and no difference in liver retention. Retention in the intestine amounted to about 50% of the gavaged dose at 1 wk and 1% at 3 wk, regardless of the oxidation state.

In an effort to demonstrate differing transport efficiencies in the various segments of the intestinal tract, we anesthetized neonates of various ages and, after laparotomy, injected nitric acid solutions (pH 2) of ^{238}Pu into 10- to 15-cm segments of the small bowel, ligated with umbilical tape. After 4 hr the animals were killed, the segments were removed, and the livers, femora and carcasses were analyzed as a measure of absorbed ^{238}Pu . Absorption from the proximal duodenum, midgut (jejunum) and the terminal ileum, calculated according to the length of the segments, are shown in Table 59. There was no appreciable difference in transport characteristics at any of the three locations. About one-third as much ^{238}Pu was absorbed/cm of gut at 3 wk as at 1 or 2 days.

FIGURE 65. Retention of Plutonium in Neonatal Swine Gavaged with Nitric Acid Solutions of Either ²³⁸Pu (IV) or ²³⁸Pu (VI) at 1 Day After Birth and Killed 7 Days Later ($\pm$ S.E.M.)

TABLE 59. Absorption of ²³⁸Pu from Segments of the Small Intestine of Neonatal Swine

Animal Age, Days	Percent of Injected ²³⁸ Pu (IV) Dose/cm of GI Tract		
	Small Intestine Segment		
	Duodenum	Jejunum	Ileum
1	0.11	0.17	0.17
1	0.07	0.09	0.16
2	0.41	0.1	0.07
2	<u>0.13</u>	<u>0.18</u>	<u>0.10</u>
Average	0.18 $\pm$ 0.08	0.14 $\pm$ 0.02	0.13 $\pm$ 0.02
19	0.07		
19	0.06		
19	<u>0.02</u>		
Average	0.05 $\pm$ 0.02		
28	0.04		

EFFECT OF OXIDATION STATE ON THE DISTRIBUTION OF INHALED ^{239}Pu

Investigators:

M. F. Sullivan, J. L. Ryan, L. S. Gorham and E. F. Blanton

Technical Assistance: B. L. Southward

Rats were exposed to aerosols of $^{239}\text{Pu}(\text{IV})$ and $\text{Pu}(\text{VI})$ to determine whether the more soluble hexavalent form was more readily absorbed from the lung and/or from the intestine after lung clearance. Some of the animals were fasted to minimize reduction of $^{239}\text{Pu}(\text{VI})$ in the gastrointestinal tract. There was a threefold increase in absorption of $^{239}\text{Pu}(\text{VI})$ over $^{239}\text{Pu}(\text{IV})$ that was not affected by fasting.

Conditions in sea and lake water may apparently result in the formation of $\text{Pu}(\text{IV})$ and/or $\text{Pu}(\text{VI})$ (Nelson and Lovett, Argonne National Laboratory Annual Report, ANL-77-65, Part III, p. 99) which, under certain conditions, might become airborne and be inhaled. We therefore exposed rats to aerosols of both ^{239}Pu IV and VI to determine if absorption was influenced by this change in valence and solubility.

Rats were divided into groups that were either fed ad libitum, fasted for 18 hr before exposure, or fasted for 18 hr prior to and 72 hr after exposure. Half of each group was exposed to aerosolized $^{239}\text{Pu}(\text{IV})$, and half to $^{239}\text{Pu}(\text{VI})$ aerosolized from a nitric acid solution. The ^{239}Pu solution was cleaned of impurities immediately before a portion was oxidized by passing a stream of O_3 , O_2 and Ar through it for several hours. Immediately after exposure, three animals from each of the $\text{Pu}(\text{IV})$ and $\text{Pu}(\text{VI})$ groups that were fed ad libitum, and two from each of the groups that were fasted for 18 or 90 hr, were killed, skinned and analyzed for ^{239}Pu deposition. The results indicated that doses of 5.44 and 5.98 μCi of $\text{Pu}(\text{VI})$ and $\text{Pu}(\text{IV})$, respectively, were retained; only about 10-15% of these amounts were found in the lungs.

Data obtained from animals killed at 5 days are compared in Figure 66. ^{239}Pu contents of both the liver and carcass of

the animals that inhaled $^{239}\text{Pu}(\text{VI})$ were about three times higher than those of animals that were exposed to $^{239}\text{Pu}(\text{IV})$, regardless of the nutritional state of the animals.

Data obtained from animals killed at 42 days are compared in Figure 67. Only about half the lung burden measured at 5 days (approximately 6%) remained; skeletal ^{239}Pu content had increased from 1% to 3 or 4%. While liver values were about the same for all groups, carcass values of the $^{239}\text{Pu}(\text{VI})$ groups were still higher than those of the $^{239}\text{Pu}(\text{IV})$ groups; the difference being most evident in the groups fed ad libitum.

The amount of $^{239}\text{Pu}(\text{VI})$ absorbed by the rats that were fasted for 18 hr prior to and for 72 hr after exposure was about 1.4% of the total dose (Figure 66). Since this value is within the range of values measured for the absorption of $^{239}\text{Pu}(\text{VI})$ from the gut, it is impossible to conclude whether lung or gut is primarily responsible for this absorption. However, since starvation had so little effect on the amount absorbed, it seems probable that most of the movement was from the lung rather than from the gut.

For reasons that are not apparent, animals that were fed ad libitum at the time of exposure retained about 25% more plutonium than those that were fasted.

FIGURE 66. Influence of Oxidation State on Absorption of Inhaled Plutonium in Rats Killed 5 Days Postexposure to ^{239}Pu Nitrate ($\pm$ S.E.M.)

FIGURE 67. Influence of Oxidation State on Retention of Inhaled Plutonium by Rats Killed 6 wk Postexposure to ^{239}Pu Nitrate ($\pm$ S.E.M.)

• Removal of Deposited Radionuclides

The objective of this project is to decrease the damage potential from inhaled, skin-, wound-absorbed, or ingested radionuclides. While primarily addressing the need for worker protection in the nuclear industry and in the laboratory and cooperating with needs of the industrial physician, we are also investigating possible treatment needs arising from exposure of larger segments of the population. We are attempting to develop methods that will prevent absorption, hasten excretion, improve decontamination, or alter translocation of the radionuclides so as to minimize radiation dose to sensitive tissue.

SOLUBILIZATION OF $^{241}\text{AmO}_2$ IN ALVEOLAR MACROPHAGE CULTURES

Investigators:

A. V. Robinson and R. P. Schneider

Technical Assistance: M. Butcher

Cultured rabbit alveolar macrophages were used to study the effect of phagocytosis on the solubilization of $^{241}\text{AmO}_2$. A comparison was made of the solubility of phagocytized AmO_2 and AmO_2 in cell-free media, in the presence and absence of 0.1 mM DTPA. A time-dependent increase of 26% in the soluble (0.1- μm filtrate) intracellular americium fraction was seen in macrophages cultured for 3 days. The addition of 0.1 mM DTPA to culture medium resulted in an increase of 36% over the same time period. In contrast, cell-free media without DTPA resulted in less than a 2% increase in solubility after 4 days of incubation, while addition of 0.1 mM DTPA resulted in a 5% increase over the same time period. These results indicate cell-mediated solubilization of phagocytized AmO_2 by macrophages.

The macrophage figures prominently in the clearance of inhaled particulates from the lung. Within minutes of inhalation, particulates deposited in the deep lung are phagocytized by macrophages. We have developed an in vitro assay system utilizing the macrophage as a test cell (Annual Reports, 1975-1978) to screen agents potentially useful in the solubilization and removal of actinide particulates from the lung. This system mimics some of the problems involved in the in vivo removal of inhaled transuranics, most notably the enclosing of the particles within cells with membranes that are nearly impermeable to the highly charged DTPA molecules commonly used in treating exposed subjects. This report describes work intended to define the role of the macrophage in solubilizing $^{241}\text{AmO}_2$, as well as the effect of DTPA on the process.

Macrophages were obtained by lung lavage from 3- to 5-lb New Zealand White rabbits (either sex). The AmO_2 suspensions were freshly prepared for each experiment. Cells and particles were incubated in Hanks + 4% BSA for 2 hr at 37°C with shaking. Cells and unphagocytized actinide particles were separated by density gradient flotation in BSA as previously described (Robinson and Schneider, Annual Report, 1978). Samples of resuspended cells were saved for subsequent counting and filtration. Macrophages (5×10^6) were added to a 25-cm² tissue culture flask containing 5 ml M-199 tissue culture medium plus 10% rabbit serum (RS). The flasks were then incubated at 37°C in a 5% CO₂ atmosphere, and the macrophages were allowed to settle and attach to the bottom of the flask for 1 to 2 hr. The media and unattached cells were then aspirated, and

5.0 ml of fresh media (M-199 + 10% RS, or media plus 0.1 mM CaNa_3DTPA) were added. The cells were incubated for a total of 3 days following attachment, and at 3, 9, 24 and 72 hr, cells were lysed by adding 0.5 ml 10% sodium dodecyl sulfate (SDS) to 4 to 5 ml of media in the flasks. The exposure duration was measured from the beginning of the phagocytosis phase. The addition of SDS dissolved the cell membrane and allowed subsequent filtration of cell lysates through a 0.1- μm polycarbonate filter (Nuclepore). Radioactivity was measured by liquid scintillation.

The data in Table 60 are expressed as the percentage of total americium that passed through a 0.1- μm filter at a given time. The data indicate a time-dependent, cell-mediated increase in the solubility of the phagocytized AmO_2 . This increase was not seen in the cell-free medium control. The use of DTPA clearly enhanced the solubility of AmO_2 in the presence of cells; however, there was no time-dependent increase in solubility in the cell-free control medium.

TABLE 60. Filterability of Intracellular $^{241}\text{AmO}_2$ as a Function of Time and DTPA Treatment

Composition of Incubation Media (a)	% of Total Activity in 0.1- μm Filtrate					
	Time in Culture, hr					
No. Hours In Culture:	0	3	9	24	72	96
Cells + 0.1 mM DTPA	14	30	48	82	89	--
Cells	14	30	41	63	67	--
Cell-Free Media	5	6	--	8	--	6
Cell-Free Media + 0.1 mM DTPA	8	10	--	12	--	12

(a) Cells were incubated in M-199 salts containing 10% rabbit serum. Cell-free samples were incubated in Hanks' salts containing 10% rabbit serum.

It is not possible to ascertain if the fraction of activity which passed through a 0.1- μm filter was truly soluble, or consisted of particles less than 0.1 μm in size. Data in the literature (Stradling, et al., *Radiat. Res.* 76: 549-560, 1978) indicate that, for AmO_2 , this distinction is not particularly important since the behavior of inhaled particles less than 0.1 μm in size approaches that of soluble actinide. There are apparently factors in the macrophage which greatly increase the disaggregation or solubility of AmO_2 over that observed in cell-free media. These factors may be naturally occurring chelators, the low pH of the lysosome, oxidation reduction reactions or surfactant action (disaggregation). Some of these mechanisms will be investigated, since understanding of these processes may provide insight into methods of enhancing naturally occurring mechanisms of solubilization and, ultimately, clearance.

Our observations in the macrophage system correlate with the results observed by others (Stradling, et al. *Radiat. Res.* 76: 549-560, 1978); i.e., that the solubility of AmO_2 in vivo is much greater than that measured in vitro (Boyd, et al., *Radiat. Res.* 76: 549-560, 1978). With regard to screening, the macrophage system correctly predicted that DTPA could be efficacious, and more closely mimics the in vivo solubilization than simple in vitro incubations in media designed to simulate body fluids, and is therefore worthy of further study.

The macrophage system will be used to screen several chelons of bacterial origin, as well as to assess the use of DTPA encapsulated in a phagocytizable, biodegradable polymer.

MIXED-CHELATE THERAPY OF INTRATRACHEALLY DEPOSITED CADMIUM OXIDE

Investigators:

A. W. Conklin and C. L. Sanders

Technical Assistance:

C. White and S. Moe

Mixed-chelate treatment with EDTA and salicyclic acid was no more effective in accelerating the removal of intratracheally instilled ^{109}CdO , or in protecting against CdO -induced mortality, than was EDTA given alone.

Schubert and Derr (Nature 275: 311, 1978) claimed a dramatic protection of mice from the effects of i.v. CdCl_2 with mixed-chelate therapy consisting of EDTA and salicyclic acid (SA).

Based upon Schubert and Derr's paper, we performed an experiment to determine the influence of combined EDTA + SA treatment upon removal of lung-deposited ^{109}CdO and on the survival of rats following inhalation of CdO .

In the removal study, two groups of 12 rats each were given an intratracheal instillation of $20\text{ }\mu\text{g}$ CdO tagged with ^{109}Cd (specific activity of $0.7\text{ nCi}/\mu\text{g}$ Cd). Treatments were started immediately after ^{109}CdO administration. Group 1 rats were killed at 24 hr, Group 2 rats at 48 hr.

The 12 rats were divided into subgroups of 3 rats each; subgroup 1 was given an i.p. injection of 0.9% NaCl ; subgroup 2 was given an i.p. injection of 2.0 mmoles/kg of SA (neutralized to pH 7.0 with NaOH); subgroup 3 was given an i.p. injection of 0.5 mmoles/kg of EDTA; and subgroup 4 was given SA + EDTA in equal amounts. The ^{109}Cd levels in tissues were determined by gamma analysis on a Beckman Gamma 310 spectrophotometer.

We found that SA was no more effective than 0.9% NaCl in accelerating the removal of ^{109}Cd found in excreta at 24-48 hr. The EDTA alone was very effective in accelerating the removal of ^{109}Cd , with over 60% of administered ^{109}Cd found in excreta. Mixed treatment with SA + EDTA was no more effective than EDTA given alone (Table 61). Although mixed SA + EDTA treatment decreased

TABLE 61. Influence of Mixed Chelates on Distribution and Excretion of ^{109}CdO Deposited in Lung

		S.A.	Na Sal	EDTA	S.A.
Lung	24 hr	31 ± 7.3	41 ± 3.7	22 ± 2.5	22 ± 3.2
	48 hr	32 ± 3.5	37 ± 0.9	22 ± 1.8	23 ± 0.1
Liver	24 hr	62 ± 6.7	54 ± 3.7	10 ± 0.28	8.1 ± 0.13 ($p < 0.001$)
	48 hr	61 ± 6.1	53 ± 6.9	13 ± 0.54	7.3 ± 1.7 ($p = 0.023$)
Kidney	24 hr	2.9 ± 0.82	2.7 ± 0.23	2.6 ± 0.36	1.7 ± 0.26 ($p = 0.054$)
	48 hr	4.6 ± 0.63	4.2 ± 0.39	4.0 ± 1.2	2.2 ± 0.06 ($p = 0.12$)
Excreta	24 hr	2.6 ± 1.5	1.2 ± 0.16	64 ± 3.1	68 ± 3.5
	48 hr	2.1 ± 1.2	1.1 ± 0.21	61 ± 0.64	66 ± 3.0

FIGURE 68. Survivors of CdO Inhalation Following Chelate Injection

retention of ^{109}Cd in liver and kidney to less than that retained after EDTA alone, whole-body retention of ^{109}Cd was not significantly different.

In the mortality study, 40 female rats were exposed for 30 min to an aerosol of CdO (MMD, $1.36\ \mu\text{m}$; Cd concentration, $110\ \mu\text{g}/\ell$) that was estimated to be about twice the LC_{50} dose. Immediately following the exposure, the rats were divided into 4 groups of 10 rats each: Group 1 was given (i.p.) 0.9% NaCl; Group 2 was given (i.p.) 2.0

mmoles/kg SA; Group 3 was given (i.p.) 0.5 mmoles/kg EDTA; Group 4 was given SA + EDTA in equal amounts. The EDTA slightly increased survival times over those seen with 0.9% NaCl or SA alone; SA + EDTA was no more effective than EDTA given alone (Figure 68).

Subsequent to these studies, Schubert acknowledged that his previously reported data were invalid (*Nature* 281: 406, 1979). He implied, however, that prompt treatment might be effective. In our hands, prompt treatment was not effective.

MIXED CHELATION THERAPY FOR REMOVAL OF PLUTONIUM AND AMERICIUM

Investigators:

V. H. Smith and R. A. Gelman

Iron-binding compounds, 2,3-dihydroxybenzoic acid (DHBA), 2-hydroxybenzoic acid (HBA) and 2-(acetyloxy)benzoic acid (ABA), were tested for their ability to remove americium and plutonium from rats following intraperitoneal injection of the radionuclides as citrates (pH 5). Treatments, 2 mmol/kg, were given on days 3, 6, 10, 12 and 14 following the actinide injection. DHBA and HBA caused about a 20% decrease in liver retention of americium compared to the control value, and DHB caused a similar effect for plutonium. The above agents, co-administered with 0.5 mmol polyaminopolycarboxylic acid (PAPCA)-type chelons, did not change tissue retention of americium and plutonium from that due to the PAPCAs alone. Administration of americium and plutonium to the same rats is useful for studying removal agents since the two actinides behave independently in their biological disposition and response to removal.

The removal of iron from man by the use of DHBA and the close relationship of iron and plutonium/americium transport in the mammal suggested that this agent might be useful in the excretion of these elements, either alone or as an adjunct to DTPA therapy. When DHBA was tested for oral effectiveness (Annual Report, 1978), it had little effect against intramuscularly injected Pu and Am nitrate, but further tests of its parenteral efficacy were needed. The possibility that DHBA might act as a mixed-ligand chelate (MLC) with DTPA was suggested by the work of Schubert and Derr (Nature 275: 311-313, 1978) on salicylic acid (HBA) administration with DTPA. HBA was also tested in rats to evaluate the claims of the authors and to observe its behavior with americium. We tested aspirin (ABA) because it has a longer circulation half-time than HBA and can be cleaved to HBA in vivo. Residence time, i.e., concentration maintenance, is an important consideration in effective chelation therapy. In theory, all eight coordination sites of plutonium and americium could be optimally occupied by bidentate HBA and a hexadentate polyaminopolycarboxylic acid (PAPCA), e.g., ethylenediaminetetraacetic acid (EDTA). Geometric configurations could lead to less strain and thus stronger MLCs for combinations of HBA and octadentate DTPA or decadentate TTHA (tetraethylenetriaminehexaacetic acid); therefore these combinations were also tested.

Adult, female, Wistar rats [weight, 360 ± 38 g ($\bar{x} \pm$ S.E.)] were injected with $0.70 \pm 0.01 \mu\text{Ci } ^{241}\text{Am}$ and $0.63 \pm 0.01 \mu\text{Ci}$ ($\bar{x} \pm$ S.E.) Pu. The plutonium was a mixture of 98.6% ^{238}Pu and 1.4% ^{239}Pu , by activity, to give a plutonium mixture with the same specific activity as ^{241}Am . The initial mole ratio of Am:Pu was 1.12:1. The isotope mixture was injected intraperitoneally in 1 ml of $5.2 \times 10^{-5}\text{M}$ citrate buffer, pH 5.0. Five rats were housed in each cage, and were fed and watered ad libitum.

Treatments were administered intraperitoneally in 3 ml of pH 7-7.2 solution on days 3, 6, 10, 12 and 14 following actinide administration. (When required, neutralization was performed just prior to use with Na_2CO_3 and NaOH.) In groups of ten, rats were injected with the following agents (doses given per kg of body weight): 1.3 mmol NaCl (PS); 0.5 mmol of Ca-DTPA or Ca-TTHA; 2 mmol of HBA, DHBA or ABA, whether alone or in combination.

Urine and feces were collected daily from each cage of rats on large sheets of Whatman 3MM filter paper. Both urine and feces samples were assayed for plutonium and americium by the methods of Keogh and Powers (Anal. Chem. 42: 421, 1970). For the first 3 days following injection of the radionuclides, excreta collections were made from five groups; when treatments were initiated, collections were made from all groups. Three days after the last treatment,

rats were killed and the liver, spleen, kidneys, ovaries and the remaining carcass assayed for americium and plutonium by the methods described above.

Tissue results were analyzed by multi-variate methods, and Duncan's New Multiple Range Test at the 0.05 significance level was used to test results. Excreta collections were not subject to statistical analyses.

The combinations of chelating agents tested were no more effective than DTPA alone in reducing plutonium and americium in the tissues (Table 62). HBA and DTPA were less effective in removing plutonium from the ovaries than DTPA. HBA significantly ($p > 0.95$) increased ovarian plutonium retention by 50% over control levels. Used alone, DHBA decreased americium and plutonium content of the liver compared to the controls ($p > 0.95$); HBA was similarly effective at $p = 0.92$. ABA was not an effective treatment for removing americium or plutonium from rats since it appeared to decrease fecal excretion of both nuclides compared to controls. ABA increases retention of americium in the carcass.

While it was generally not detected in the tissue measurements, excretion data showed the benzoic acid derivatives affected excretion of americium and plutonium relative to controls (Table 62). DHBA or HBA, together with DTPA, increased fecal and urinary excretion of americium over that of DTPA alone during the first treatment week (as shown for DHBA in Figure 69). In contrast, administering a second chelate with DTPA had no apparent effect on fecal and urinary excretion of plutonium. The hydroxybenzoic acid derivatives by themselves caused a much smaller increase in excretion of plutonium in urine and in feces than did DTPA but did have an effect as illustrated for DHBA and Pu (Figure 69). Similar results were obtained for americium. All the hydroxybenzoic acid derivatives caused a lesser augmentation compared to controls of americium than of plutonium excretion in urine (Table 62). DHBA caused the largest actinide removal via feces: 150% of control rats' excretion of plutonium.

Because the combination of EDTA and HBA provides the exact number of ligands needed for the 8-coordinate americium and plutonium, it should (according to Schubert) form a

TABLE 62. Retention and Excretion of Am and Pu by the Rat 17 Days After Intraperitoneal Injection of Am/Pu Citrate and Efficacy of Various Treatments Given on Days 3, 6, 10, 12 and 14 Afterwards.

Tissue/ Excreta	Percent of Injected Radionuclide in Control Rats ($\bar{x}$ + S.E.)	Treatment Effect as Percentage of Corresponding Control Values ^(a)									
		DTPA ^(b)	HBA+ DTPA	DHBA+ DTPA	ABA+ DTPA	HBA+ TTHA	TTHA	HBA+ EDTA	HBA	DHBA	ABA
Americium:											
Carcass(c)	23 ± 1	59	63	57	61	60	55	91	110	93	150
Liver	18 ± 2	3.8	5.8	4.3	3.8	22	26	84	81	53	94
Kidneys	0.32 ± 0.02	82	79	108	97	103	139	148	108	91	124
Spleen	0.038 ± 0.007	47	56	66	72	72	102	133	86	105	96
Ovaries	0.0066 ± 0.0006	41	62	86	98	65	69	67	67	100	100
Urine	12(d)	280	280	270	350	240	230	170	120	140	130
Feces	35(d)	110	150	120	92	153	130	110	110	100	69
Plutonium:											
Carcass(c)	56 ± 3	62	60	54	63	61	66	89	87	91	95
Liver	8.5 ± 0.8	13	18	16	14	40	49	84	85	78	110
Kidneys	0.64 ± 0.03	68	47	53	60	68	94	100	92	99	110
Spleen	0.33 ± 0.04	56	56	69	63	49	49	85	89	110	100
Ovaries	0.012 ± 0.001	67	102	58	65	112	94	120	150	133	100
Urine	4.2(d)	680	620	710	720	650	550	210	200	230	210
Feces	23(d)	120	110	130	110	100	110	100	92	150	81

(a) Duncan's New Multiple Range Test on the $\sin^{-1} \sqrt{\text{percent of injected dose}}$ transformed data shows that values differing by $> 20\%$ in this table were different at the 0.05 significance level.

(b) DTPA = CaNa_2 diethylenetriaminepentaacetate; TTHA = HCaNa_3 triethylenetetraaminehexaacetate; EDTA = CaNa_2 ethylenediaminetetraacetate. Dose of these three agents was 0.5 mmol/kg body weight. HBA = 2-hydroxybenzoic acid; DHBA = 2,3-dihydroxybenzoic acid and ABA = 2-(acetyloxy) benzoic acid. Dose of these three agents was 2 mmol/kg; all were administered as pH 7-7.2 sodium salts.

(c) Carcass includes remains of rat less the tissues shown. For Am and Pu it has been shown to be essentially equivalent to the skeletal burden of these actinides.

(d) Excreta were collected from groups of rats and no statistical inferences were made.

FIGURE 69. Excretion by Rats of Intraperitoneally Administered Am and Pu Citrate as a Function of Time and Treatments with DHBA + DTPA, DTPA and DHBA. See footnotes to Table 1 for the identity of abbreviated designations assigned to the excretion curves.

strong chelate, and therefore be a superior removal agent; no such effect was observed. TTHA, which is higher dentate than DTPA and has greater geometrical possibilities for occupying the metal sites than EDTA or DTPA, showed no additional actinide removal when administered with HBA (Table 62). We conclude there was no measurable mixed-ligand effect or cooperative action of the iron-binding, hydroxybenzoic acid derivatives with the PAPCA chelons *in vivo*.

With the exception of liver, organ retention of plutonium was greater than that for americium by at least a factor of two in the control animals (Table 62). Less plutonium than americium was retained in the livers of control animals but more americium was removed by the chelators.

The tissue distribution of americium and plutonium in control rats was consistent with parenteral injections of the nuclides separately (J. Vaughan in Hodges, H. C., J. N. Stannard and J. B. Hursh, eds., *Uranium-Plutonium Transplutonic Elements*, Springer-Verlag, New York (1973), pp 357-359, P. Durbin, *ibid.*, pp. 779-784). Excretion of americium and plutonium in urine were similar under the influence of DTPA, although enhancement of control rats' output in urine was only 2-3 times for americium compared to 6-7 times for plutonium. Over 30% of each injected radionuclide was removed by DTPA. Thus, the DTPA-enhanced excretion of the two actinides is similar and consistent with the comparable stability constants of americium and plutonium with DTPA and the bioavailability of the two

nuclides for chelation. Tests of treatment agents in the rat for simultaneously injected americium and plutonium will be valid for either nuclide tested alone.

The iron-binding agents, HBA and DHBA, slightly enhanced plutonium and americium removal from rat liver; DHBA is the more

effective of the two agents. However, neither of the two agents. However, neither of these is nearly as efficient as DTPA. They do not appear to be tapping an actinide pool not available to DTPA, and do not enhance the removal effectiveness of DTPA by mixed-ligand formation, or synergistic or cooperative action.

SOLUBILIZATION OF $^{241}\text{AmO}_2$ IN SIDEROPHORE SOLUTIONS

Investigator: A. V. Robinson

The solubilization of $^{241}\text{AmO}_2$ in tissue culture medium was measured. Variables included serum concentration, chelon type and time. Serum was found to affect the soluble (0.1 μm filterable) fraction more than any other variable.

Macrophages play a central role in the removal of inhaled particles and are thereby exposed to many insults. Inhaled metal oxides deposited in the lung are phagocytized by macrophages, and exert cytotoxic effects on macrophages and surrounding tissue. It is of interest, therefore, to assess the ability of various potentially therapeutic agents to mobilize metal oxides from the macrophage, thus possibly enhancing subsequent clearance from the lung. Before solubility measurements can be made, baseline data must be accumulated regarding the solubility of metal particulates in cell-free culture fluid. The solubility of AmO_2 particles in tissue culture medium, with and without serum, was measured as a function of time, chelon, and siderophore concentration. Solubility was defined as the ability to pass through a 0.1- μm filter.

Americium oxide particles were resuspended in either M-199 or M-199 containing 10% heat-inactivated rabbit serum (RS). Suspensions were centrifuged for 15 min at 1000 x g; the supernatant was aspirated; and the remaining activity was resuspended in appropriate medium. The following day, dilutions of stock were made into appropriate media containing various combinations of siderophore, DTPA (CaNa_3DTPA) and salicylic acid. Concentrations of all agents were adjusted to 0.1 mM and when two agents were combined, each was maintained at 0.1 mM. At appropriate intervals, a sample of each solution was filtered through a 0.1- μm Nuclepore filter, and the fraction of activity in the filtrate (soluble fraction) was counted by liquid scintillation.

The results in Table 63 reveal that, in the absence of serum or chelon, less than 0.1% of the americium was soluble. The addition of various chelons, alone or in concert, reveal some small increases in solubility. The DTPA alone increased the

TABLE 63. In Vitro Solubilization of AmO_2 in Serum-Free Media by Various Chelons

Chelon	% of Initial Activity in 0.1- μm Filtrate		
	Days of Incubation		
	0	4	8
None	0.04	0.05	0.05
DTPA ^(a)	0.05	0.94	1.19
SA ^(b)	0.03	0.05	--
SA + DTPA	0.06	0.95	1.31
SP ^(c)	0.04	0.08	--
SP + DTPA	0.05	1.64	2.09
DHBO ^(d)	0.01	0.02	--
DHBP ^(e)	0.06	0.09	--
DHBP + DTPA	0.06	0.15	--
CA ^(f)	0.13	1.95	0.99
CA + DTPA	0.02	2.66	3.17

^(a) Diethylenetriaminepentaacetic acid

^(b) Salicylic acid

^(c) Bis-salicylputrescine

^(d) Bis-dihydroxybenzoic acid octanediamine

^(e) Bis-dihydroxybenzoic acid putrescine

^(f) Cheladamic acid monohydrate

soluble fraction to about 1% of the total activity, whereas DTPA plus SP, or DTPA plus CA gave values as high as 2% and 3%, respectively. Cheladamic acid alone gave a 4-day solubility of 2%; however, this value dropped to 1% by day 8.

Table 64 lists data obtained in medium containing 10% RS. The most striking aspect of this data is the fact that RS increased the solubility of AmO_2 to such an extent that any contribution by the chelons was insignificant. Dialysis of selected samples on day 8 revealed a distinct component of non-protein-bound soluble activity

TABLE 64. In Vitro Solubilization of AmO₂ in Serum-Containing Media by Various Chelons

Chelon	% of Initial Activity in 0.1- μ m Filtrate Days of Incubation			% Dialyzable at 8 Days
	0	4	8	
None	63	65	63	0
DTPA	60	65	65	11
SA	60	63	--(a)	--
SA + DTPA	59	65	62	10
SP	61	62	--	--
SP + DTPA	60	62	63	11
DHBO	63	64	--	--
DHBP	60	61	--	--
DHBP + DTPA	63	63	--	--
CA	60	63	61	0.03
CA + DTPA	58	61	64	8

(a) Not sampled

in the presence of DTPA. None of the mixed-chelon systems appeared to increase the small-molecular-weight fraction over that of DTPA alone.

These preliminary results tend to confirm the results of Smith and Gelman (Annual Report, 1979), Smith (Annual Report, 1979) and Conklin (Annual Report, 1979), which showed that the use of mixed chelates does not produce a great increase in solubility. The serum-free data indicate the presence of a small component of activity that is available to mixed chelons but not to individual chelons. Further evidence of an interaction between americium and the chelons was found in the total activity (soluble plus suspended particles) measurable in each solution. This total varied from 55% (control, serum-free medium) to 100% (CA + DTPA, DHBO, DHBP + DTPA, serum-free medium). The serum-containing solutions gave a recovery of $86 \pm 5\%$.

Work is underway to more closely define the solubilization of AmO₂ in media and in chelon solutions, and to measure the ability of these chelons to solubilize americium in the in vitro macrophage culture system.

● Viral and Radiation Carcinogenesis

The studies included under this project are concerned with basic biological and biochemical indices that may aid in the detection and understanding of the primary effects of radiation insult and the initiation of the observed malignancies. A primary objective is to determine the role of virus in "radiation-induced" malignancies and in the process to identify those changes (biochemical, virological, immunological, etc.) which might serve to monitor the oncogenic process. Specific efforts include studies of leukemia induced by the β -emitter ^{90}Sr , and lung and bone tumors induced by inhalation of the α -emitters, ^{238}Pu and ^{239}Pu .

CULTURE CONDITIONS AFFECTING THE INCORPORATION OF ^{125}I -LABELED IODODEOXYURIDINE INTO DNA OF MITOGEN-STIMULATED CANINE LYMPHOCYTES

Investigators:

J. E. Morris and T. Graham

A series of experiments have been conducted to define and describe the response of canine lymphocytes in the mitogen stimulation assay systems. These studies include the effects of serum, mitogen, and FUDR concentrations on the incorporation of ^{125}I -IUDR by canine lymphocytes.

In vitro mitogen-induced activation of canine lymphocytes is being used as a method of evaluating cell-mediated immunity in plutonium-exposed dogs.

This study was performed in order to characterize the methods required and to establish base-line data for comparison with those from exposed dogs. We examined the effects of homologous, autologous and heterologous serum concentrations; of fluorodeoxyuridine (FUDR) concentrations; and of mitogen concentration on the incorporation of ^{125}I -iododeoxyuridine (^{125}I -IUDR) by canine lymphocytes from whole blood or density-gradient-isolated peripheral-blood lymphocytes (PBL).

The assay systems used for these studies are outlined in Figure 70. In order to establish whole-blood cultures, canine blood from the external jugular vein was collected in tubes containing heparin, the numbers of leukocytes were counted and differential counts were performed. The whole blood was diluted 1:20 (approximately $1-2 \times 10^5$ lymphocytes per ml) with tissue culture medium and 2 ml of this was cultured with various mitogens for 4 days.

For the isolated peripheral-blood lymphocyte assays, canine blood was collected in tubes containing ethylenediamine tetraacetic acid (EDTA). The lymphocytes were isolated on a density gradient, washed, and viability was determined with trypan blue. Total leukocyte and differential counts revealed that 90% or more of the cells were lymphocytes. The isolated cells were diluted in RPMI 1640 tissue culture medium (containing 10% heat-inactivated fetal bovine serum), mixed with the various mitogens, and incubated for 3 days.

In both culture systems, lymphocyte activation was measured by assessing the incorporation of ^{125}I -IUDR into newly synthesized DNA. The data were reported as a stimulation index (SI); obtained by dividing the total cpm of ^{125}I -IUDR incorporated by stimulated cultures by the total ^{125}I -IUDR incorporated by unstimulated lymphocytes.

Substitution of 10% or 20% autologous or homologous dog serum for 10% fetal bovine serum in the culture medium inhibited the response (Figure 71). Autologous serum

FIGURE 70. Flow Diagram of Peripheral Blood Lymphocyte Activation Studies (WBL, Whole Blood); (PBL, Peripheral Blood Lymphocytes); (IPBL, Isolated PBL).

FIGURE 71. Effect of Serum Source and Concentration on the Incorporation of ^{125}I -IUDR by Mitogen-Stimulated Canine Lymphocytes

appeared to be more inhibiting than homologous serum; 5% homologous serum showed approximately the same amount of incorporation of ^{125}I -IUDR as 10% fetal bovine serum. Similar results have been seen with other dogs in both whole-blood and isolated peripheral-blood lymphocyte studies.

As noted previously, lymphocyte activation with mitogen was measured by the incorporation of ^{125}I -IUDR, a gamma-emitting thymidine analog, into newly synthesized DNA. Two μCi of ^{125}I -IUDR was added to all cultures for the final 20 hr of incubation. Fluorodeoxyuridine (FUDR), a known inhibitor of thymidylate synthetase, was added simultaneously with the ^{125}I -IUDR pulse to preferentially increase the incorporation of the labeled IUDR into DNA.

Concentrations of FUDR were from 10^{-8} to 10^{-6}M for incorporation of ^{125}I -IUDR by lymphocytes in whole-blood cultures stimulated with $74\text{ }\mu\text{g}$ of Con A per culture. In isolated PBL studies stimulated with $2\text{ }\mu\text{g}$ of Con A per culture, maximum incorporation of ^{125}I -IUDR occurred at the same FUDR range.

In general, the characteristics of mitogen stimulation of canine lymphocytes are similar to those of other species. The one exception is the biphasic response to Con-A concentration in whole-blood cultures (Figure 72), which has been observed in all the dogs we have studied thus far. The

FIGURE 72. Effects of Fluorodeoxyuridine Concentrations on the Incorporation of ^{125}I -IUDR by Canine Lymphocytes in Whole Blood Cultures.

responses obtained from isolated peripheral-blood lymphocytes (from the same dogs) and for rat lymphocytes in whole-blood cultures were not biphasic (Figure 73). We do not know whether this biphasic response was a result of a cooperation between lymphocytes

and other cells in the peripheral blood, or due to the presence of serum factor(s) removed upon isolation of the lymphocytes. Additional studies will be necessary to define this point.

FIGURE 73. Stimulation Indices of Concanavalin-A-Induced Activation of Peripheral-Blood Lymphocytes

CHARACTERIZATION OF PERIPHERAL-BLOOD LYMPHOCYTES
IN LYMPHOCYTOPENIC DOGS EXPOSED TO $^{239}\text{PuO}_2$

Investigators:

J. E. Morris, T. Graham and J. F. Park

Mitogen stimulation assays of canine peripheral-blood lymphocytes in whole-blood and density-gradient-isolated lymphocyte cultures showed that a functional decrease had occurred in these lymphocyte populations from plutonium-exposed dogs, suggesting an alteration in immunocompetence.

Previous studies at this laboratory have established that inhalation exposure of dogs to plutonium oxide (at doses between 0.2 and 3 μCi) results in a dose- and time-dependent lymphocytopenia that persists through the life span of the animal. Subsequent studies have shown that the distribution of T and B lymphocytes in the peripheral-blood of these lymphocytopenic dogs has shifted, with a greater reduction in B lymphocytes occurring in exposed dogs when compared to controls (Annual Report, 1978).

To obtain additional information for describing the functional capacity of peripheral-blood lymphocyte populations in exposed dogs, a series of mitogen stimulation assays of whole-blood and isolated peripheral-blood lymphocyte cultures were conducted as in vitro correlates of cell-mediated immunity (Annual Report, 1979).

For the mitogen response studies, we used three groups of dogs (Table 65), matched for age and time postexposure. The three groups included one control (Group I) and two exposed (Groups II and III) groups. The average age and time on experiment were 9.7 and 8.2 yr, respectively. Mean initial deep lung depositions were 128.6 ± 8.9 nCi (Group II) and 738.0 ± 44.4 nCi (Group III). Average number of lymphocytes per ml of blood were $2.73 \pm 0.54 \times 10^6$ (Group I), $2.18 \pm 1.19 \times 10^6$ (Group II; approximately 80% of control values) and $2.07 \pm 0.15 \times 10^6$ (Group III; approximately 75% of control values).

Concanavalin-A (Con A), phytohemagglutinin (PHA) pokeweed mitogen (PWM) and lipopolysaccharide (LPS) were used to induce activation of canine lymphocytes. Lymphocyte activation was measured by the

TABLE 65. Initial Deep Lung Deposition and Peripheral Blood Lymphocyte Values in $^{239}\text{PuO}_2$ -Exposed and Control Dogs

Group	Dog No.	Sex	Age, yr	Years After Exposure	Initial Deep Lung Deposition, nCi	Lymphocytes, cells $\times 10^6/\text{ml}$
I	775	F	10.3	8.7	Control	3.5
	789	M	10.2	8.6	Control	2.4
	801	M	10.0	8.3	Control	2.9
	868	F	9.7	8.3	Control	2.0
	878	M	9.5	7.9	Control	2.5
	903	F	9.3	7.9	Control	3.1
II	750	M	10.3	8.7	118	2.3
	814	F	9.9	8.6	140	4.1
	844	F	9.8	8.3	135	1.0
	884	M	9.5	8.0	123	1.4
	905	F	9.3	7.9	127	2.1
III	840	F	9.8	8.3	703	2.2
	864	F	9.8	8.3	801	1.7
	898	M	9.3	7.9	711	1.9
	909	M	9.3	7.9	737	2.1

incorporation of ^{125}I -iododeoxyuridine (^{125}I -IUDR) in newly synthesized DNA.

In the whole-blood culture studies (Table 66), a definite reduction in the mitogen responsiveness of lymphocytes to Con A, PHA and PWM was observed in both exposure groups compared to control animals. Part of this reduction is obviously due to the lymphopenic state of the exposed dog. However, upon stimulating a constant cell number of 0.1×10^6 isolated peripheral-blood lymphocytes (obtained by density-gradient technique) from exposed or control dogs, the general mitogen response (Con A, PHA and PWM) of cells from the exposed animals was still reduced (Table 67). The

decrease in response of peripheral-blood lymphocytes to the mitogens employed in both assay systems was similar, with a greater reduction in response observed with Con A and no change in response to LPS. This information suggests a possible impairment in immune functions.

Data presented in this report indicate that the inhalation exposure of dogs to plutonium oxide resulted not only in a reduction in peripheral-blood lymphocyte levels, but also in a definite reduction in the functional capacity of the lymphocytes present as measured by mitogen-induced stimulation assays.

TABLE 66. Mitogen Stimulation of Whole-Blood Lymphocytes From Control and Exposed Dogs

Group	Lymphocytes, Cells/ml $\times 10^6$ $\pm$ S.D.	Mitogen Stimulation Index ^(a) $\pm$ S.D.		
		Con-A	PHA	PWM
I	2.73 $\pm$ 0.54	11.53 $\pm$ 5.70	3.63 $\pm$ 1.31	4.22 $\pm$ 1.03
II	2.18 $\pm$ 1.19	5.62 $\pm$ 5.52	1.73 $\pm$ 0.75	1.83 $\pm$ 0.86
III	2.07 $\pm$ 0.15	5.60 $\pm$ 3.56	2.12 $\pm$ 0.24	2.91 $\pm$ 0.60

(a) Stimulation Index = $\frac{\text{cpm Incorporated in Stimulated Cells}}{\text{cpm Incorporated in Unstimulated Cells}}$

TABLE 67. Mitogen Stimulation of Isolated Peripheral-Blood Lymphocytes From Control and Exposed Beagle Dogs

Group	Mitogen Stimulation Index ^(a) $\pm$ S.D.			
	Con-A	PHA	PWM	LPS
I	11.18 $\pm$ 7.32	9.84 $\pm$ 7.44	5.39 $\pm$ 2.96	1.30 $\pm$ 0.40
II	7.25 $\pm$ 3.74	5.82 $\pm$ 3.94	4.49 $\pm$ 2.90	1.60 $\pm$ 0.68
III	4.89 $\pm$ 3.09	3.50 $\pm$ 2.48	3.47 $\pm$ 1.81	1.32 $\pm$ 0.26

(a) Stimulation Index = $\frac{\text{cpm Incorporated in Stimulated Cells}}{\text{cpm Incorporated in Unstimulated Cells}}$

IN VITRO CELL-MEDIATED IMMUNITY STUDIES OF PLUTONIUM-EXPOSED BEAGLE DOGS

Investigators:

J. E. Morris, T. Graham and J. F. Park

Mitogen-induced activation was measured in spleen and mesenteric lymph node cell preparations from dogs exposed to a single inhalation exposure of plutonium oxide (^{238}Pu or ^{239}Pu). Reduced stimulation indices of splenic lymphocytes from exposed animals suggest that a reduction in lymphocyte function has occurred in this tissue. No apparent reduction in mitogen stimulation indices was observed in mesenteric lymph node cultures.

Previous studies on the biological effects of inhaled $^{239}\text{PuO}_2$ or $^{238}\text{PuO}_2$ in beagle dogs have indicated that the most significant effects were the appearance of chronic lymphocytopenia, respiratory insufficiency, and pulmonary neoplasia after initial alveolar deposition of 0.2 to 3.0 μCi . In these studies, lymphocytopenia persisted throughout the life span of the animals and was often the only observed effect of the single plutonium exposure. Subsequent studies have established that the lymphocytes in peripheral blood of plutonium-exposed dogs were not only reduced in number per unit volume of blood but also in functional capacity, as monitored with mitogen stimulation studies (Annual Report, 1979).

To determine if the loss in mitogen responsiveness of lymphocytes had occurred in other lymphoid tissues, mitogen studies have been conducted on spleen and mesenteric lymph node cells obtained from long-term plutonium-exposed dogs at sacrifice.

The assay system used in these studies is outlined in Figure 74. Basically, spleen and mesenteric lymph nodes of dogs were removed aseptically. Cell suspensions of the tissues were prepared, total and differential counts were made, and viability was checked with trypan blue. The cells were diluted with supplemented RPMI 1640 tissue culture media (containing 10% heat-inactivated fetal bovine serum) to give a final density of 2.2×10^6 viable cells per ml in the assay.

The mitogens used were: Concanavalin-A (Con-A) and phytohemagglutinin (PHA), plant lectins that stimulate T lymphocytes; lipopolysaccharide (LPS, a B-cell-activating endotoxin); and pokeweed mitogen (PWM), a plant lectin, mainly specific for B lympho-

cytes, but which also stimulates a small portion of the T-cell population.

Lymphocyte activation was estimated by measuring the incorporation of ^{125}I -iododeoxyuridine (^{125}I -IUDR) into newly synthesized DNA. The extent of ^{125}I -IUDR incorporated by lymphocytes was measured. The data are reported as a stimulation index (SI); after 3 days of cell incubation in the presence of the mitogens at selected concentrations, obtained by dividing the total cpm of ^{125}I -IUDR incorporated by stimulated cultures by the total ^{125}I -IUDR incorporated by unstimulated lymphocytes.

In spleen culture studies (Table 68), we have observed a reduced mitogen response with lymphocytes from severely lymphopenic dogs. Specifically, the SI of splenic lymphocytes from $^{238}\text{PuO}_2$ -exposed dogs showed reduced mitogen stimulation which is related to both the initial deep lung deposition of plutonium oxide and the presence of bone tumors. The SI of two low-level ^{238}Pu -exposed dogs (#1189, #1312), with no diagnosed tumors, was high. In $^{239}\text{PuO}_2$ -exposed dogs, the Con-A-induced stimulation of spleen lymphocytes was consistently repressed and correlated with the presence of lung tumors. However, apparent correlation was observed between SIs of spleen lymphocytes and initial deep lung depositions of $^{239}\text{PuO}_2$ -exposed dogs. Thus far, we have analyzed the spleen data from one control animal, which was 10 yr old at death. It should be noted that we do not know the specific effects that aging may have on immune functions estimated by mitogen stimulation assays.

Relatively high and variable SIs have been observed with mesenteric lymph node cells from exposed dogs (Table 69). No

FIGURE 74. Flow Diagram of Tissue-Derived Lymphocyte Activation Studies

TABLE 68. Mitogen Stimulation of Spleen Lymphocytes from Control and Exposed Beagle Dogs

Dog No.	Sex	Age, yr	Years After Exposure	Inhaled Plutonium Oxide	Initial Alveolar Deposition, μCi	Tumor	Mitogen Stimulation Index ^(a) $\pm$ SD			
							Con-A	PHA	PWM	LPS
1037	M	7.4	5.8	^{238}Pu	4.854	Bone	1.7 ± 0.5	2.0 ± 0.2	1.1 ± 0.04	2.1 ± 0.5
1042	F	7.3	5.7	^{238}Pu	2.959	Bone	6.4 ± 1.0	4.2 ± 0.2	4.5 ± 0.5	1.9 ± 0.2
1027	M	7.5	5.9	^{238}Pu	2.148	Bone	1.7 ± 0.01	<1	<1	<1
1092	M	7.0	5.4	^{238}Pu	1.848	Bone	2.8 ± 0.5	1.7 ± 0.2	1.2 ± 0.1	<1
1189	M	6.6	5.0	^{238}Pu	0.071	N ^(b)	16.0 ± 1.6	9.0 ± 0.2	11.0 ± 2.4	1.0 ± 0.2
1312	M	5.7	4.1	^{238}Pu	0.058	N	26.7 ± 9.9	14.2 ± 1.2	21.5 ± 1.9	3.4 ± 0.7
1328	F	5.1	3.5	^{239}Pu	3.440	ND ^(c)	8.8 ± 0.9	5.0 ± 0.1	1.2 ± 0.3	0.9 ± 0.1
1371	M	5.0	3.0	^{239}Pu	3.060	ND	8.7 ± 0.7	2.0 ± 0.4	3.4 ± 0.2	<1
1377	M	5.1	3.7	^{239}Pu	2.260	ND	9.0 ± 0.3	8.9 ± 0.1	4.2 ± 0.8	<1
1276	F	5.7	4.1	^{239}Pu	2.957	ND	3.4 ± 1.0	NT ^(d)	NT	NT
864	F	9.8	8.2	^{239}Pu	0.801	Lung	3.9 ± 0.3	1.8 ± 0.5	2.4 ± 0.1	<1
880	F	8.8	7.2	^{239}Pu	0.840	Lung	2.7 ± 0.6	1.8 ± 0.3	1.4 ± 0.3	<1
888	M	8.8	7.2	^{239}Pu	0.274	Lung	5.1 ± 0.6	3.8 ± 0.7	3.4 ± 0.2	1.2 ± 0.1
752	M	9.8	8.2	^{239}Pu	0.062	Bone	2.4 ± 0.3	1.5 ± 0.02	1.5 ± 0.1	<1
701	F	10.1		C ^(e)			10.7 ± 0.8	9.1 ± 0.7	5.4 ± 0.4	1.5 ± 0.1

^(a) Mitogen Stimulation Index = $\frac{\text{incorporated cpm of stimulated lymphocytes}}{\text{incorporated cpm of unstimulated lymphocytes}}$

^(b) N - None detected by histological examination

^(c) ND - Not determined

^(d) NT - Not tested

^(e) C - Unexposed controls

TABLE 69. Mitogen Stimulation of Mesenteric Lymph Node Lymphocytes from Control and Exposed Beagle Dogs

Dog No.	Sex	Age, yr	Years After Exposure	Inhaled Plutonium Oxide	Initial Alveolar Deposition, μCi	Tumor	Mitogen Stimulation Index ^(a) ± SD			
							Con-A	PHA	PWM	LPS
1037	M	7.4	5.8	^{238}Pu	4.854	Bone	44.5 ± 5.4	21.7 ± 3.5	30.2 ± 4.4	< 1
1042	F	7.3	5.7	^{238}Pu	2.959	Bone	67.9 ± 7.6	44.7 ± 7.7	43.5 ± 3.7	1.1 ± 0.1
1027	M	7.5	5.9	^{238}Pu	2.148	Bone	208.4 ± 3.4	130.3 ± 1.0	97.0 ± 5.4	<1
1092	M	7.0	5.4	^{238}Pu	1.848	Bone	58.5 ± 5.8	23.8 ± 8.4	21.7 ± 4.5	1.2 ± 0.2
1189	M	6.6	5.0	^{238}Pu	0.071	N ^(b)	49.1 ± 6.8	24.0 ± 5.1	20.6 ± 1.5	1.2 ± 0.1
1312	M	5.7	4.1	^{238}Pu	0.058	N	67.3 ± 12.7	35.4 ± 0.6	15.5 ± 0.3	3.1 ± 0.8
1328	F	5.1	3.5	^{239}Pu	3.440	ND ^(c)	163.1 ± 18.9	57.4 ± 6.6	1.3 ± 0.3	2.2 ± 0.5
1371	M	5.0	3.0	^{239}Pu	3.060	ND	173.9 ± 11.8	67.9 ± 3.4	68.0 ± 20.3	1.7 ± 0.2
1377	M	5.1	3.7	^{239}Pu	2.260	ND	64.8 ± 11.5	22.6 ± 5.0	28.6 ± 2.8	1.4 ± 0.2
1276	F	5.7	4.1	^{239}Pu	2.957	ND	33.7 ± 2.6	NT ^(d)	NT	NT
864	F	9.8	8.2	^{239}Pu	0.801	Lung	77.3 ± 3.9	35.6 ± 4.4	62.5 ± 7.3	2.2 ± 0.3
880	F	8.8	7.2	^{239}Pu	0.840	Lung	16.4 ± 1.0	11.9 ± 0.1	9.8 ± 1.1	<1
888	M	8.8	7.2	^{239}Pu	0.274	Lung	70.7 ± 3.2	17.1 ± 4.6	26.5 ± 3.9	1.9 ± 0.6
752	M	9.8	8.2	^{239}Pu	0.062	Bone	94.9 ± 22.2	35.9 ± 12.9	27.1 ± 1.2	2.2 ± 0.1
701	F	10.1		C ^(e)			80.4 ± 12.2	33.0 ± 7.1	34.2 ± 1.7	2.1 ± 0.5

(a) Mitogen Stimulation Index = $\frac{\text{incorporated cpm of stimulated lymphocytes}}{\text{incorporated cpm of unstimulated lymphocytes}}$

(b) N - None detected by histological examination

(c) ND - Not determined

(d) NT - Not tested

(e) C - Unexposed controls

apparent trend in the SIs of these lymphocytes with Con-A, PHA, PWM or LPS have been observed in dogs exposed to high (2 to 4 μCi) or low (<0.1 μCi) levels of plutonium oxide (^{238}Pu or ^{239}Pu).

The reduction in stimulation indices of spleen cells correlates with the reduced responsiveness observed with peripheral-blood lymphocytes, and suggests that immunocompetence of plutonium-exposed dogs is impaired at the cellular level.

FURTHER CHARACTERIZATION OF THE REVERSE TRANSCRIPTASE
ASSOCIATED WITH PORCINE (RADIATION-INDUCED) RETROVIRUSES

Investigators:

M. E. Frazier, L. P. Mallavia, and M. J. Hooper

Technical Assistance:

T. K. Andrews, Jr. and J. E. Samuels

Differential centrifugation and affinity chromatography were used to purify the porcine, radiation-induced retrovirus, RNA-dependent DNA polymerase. The purified RDDP was then differentiated from cellular DNA polymerase γ by its template specificity, chromatographic characteristics, and the optimum reaction conditions required for DNA synthesis.

Not until the 1970s was it known that normal animal cells contained several DNA polymerases. Since that time, the study of DNA polymerases in normal and neoplastic cells has helped to elucidate the mechanism of oncogenesis by RNA tumor viruses (retroviruses). Reverse transcriptase, the RNA-dependent DNA polymerase (RDDP) associated with retroviruses is now recognized as a unique biochemical marker of the presence of these agents. It is therefore important to be able to distinguish RDDP activities from those of normal cellular polymerases. These include RNA-primed DNA-dependent DNA polymerases, DNA polymerase γ , and terminal transferase. DNA polymerase γ and reverse transcriptase are frequently confused because they have similar template specificities, molecular weights, and chromatographic characteristics. Nonetheless, they can be distinguished from one another on the basis of these same properties.

Earlier studies in this laboratory have provided four lines of evidence that the DNA polymerase from leukemic swine is indeed a viral reverse transcriptase: 1) The DNA polymerase is associated with a cytoplasmic particulate fraction the size, density and morphology of a retrovirus; 2) the endogenous synthesized DNA by this enzyme is sensitive to RNase, requires a divalent cation, and needs all four deoxynucleoside triphosphates; 3) the product of the endogenous reaction is newly synthesized ^3H -DNA; 4) the product of the endogenous reaction is associated with a 70S RNA molecule (characteristic of the retrovirus genome) in an RNA:DNA hybrid.

In this report we provide the fifth, and final, piece of evidence: the purified enzyme can be differentiated from "normal" cellular DNA polymerases.

The purification scheme (Figure 75) for RDDP derived from cultured leukemic cells was designed to isolate all particulate matter of the size of porcine radiation-induced retrovirus (PORRV) by differential centrifugation. We have found this procedure very effective in removing contaminating cellular DNA polymerases (data not shown) from the virus pellet. In fact, the virus pellet contains both virus and cellular debris with a sedimentation coefficient from 400 to 900S. Following solubilization of viral protein, and centrifugation to remove any remaining particulates, the viral DNA polymerase activity is applied to an affinity chromatography column containing oligo dT₁₂₋₁₈ covalently bound to cellulose, which is used to provide one-step purification of the PORRV DNA polymerase.

Each step of the purification process was followed by monitoring the ability of the DNA polymerases to use various primers (rAdT₁₂₋₁₈, dAdT₁₂₋₁₈, or nicked DNA) as templates for initiating synthesis. In these assays, incorporation of ^3H -TTP into an acid-insoluble product by PORRV DNA polymerase was linear for over an hour (Figure 76) when rAdT was the template. The enzyme was unable to use the other two templates.

Two peaks of viral RDDP activity were observed from the oligo dT cellulose column:

FIGURE 75. Porcine Retrovirus (PR) Purification Scheme

FIGURE 76. Enzyme Assays were Carried out in a 100- μ l Reaction Mixture Containing 18 μ l Purified PoRRV-DNA Polymerase; 200 mM Tris-HCL, pH 8.3; 1 mM DTT; 50 mM KCl ; 0.2 mM Mn(CH₃CO₂)₂; 0.8 μ M dTTP; 2 μ Ci [³H] dTTP (40 Ci/mM) and 5 μ g rAdT 12-18. The reaction mixture was stored at 37°C; 10- μ l aliquots were withdrawn at the indicated intervals and assayed for trichloroacetic-acid-precipitable reactivity.

the first peak was eluted by 0.1M KCl and the second eluted by about 0.3M KCl (Figure 77). While the enzyme activities in both peaks appear to be identical, the second peak was of considerably higher specific activity, and was used in the assays to characterize enzyme activity. The increase of specific activity obtained at each step of purification indicates that our purification scheme provides a highly purified RDDP preparation for characterization (Table 70a).

Divalent cation preferences of viral DNA polymerases are commonly used to differentiate between RNA tumor virus types. Therefore, we assayed the response of the enzyme to the concentration of magnesium and manganese (Figure 78). The PORRV DNA polymerase clearly prefers Mn^{++} , showing a 2-fold increase in synthesis with Mn^{++} vs Mg^{++} at optimal concentration for both divalent cations. Earlier experiments have indicated that the endogenous DNA polymerase activity of PORRV (viral RNA serves as the template) shows a similar preference for Mn^{++} , although

these reactions require 10-fold (2 to 5 mM) higher concentrations of divalent cations for optimal synthesis. These results help to substantiate morphologic data indicating that PORRV is a type C virus.

Earlier data (Annual Report, 1978) clearly showed that the PORRV DNA polymerase from swine leukemia cells was different from terminal transferase, DNA polymerase α , and DNA polymerase β . However, we felt that further evidence was required to substantiate our claims that the observed activity was not DNA polymerase γ . Table 70b lists several differences between these two enzymes that we have observed in experiments comparing DNA polymerase γ with PORRV-RDDP. These data constitute evidence that we have obtained highly purified reverse transcriptase from PORRV. Future studies will include molecular weight determination, immunological characterization, and comparison with similar enzymes isolated from other retroviruses.

FIGURE 77. Chromatography of PoRRV DNA polymerase of dT 12-18 Cellulose. Approximately 650 µg of protein were applied to the column and eluted with a linear KCl gradient containing 0.1% Triton X-100 and 1 mM dithiothreitol. Fractions were collected and 25-µl aliquots were assayed for activity with rAdT 12-18 as a primer under the conditions described in Figure 76. The reaction mixtures were assayed for trichloroacetic-acid-precipitable activity after 30 min of incubation at 37°C. (●) cpm ³[H] TTP incorporated with rAdT 12-18 assay; (▲) m KCl; (x) µg protein based fluorescamine method.

TABLE 70a. Purification of PoRRV DNA Polymerase^(a)

Fraction	Total Protein, mg	Total ^(b) Units	Specific Activity, Units/mg	% Yield
19,000 rpm Pellet	17.8 (c)	30972	1740	--
Total Glycerol Gradient	8.5 (c)	36211	4260	100
Fraction B Glycerol Gradient	2.2 (d)	27484	12493	76
Solubilized Enzyme (50,000 rpm Supernate)	0.9 (d)	27863	30959	77
Oligo dT Column				
Fractions 3-8	0.6 (d)	14145	23575	39
Fractions 21-24	0.016 (d)	7488	468000	21

- (a) The PoRRV was obtained from a cell line derived from a miniature swine with radiation-induced myelogenous leukemia
(b) One unit is defined as the amount of enzyme that converts 1 pmole of ³H-TTP per hour at 37° to acid precipitable activity (rAdT₁₂₋₁₈ templated reaction)
(c) Protein determination by method of Lowry
(d) Protein determination by fluorescamine method

FIGURE 78. Dependence of Reaction Velocity of PoRRV DNA Polymerase on Divalent Cation Concentration. The divalent cations were tested as manganese acetate and magnesium acetate. DNA polymerase was assayed as described in Figure 76, using rAdT 12-18 as template. Kinetics of incorporation at each point were monitored every 15 min and found to remain linear for at least 60 min. The values plotted represent the cpm value from a 10-μl sample at 60 min.

TABLE 70b. Comparative Properties of PoRRV DNA Polymerase and Cellular DNA Polymerase α

Property	DNA PoRRV	Polymerase γ
Primer Template Preference ^(a)		
rAdt ₁₂₋₁₈	100	33
dAdt ₁₂₋₁₈	< 0.1	100
Activated DNA	< 0.1	5
rCmdG ₁₂₋₁₈	1	0.1
rCdG ₁₂₋₁₈	9	0.1
$\frac{\text{dAdt}_{12-18}}{\text{rAdt}_{12-18}}$ Ratio	< 0.001	3
Divalent Cation Preference	Mn ⁺⁺ , 0.2 mM	Mn ⁺⁺ , 0.5 mM
N-Ethylamide Inhibition	Sensitive	Sensitive
pH Optimum	7.9-8.4	7-8
Chromatographic		
DEAE Cellulose	Binds Poorly/Elutes at <0.05 M	Binds/Elutes <0.3 M
Phosphocellulose	Binds/Elutes at ~0.3 M	Binds/Elutes ~0.3 M
Oligo dT Cellulose	Binds/Elutes at ~0.3 M	Does Not Bind

^(a) Expressed as percentage of the preferred template

USE OF NUDE MICE FOR GROWTH AND EVALUATION OF RADIATION-INDUCED TUMORS

Investigators:

M. E. Frazier, T. K. Andrews and K. E. McDonald

Technical Assistance:

B. B. Thompson and M. J. Hooper

Heterotransplantation of cell cultures, derived from radiation-induced tumors, into nude mice indicates that this technique can be a useful tool in determining the malignancy potential of these cells. However, the route of inoculation of these tumor cells is an important factor in determining the transplantability of such cells.

In general, malignant tumor cells cause tumors when injected into nude (immune-deficient) strain of mice. The ability of cells to form tumors in these mice is commonly used as an assay for malignant transformation. However, special considerations have been noted which can cause difficulties in interpreting the results of these assays: 1) normal tissues can be transplanted to nude mice, 2) tumors sensitive to natural killer cells (natural immunity) cannot be transplanted to nude mice, 3) when injected subcutaneously, transplanted tumors usually do not metastasize in nude mice, and 4) cultured tumor cells give higher percentages of successful transplants with fewer cells than do tissue slices. Nevertheless, the nude mouse remains the simplest system for studying the heterotransplantability of malignant tumors. We routinely use this technique in our laboratory for evaluating the malignancy potential of cell cultures derived from tumors induced in experimental animals following exposure to internally deposited radionuclides. In the course of these studies we have made some observations which have improved the chances of successful transplantation.

The most important factor in obtaining successful transplantation is the route of inoculation. All previous studies using

nude mice to evaluate the malignant potential of cell lines call for the subcutaneous injection of the tumor cells. We have, however, obtained much better results using intraperitoneal injections of these same cell lines. The data in Table 71 are representative of observations for a number of cell lines: intraperitoneal inoculation of malignant cells gives higher percentages of tumors in shorter time periods, and with fewer cells in the initial inoculum than are obtained using the more conventional (subcutaneous) routes of inoculation.

In addition, tumor cells inoculated into the peritoneal cavity of nude mice readily metastasize to the lymph nodes and other organs, providing added proof of their malignant potential.

Another advantage to this route of inoculation is that the tumor cells grow in the peritoneal cavity as single cells or in small clumps of cells suspended in ascites fluid (Figure 79). This allows the investigator to obtain large numbers of monodispersed tumor cells in excellent physiological condition. In fact, if we use aseptic techniques to avoid infection, the ascites fluid can be harvested every 4 to 5 days for up to 3 wk, at which time the animals usually die from metastases.

TABLE 71. Effect of Inoculation Route on Transplantability of Cultured Tumor Cells in Nude Mice

Cell Line	Number of Cells Inoculated	Route of Inoculation	Average Time To Tumor Development	Mice with Tumors/ Mice Inoculated
PNL-P2 Clone 1 ^(a)	1.3×10^7	S.C. ^(b)	~60 days	5/5
	6.7×10^6	S.C.	~80 days	3/4
	1.3×10^6	S.C.	~90 days	5/9
	1.3×10^6	I.P. ^(c)	~30 days	5/5
D1064 ^(d)	1×10^7	S.C.	~80 days	5/5
	5×10^6	S.C.	~100 days	4/5
	2×10^6	I.P.	~60 days	5/5

(a) PNL-P2 Clone 1 is a cell line derived from a miniature swine with radiation induced myelogenous leukemia

(b) Subcutaneous

(c) Intraperitoneal

(d) D1064 is a cell line derived from a plutonium-induced lung tumor in a beagle dog.

FIGURE 79. Nude Mouse 35 Days after Intraperitoneal Injection of 1.3×10^6 PNL-P2 clone cells. The peritoneal cavity contained more than 50 ml of ascitic fluid. Lactic acid dehydrogenase isoenzyme analysis of the cells present in the ascitic fluid (following removal of the red blood cells) indicated that more than 95% of the cells were miniature swine (porcine) in origin.

• Development of Blood Irradiators

Extracorporeal irradiation of blood, using repeated brief exposures, has been shown to suppress rejection of tissue transplants and to inhibit progression of chronic lymphocytic leukemia. This project is designed to study the basic processes by which blood irradiation produces such effects, to establish the conditions of dose administration which optimize therapeutic effect, to improve the techniques of blood irradiation through the development of improved and portable blood irradiators, and to move this technique toward clinical applications.

A FULLY PORTABLE BLOOD IRRADIATOR

Investigators:

F. P. Hungate, W. F. Riemath and L. R. Bunnell

A fully portable blood irradiator has been developed using the beta emitter thulium-170 as the radiation source and vitreous carbon as the body of the irradiator, matrix for isotope encapsulation and blood interface material. These units have been placed in exteriorized arteriovenous shunts in goats, sheep and dogs and the effects on circulating lymphocytes and on skin allograft retention times measured. The present work extends these studies by establishing baseline data for skin graft rejection times in untreated animals.

Dogs selected for reciprocal grafting were paired on the basis of having no common ancestor in the prior two generations. Following removal from the anesthetized dog, skin patches, uniformly 2 cm x 2 cm, were prepared by slicing with a sharp blade through the skin patch at the level of the hair follicle. In this way, there was minimal damage to the dermal and epidermal tissues transplanted. Skin patches were briefly held in normal saline prior to application to the lateral rib cage area of the host. Bleeding of small vessels in the transplant area was suppressed by applying a small amount of 1:50,000 epinephrine in saline and by clamping large vessels. Typically, two identical allografts were placed on each animal. In some dogs, an autograft was also applied, making three patches on each animal. Patches were sutured into place with 3-0 silk and covered with a gauze pad held in place by a bandage wrap around the animal. The gauze pad, which provided initial pressure to assist in initiating vascular growth, was removed a day or two after surgery; sutures were

removed about 1 wk following graft placement. Animals received systemic antibiotics but no local treatment of the graft areas.

Table 72 gives the results of the skin graft tests. Dogs 1098 and 1075 were regrafted 1 mo following placement of the initial grafts to confirm that an immune reaction had been initiated, and to observe the course of a second rejection. It was clear that the second set of skin patches provoked immediate vascular congestion, so that rejection depended only on the time necessary for the skin to become distinctly necrotic. Animal numbers grouped by two in the table indicate that skin grafts were reciprocal between the pairs. While aseptic techniques were used, some of the patches (Dogs 1142, 1138, 1295) appeared to be infected, resulting in early loss. This was not confirmed by direct culture of the organisms.

The biopsy samples taken from dogs on day 7 (1137, 1156, 1159, 1169), on day 5 (1142, 1122, 1100, 1073), or on day 4 (1138,

TABLE 72. Full-Thickness Skin Grafting on Beagle Dogs

Dog Number	Allograft Rejection Time, Days	Autograft Retention Time, Days
1098	15	NA
1075	10	NA
1098 regraft	7	Take
1075 regraft	7	Take
1076	13	Take
1099	14	Take
1137	10, 11 ^(a)	NA
1156	11, 11	NA
1159	10 ^(a) , 10	NA
1169	11, 11	NA
1073	15 ^(a) , 8	NA
1100	6 ^(a) , 14	Take
1142	6 ^(a) , 6	9
1122	9 ^(a) , 12	NA
1138	9 ^(a) , 9	4
1285	14 ^(a) , 14	Take
1259	14, 14	Take
1281	9, 9	9
1295	7, 7	7
1163	7, 11	Take
1665 (1161)	29, 28	NA

^(a) Biopsies removed for histologic examination
 NA - not applicable, no autograft was placed

1285, 1259, 1281) were not helpful in interpreting the rejection reaction. Overall, the extent of necrosis was moderately severe; mild neutrophilic cell infiltration and hemorrhage was apparent; and there was little evidence of fibrocytic proliferation. There was no clear distinction between the histology of the allografts as compared to the autografts.

Dog 1665 received a pair of skin patches from dog 1161 following 12 days of treatment with a vitreous carbon-thulium 170 (VCTm) irradiator that delivered a transit dose of 30 rad. The irradiator remained in place, continuing to give a daily dose to blood of about 4500 rad during the entire period. It is clear that rejection of the skin patches was delayed well beyond any of the times observed for previously treated animals. Lymphocyte levels in Dog 1665 were unusually low ($1.7 \times 10^3/\text{mm}^3$) prior to any treatment, and fell to only $0.5\text{--}0.7 \times 10^3/\text{mm}^3$ following continued treatment with the VCTm unit. Therefore, it would be difficult to conclude from the findings for this dog alone that prolonged retention resulted from the blood irradiation. However, this prolonged retention is consistent with earlier data reported for goats, a baboon and dogs.

The unit designed last year, which was used to hold the irradiator on dog 1665, is difficult to use because of limited accessibility to the irradiator and its connections to the shunt tubing. An alternative system, using a nylon jacket designed for beagle dogs (Alice Chatham, 5043 Oaknoll Ave., Los Angeles, CA) was used to stabilize the irradiator, which was fastened to the jacket dorsally, at the base of the neck. A thermoplastic cover surrounded the irradiator and the exposed shunt tubing. The shunt tubing was tunneled under the skin from the cannulas leading from the carotid artery and the jugular vein to points on the midlateral surface of each side of the neck. While this approach offers many potential advantages, more tests will be needed to establish our ability to maintain prolonged flow through shunts routed in this way.

We anticipate augmenting our information about the immune reaction and the skin graft response by pretesting the animals for their mixed-lymphocyte response in order to assure that donor and recipient animals are immunologically dissimilar. The mixed-lymphocyte response can also be used to evaluate the extent of recovery in the cellular immune system associated with fractionated as compared to acute exposures.

Fusion

FUSION

Limits on human exposure to magnetic fields have been established in most high-energy particle accelerator facilities. It is recognized, however, that these limits are almost completely arbitrary because knowledge of the potential for magnetic-field bioeffects is extremely meager. Certain advanced technological applications of high-strength magnetic fields will be accompanied by occupational exposure to fields one hundred or more times that of the geomagnetic field, examples being magnetic-confinement fusion reactors, superconducting magnetic energy storage and magnetohydrodynamic power generation. Magnetically levitated vehicles will expose both occupational groups and public passengers. Magnetic shielding of space to be frequented by people has practical limitations and constitutes a significant cost factor in all applications. Moreover, the fundamental criterion of shielding design—the maximum acceptable residual magnetic field strength, from the point of view of human safety—is not known.

In this section we report on subprojects which comprise our current program on magnetic-field bioeffects. The field strengths used are high, usually of the order of 1 tesla, and are static or ramped. The experimental systems range from biophysical and cellular preparations in small-volume magnetics to laboratory mice in large accelerator beam-bending magnets.

• Biological Effects of Magnetic Fields

The development of magnetic fusion power sources, as well as other technologies such as superconducting magnetic energy storage, magnetohydrodynamic power generation and magnetically levitated vehicles, increases the probability of exposure of people to magnetic fields. The lack of a credible data base from which exposure limits can be established led to the initiation of a broadly based program to study possible effects in a number of biological systems under carefully controlled conditions. Studies using cultured mammalian cells, pregnant mice, trout eggs, male mice and biologically derived polymers exposed to magnetic fields are reported.

DOMINANT LETHAL STUDIES IN MICE EXPOSED TO MAGNETIC FIELDS

Investigators:

L. D. Montgomery, D. D. Mahlum, and J. R. Decker

Technical Assistance: E. G. Kuffel

An experiment was performed to evaluate the effects of exposure to magnetic fields on the production of dominant lethals in male Swiss-Webster mice by ethylmethane sulfonate (EMS). Mice were administered EMS and exposed to either a homogeneous or inhomogeneous magnetic field. After exposure, the males were assessed for dominant lethal mutations by breeding to untreated females. A dose-related response to EMS was observed; however, the breeding performance and the incidence of dominant lethal mutations of the male mice injected with EMS was not affected by exposure to a magnetic field.

Studies in progress with cultured mammalian cells have suggested that magnetic fields increase the effectiveness of the known mutagen, ethylmethane sulfonate (EMS; Frazier, this Annual Report). A study was therefore undertaken to determine if exposure to a magnetic field would affect the mutagenicity of EMS in the mouse.

In the first portion of the study, adult male, Swiss-Webster mice were purchased from Hilltop Laboratories and randomly assigned to five dose groups. Each animal was individually numbered and weighed prior to a single injection of 100, 200, 300 or 400 mg/kg body weight of EMS, or 400 mg/kg of saline only (non-EMS control). After injection, each male was housed in a solid-bottom cage with three untreated female mice. Females were replaced weekly. Females were sacrificed by cervical dislocation 10 days after separation from the males (i.e., at 10-17 days of gestation (dg), if mating had

occurred). The uterus was exteriorized for examination, and the total number of implantations, fetal viability and the incidence of resorptions noted.

All groups showed a decreased fertility rate during the second week of breeding (Figure 1). Only one of the 15 females available for breeding in the non-EMS group was pregnant; she had 12 implants. In the 400-mg/kg group, three were pregnant, with a combined total of 9 implants. In weeks 3 through 6, the 300-mg/kg group also had a reduced incidence of pregnancies.

The incidence of early resorptions was used as the measure of dominant lethality (Figure 2). The administration of EMS resulted in a dose-related increase in the incidence of early resorption. This increase was particularly apparent in the 300- and 400-mg/kg groups during the first and second weeks of breeding, after which the incidence

FIGURE 1. Effect of Ethylmethane Sulfonate (EMS) on Fertility

FIGURE 2. Effect of Ethylmethane Sulfonate (EMS) on the Incidence of Early Resorptions

returned to control levels. The incidence of early resorptions was also increased in the 200-mg/kg group, although the response was substantially lower than in either the 300- or 400-mg/kg group. The 100-mg/kg group showed no effect.

In the second part of the study, male mice were injected with either 200 or 300 mg/kg of EMS, or with saline. They were then sham-exposed or exposed to either a static homogeneous field of 1 Tesla (T) or to a static inhomogeneous field of 2 T/meter (range 0.1-1.0 T). Exposures were performed at the Stanford Linear Accelerator Center, Palo Alto, CA, using the system described by Decker et al. (Annual Report, 1978). After exposure, the mice were transported to the Pacific Northwest Laboratory for the dominant lethal assay.

In this part of the study, the fertility rate was decreased by the 300-mg/kg dose of EMS, but only during the first week of breeding (Figure 3). The 200-mg/kg dose had no effect. Exposure to either a homogeneous or inhomogeneous magnetic field did not

affect the fertility rate. The effect of EMS treatment combined with magnetic field exposure was not significantly different from that observed with EMS alone.

The incidence of early resorptions was increased during the first week of breeding by both the 200- and 300-mg/kg doses of EMS (Figure 4). The incidence remained elevated during the second week in the 300-mg/kg group. Exposure of males to either a gradient or a homogeneous field alone did not increase the incidence of early resorptions. The response to EMS was not influenced by exposure to an inhomogeneous magnetic field. Exposure to a homogeneous field, however, appeared to decrease the incidence of early resorptions in mice which received 300 mg/kg of EMS. Since the incidence was unaffected in the 200-mg/kg group, it is unknown whether the decrease seen in the 300-mg/kg group is artifactual or not.

No evidence was obtained in these experiments for an enhancement of the mutagenic effect of EMS in mice by exposure to magnetic fields.

FIGURE 3. Effect of Ethylmethane Sulfonate (EMS) and Magnetic Field Exposure on Fertility

FIGURE 4. Effect of Magnetic Field Exposure and Ethylmethane Sulfonate (EMS) on Early Resorptions

DEVELOPMENT OF MICE AFTER INTRAUTERINE EXPOSURE TO DC MAGNETIC FIELDS

Investigators:

L. D. Montgomery, M. R. Sikov, D. D. Mahlum and J. R. Decker

Technical Assistance:

E. G. Kuffel and J. D. Stearns

Experiments were performed to evaluate the effects on fetal development of exposure of pregnant mice to time-varying, inhomogeneous magnetic fields. No consistent or significant differences were observed between exposed fetuses and fetuses which were sham exposed in a similar environment.

A system was established at the Stanford Linear Accelerator Center, Palo Alto, CA, to expose mice to homogeneous and gradient magnetic fields. Subsequent studies have been performed using a gradient field (0.1-1.0 T; gradient ~ 2 T/m), the strength of which was cycled as (approximately) a trapezoidal waveform. Rise time was 2.8 sec (10%-90%), plateau time was 62 sec, fall time (90%-10%) was 15.6 sec, and OFF time was 29 sec. As in the earlier experiments, control groups were contemporaneously sham exposed in a similar magnet, degaussed to produce an ambient field of less than 10^{-4} T. A description of the housing system and environmental conditions for experimental and control groups was reported previously (Annual Report, 1978).

For the first experiment (I), pregnant Swiss-Webster mice of known gestational stages were obtained from Simonsen Laboratories, Inc. Fetuses at 5-7 days of gestation (dg; early), 7-15 dg (middle), and 12-14 dg (late) were exposed or sham exposed to the above field for 42 hr. After exposure, the animals were transported to PNL and sacrificed at 18 dg for fetal examinations.

Two additional experiments (IIa and IIb) were performed using Swiss-Webster mice which were purchased from Hilltop Laboratories, Inc. and bred at Laboratory Animal Medicine, Stanford, CA. Following a night of cohabitation (3 females/male), the females were inspected for sperm plugs; all females with plugs were considered pregnant and separated for the study. An exposure period of 8 days,

extending from 7-15 dg, was used in both experiments. All animals were sacrificed for fetal examinations at 18 dg.

Other mice, mated at the same time as those of Experiment IIa, were exposed from 0-7 dg. These mice (Experiment III) were sacrificed at 12 dg and the fetuses examined for gross external abnormalities.

There was little effect of magnetic field exposure on prenatal mortality in any of the groups in Experiment I (Table 1). cursory examination of the data may suggest that exposure from 7-9 dg increased, while exposure from 12-14 dg decreased prenatal mortality. However, the differences between exposed and sham-exposed were the result of variations in the sham-exposed groups rather than changes due to exposure.

There are large variations in the pregnancy rates between groups in Experiment I. The reason for this is unknown.

Longer exposure to the field (Experiment IIa and IIb) had no effect upon prenatal mortality. Again (IIb), an increase in the percentage of prenatal dead was seen in the exposed group; however, there was large variability (as indicated by the standard deviation), so the difference was not statistically significant.

There were no statistically significant differences in the incidence of visceral or skeletal anomalies between exposed and sham-exposed groups in any of the experiments,

TABLE 1. Effects of Exposure of Mice to a Ramped Gradient Magnetic Field on Prenatal Survival, Weight

Exposed at:	I ^(a)						IIa ^(b)		IIb ^(b)		III ^(c)	
	5-7 dg		7-9 dg		12-14 dg		7-15 dg		7-15 dg		0-7 dg	
	Exposed	Sham	Exposed	Sham	Exposed	Sham	Exposed	Sham	Exposed	Sham	Exposed	Sham
Number Females	22	22	21	20	19	22	16	15	37	37	16	16
Number Pregnant (%)	6 (27.3)	8 (36.4)	15 (71.4)	5 (25.0)	4 (21.1)	8 (36.4)	16 (100)	15 (100)	28 (75.7)	30 (81.1)	15 (93.8)	14 (87.5)
Number Implants	67	95	184	71	44	77	208	197	345	358	198	200
Number Live	62	88	171	69	40	55	179	170	300	328	180	173
Number Dead	5	7	13	2	4	22	29	27	45	30	18	27
Percent Dead ($\bar{x} \pm 5D$) ^(d)	7.0 $\pm$ 9.8	7.4 $\pm$ 9.6	7.1 $\pm$ 6.9	2.9 $\pm$ 3.9	10.4 $\pm$ 15.8	25.0 $\pm$ 46.3	14.0 $\pm$ 13.3	14.9 $\pm$ 18.2	12.9 $\pm$ 10.4	8.6 $\pm$ 8.5	9.8 $\pm$ 3.0	15.0 $\pm$ 4.2
Number Live/Litter ^(d)	10.3 $\pm$ 2.7	11.0 $\pm$ 1.7	11.4 $\pm$ 1.8	13.8 $\pm$ 1.5	10.0 $\pm$ 2.7	9.2 $\pm$ 3.6	11.2 $\pm$ 2.2	11.3 $\pm$ 3.3	10.7 $\pm$ 1.9	10.9 $\pm$ 2.2	12.0 $\pm$ 3.2	12.4 $\pm$ 3.7
Total Weight ^(d)	1.27 $\pm$ 0.11	1.21 $\pm$ 0.09	1.28 $\pm$ 0.10	1.25 $\pm$ 0.18	1.17 $\pm$ 0.15	1.30 $\pm$ 0.10	1.21 $\pm$ 0.05	1.22 $\pm$ 0.14	1.25 $\pm$ 0.09	1.28 $\pm$ 0.11	0.089 $\pm$ 0.003	0.086 $\pm$ 0.00
Number with External Visceral Anomalies	5	6	10	3	1	2	15	14	17	21	6 ^(e)	4 ^(e)
Fraction of Fetuses Affected	6/29	9/42	13/82	5/34	0/20	2/28	20/90	18/85	23/156	27/171	6/180	4/173
Number of Skeletal Anomalies	5	5	7	5	4	4	14	12	23	24	--	--
Fraction of Fetuses Affected	8/34	7/46	12/88	15/35	3/20	7/27	13/89	11/85	15/144	13/157	--	--

(a) 42 hr exposure, sacrificed at 18 dg
(b) 5 days exposure, sacrificed at 18 dg
(c) 7 days exposure, sacrificed at 12 dg
(d) Mean of individual litter means
(e) Gross external examination only

except for the 7-9 dg groups in Experiment I. In this case, the sham exposed had a significantly higher incidence of skeletal anomalies than the exposed. There were no differences in the incidences of skeletal anomalies between exposed and sham-exposed groups in subsequent Experiments IIa and IIb, even

though these included exposure through 7-9 dg. We have no explanation for the results of the 7- to 9-dg group of Experiment I.

No consistent or significant differences attributable to magnetic field exposure were seen in these experiments.

INFLUENCE OF MAGNETIC FIELDS ON DEVELOPMENT OF RAINBOW TROUT (*Salmo gairdneri*)

Investigators:

J. A. Strand, C. S. Abernethy, R. G. Genoway,
J. R. Skalski, and J. C. Montgomery

The effects of a magnetic field were assessed on development in rainbow trout eggs. Particular interest is in the immediate effects of inhibited fertilization, delayed or arrested development, and inhibition of hatching.

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

In the first approach (previously used), approximately 4,500 eggs were exposed from fertilization through 21 days in an oscillating field (0.28 to 1.12 T) within a 1-min period) of a Varian Model V-3603 electromagnet. An additional 4,500 eggs maintained outside the magnetic field served as controls.

Incubation chambers for exposed and control embryos were identical. Subsampling of 150 embryos from both exposed and control chambers was conducted on days 7, 14, and 21. At 21 days (eyed stage), the embryos were transferred to standard drip incubators where hatching occurred in 28 ± 1.5 days. The fry were transferred to standard hatchery troughs 2 wk after hatching to detect potential latent mortality and impaired growth. A chi-square statistic was applied to each index of effect to determine differences between exposed and paired control groups. Evaluation of each index was conducted "in the blind" to decrease the possibility of investigator bias.

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

control groups, either for percent hatchability or percent survival of hatched fry.

Results of subsampling are presented in Table 3. Combining all data for 7, 14 and 21 days indicated no significant difference in fertilization success between the exposed and control groups, a result that contradicts enumeration of nonsubsamped embryos. Subsampling on day 7 revealed a higher incidence of abnormal embryos in the control group; however, this observation was not confirmed by subsampling at 14 and 21 days.

A second approach was designed to examine the fertilization process itself and to determine the relative susceptibility of egg and sperm to magnetic-field effects. Accordingly, approximately 5,000 eggs and 50 ml of sperm were separately exposed in a homogeneous 1.12-T magnetic field for 1 hr. A similar number of eggs and aliquots of sperm were maintained outside the magnetic field. After exposure, eggs and sperm were placed in standard hatchery drip incubators in the following combinations: 1) egg unexposed, sperm unexposed; 2) egg exposed, sperm unexposed; 3) egg unexposed, sperm exposed; and 4) egg exposed, sperm exposed. Subsampling was again performed on days 7, 14 and 21. Hatching occurred in 28 ± 1.5 days and fry were transferred to standard hatchery troughs 2 wk after hatching.

Results (Table 4) suggested that there was a significant difference in fertility among the four treatment groups; however, the lower percentage was again encountered in the control group. While no significant difference in hatchability was found, these data suggested that survival through 10 wk was significantly greater in the control group. A homogeneity χ^2 test of all data ($p = 0.05$) supported these interpretations.

TABLE 2. Effect of Magnetic Exposure of Ova on Early Life Stages of Rainbow Trout

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

(a)Significant χ^2 at 0.05 level(b)Nonsignificant χ^2 at 0.05 level**TABLE 3.** Effect of Magnetic Exposure on Incidence of Abnormal Embryos

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

(a)Significant χ^2 at 0.05 level(b)Nonsignificant χ^2 at 0.05 level**TABLE 4.** Effect of Magnetic Exposure of Ova or Sperm on Early Life Stages of Rainbow Trout

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

*Gamete exposed to magnetic field

Results of statistical analyses to determine the relative influence of magnetic exposure on eggs or sperm are shown in Table 5. Although these data suggested that magnetic exposure of either egg or sperm significantly influenced fertility and survival, the finding of a significant interaction diminished interpretation of main effects; that is, an additional influence on fertility and survival occurred that was not attributable to magnetic exposure of eggs and sperm alone.

The findings reported in Table 6 suggested that a significant increase in the incidence of abnormal embryos occurred in subsampling

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

These data are in direct conflict with those reported last year. The explanation for the difference in results is unclear at this time.

TABLE 5. Relative Influence of Magnetic Exposed Gamete on Fertility, Hatchability and Survival

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

(a) Significant χ^2 at 0.05 level

(b) Nonsignificant χ^2 at 0.05 level

*Gamete exposed to magnetic field

TABLE 6. Effect of Magnetic Exposure on Incidence of Abnormal Embryos, Day 21

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

*Gamete exposed to magnetic field

TABLE 7. Relative Influence of Magnetic Exposed Gamete on Incidence of Abnormal Embryos, Day 21

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

(a) Significant χ^2 at 0.05 level

(b) Nonsignificant χ^2 at 0.05 level

*Gamete exposed to magnetic field

VISCOSITIES OF BIOPOLYMERS IN LARGE MAGNETIC FIELDS

Investigator: D. R. Kalkwarf

Viscosities of representative biopolymers in aqueous solution were found to be unaffected by magnetic fields of up to 1 T even when measured in a configuration designed to maximize interaction of the field with the flowing solutes.

Magnetic fields have been reported to slightly increase the viscosity of water (Lielmezs et al., *Z. Physik. Chem.* NF 99: 117-130), and it seemed possible that they might increase the viscosities of aqueous biopolymer solutions more significantly. Such an effect could cause severe health problems to persons exposed to large magnetic fields. Results reported last year (Annual Report, 1978) indicated that no changes in the viscosities of several biopolymer solutions could be detected even at fields of 1 T. Because of the viscometer and magnet designs, however, it was not possible to arrange for maximum interaction between the field and the flowing solutions. This arrangement was achieved this year with a newly designed viscometer; again, the viscosities of several biopolymer solutions were found to be unaffected by fields up to 1 T.

The arrangement of the flowing solution with respect to the field is important, since most biopolymers are expected to be diamagnetic, with maximum moments parallel to their long axes. As a consequence, the biopolymer chains tend to orient themselves with these axes perpendicular to an applied field. Maximum viscosity can thus be expected if the direction of flow is parallel to the field. Since the usual Ostwald viscometer cannot be positioned this way in a horizontal field, a modified version (Figure 5) was designed and fabricated.

Aqueous solutions containing either 1% bovine serum albumin in 8 M urea, 1% polyvinylpyrrolidone or 0.5% sodium polypectate were prepared, and their viscosities were measured in the new viscometer. The temperature of the solutions was maintained at 25.0°C by means of a water bath around the viscometer. Series of successive viscosity measurements were made alternately at 0 and 1 T, and the effect of the field was evaluated statistically by the method of paired comparisons. No significant effect could be

FIGURE 5. Modified Ostwald Viscometer to Measure Viscosities of Solutions Flowing Parallel to a Horizontal Magnetic Field

seen at the 95% confidence level, even though differences as small as 0.4% could have been detected.

Oil Shale

OIL SHALE

Potential biomedical concerns in the area of oil shale fuel technology are being investigated, in part, by a tiered testing approach utilizing the Ames assay and mammalian cell transformation, followed by more standard toxicologic procedures. Fractions of potential importance, as defined by the Ames assay, were assayed for pulmonary carcinogenesis using the lung pellet implantation technique.

• Late Effects of Oil Shale Pollution

The purpose of this project is to investigate the potential health hazards associated with human exposure to dusts and other effluents released during the processing of oil shale, and to crude and refined shale oil materials. Potential exists for occupational or environmental exposure to raw or spent oil shale particulates, crude or refined shale oil, and waste retort water, all of which contain polycyclic aromatic hydrocarbons, including known carcinogens. Present and past studies at PNL have examined the fibrogenic and carcinogenic potential of raw or spent oil shale from experimental retort technologies (Annual Report, 1977 and 1978). Current studies are examining the epidermal and respiratory carcinogenic potential of crude shale oil and its chemical fractions using mouse skin-painting assays and lung pellet implant studies in rats.

A large portion of the work done in this program is a comparative epidermal carcinogenesis study of several fossil fuel materials, using skin-painting in mice; this work is reported in another section of this report (see Renne et al., Epidermal Carcinogenesis Studies in Mice of Crude Materials from Several Fossil Fuel Technologies).

LUNG PELLET CARCINOGENESIS ASSAY OF SHALE OIL IN RATS

Investigators:

G. E. Dagle, F. G. Burton, K. E. McDonald,

J. F. McShane, L. G. Smith, and D. L. Stevens

Technical Assistance: J. Meheen

Beeswax pellets impregnated with crude shale oil or its fractions were implanted into the lungs of rats for carcinogenesis studies. Pellets containing the polynuclear aromatic fraction (PNA) were the most carcinogenic, followed by those containing the basic and neutral fraction pellets. There was no difference in effects of crude shale oil and Wilmington crude petroleum.

Lung pellet implantation has emerged as a useful and relatively inexpensive procedure for evaluating potential pulmonary carcinogens. The material to be evaluated is mixed with a beeswax-tricaprylin vehicle which, when implanted in the lungs of rats, forms a pellet, with potential carcinogens slowly leaching into the surrounding pulmonary parenchyma.

In the present study, crude shale oil (LOI) from the experimental retort at Lawrence Livermore Laboratory (LLL) or its neutral, basic, and polynuclear aromatic (PNA) fractions, were mixed with equal parts of melted beeswax and tricaprilyn. For comparison, samples of California (Wilmington) crude

petroleum were similarly prepared. Methylcholanthrene (MCA) was used as a positive control. Young adult, female Wistar rats were anesthetized with ether; 0.2 ml of pellet material, melted at 76°C, was implanted in the left lung, which was exposed by thoracotomy. Rats were scheduled for sacrifice at 6, 12, 18 or 24 mo postimplantation (Table 1).

Clinically, the rats tolerated all levels of the test materials well. During tissue processing, microscopic examination of the implant site revealed that the vehicle alone produced a granulomatous response surrounding the cavity where the pellet had dissolved during tissue processing. It appeared that

the pellet material interrupted the continuity of a bronchiole at some points, resulting in a tendency for growth and extension of bronchiolar epithelium along the inside lumen of the cavity. This epithelium then underwent squamous metaplasia. There was a trend toward increased squamous metaplasia in response to crude shale oil, compared with crude petroleum, and increased squamous metaplasia in response to the basic and PNA fractions of shale oil when compared with the neutral fraction.

Pulmonary carcinomas developed around the pellet site with a positive dose-response relationship (Table 2). These were epidermoid carcinomas composed of keratinizing, stratified, squamous epithelium that invaded blood vessels and extended into adjacent

areas of lung and pleura. Metastases to distant sites such as the kidney were frequently observed.

At the highest and lowest doses (60 and 0.6 mg), no significant differences in the incidences of carcinomas were observed among the groups receiving crude shale oil, shale oil fractions or crude petroleum (Table 2). However, at the intermediate dose (6 mg), the group that received the PNA fraction of shale oil had significantly more tumors than did any of the other groups. Exposure to methylcholanthrene, intended as a positive control, resulted in pulmonary carcinomas earlier, and at a higher incidence, than did exposure to the crude oils or shale oil fractions. Lung tumors did not develop in the vehicle-treated or in untreated control rats.

TABLE 1. Protocol for Chronic Lung Pellet Study in Rats

Treatment	Dose, mg	No. Rats Per Scheduled Sacrifice			
		6 mo	12 mo	18 mo	24 mo
Livermore Crude Shale Oil	60	5	5	5	15
	6	5	5	5	15
	0.6	5	5	5	15
Shale Oil Fractions					
Neutral Fraction	60	5	5	5	15
	6	5	5	5	15
	0.6	5	5	5	15
Basic Fraction	60	5	5	5	15
	6	5	5	5	15
	0.6	5	5	5	15
PNA Fraction	60	5	5	5	15
	6	5	5	5	15
	0.6	5	5	5	15
Wilmington Crude Petroleum	60	5	5	5	15
	6	5	5	5	15
	0.6	5	5	5	15
Methylcholanthrene	1	5	5	5	15
Vehicle		5	5	5	15
Control		5	5	5	15

TABLE 2. Pulmonary Carcinomas Produced in Chronic Lung Pellet Study in Rats

Treatment	Dose, mg (30 Rats Each Group)		
	60 mg	6 mg	0.6 mg
Shale Oil Fractions			
Neutral	6	1	0
Basic	8	2	2
PNA	10	9	1
Livermore Crude Shale Oil	8	3	1
Wilmington Crude Petroleum	9	1	1
Methylcholanthrene	--	--	24

Multitechnology

• Toxicology of Inhaled Acid Aerosols

This project is concerned with determining the long-term biological effects of exposure to inorganic acid aerosols, which are common to many industrial processes and to fossil fuel plant effluents. Rats were exposed to graded, subacutely toxic doses of HNO_3 , HCl and H_2SO_4 to determine dose-related tumorigenesis. The study is scheduled for completion in FY 1980.

LATE EFFECTS FOLLOWING EXPOSURE TO GRADED DOSES OF HNO_3 , HCl and H_2SO_4

Investigators:

J. E. Ballou, R. A. Gies and G. E. Dagle

Osteosarcomas of the skeleton have been tentatively identified in rats exposed to HNO_3 , HCl and H_2SO_4 aerosols, and in control rats exposed to water vapor. The preliminary diagnosis is based on examination of radiographs obtained at necropsy. The tumor incidence apparently represents the spontaneous rate for male Wistar rats under our experimental conditions.

Rats were exposed to aerosols of mineral acids as a follow-up investigation of an earlier finding of bone tumors in rats exposed to 0.27N nitric acid aerosols. The experimental protocol and preliminary results have been reported in earlier Annual Reports (1976-1978). The objective of the study is to establish the causal relationship between acid exposure and bone tumor induction. Graded doses of HNO_3 , HCl and H_2SO_4 were administered to male Wistar rats exposed 6 hr per day on alternate weekdays for a total of six exposures. The experimental protocol and progress to date are summarized in Table 1.

Although the histopathologic examination of tissues has not been completed, there is good evidence of bone tumor induction, obtained from radiographs taken at necropsy.

Table 1 lists what is probably a minimum number of bone tumors, since questionable lesions have been excluded.

These preliminary results suggest that there is no dose-related induction of tumors following acid inhalation. The finding of at least one bone tumor in a treated control rat indicates that the number of bone tumors we observed are probably the spontaneous incidence of this lesion in our male Wistar rats. Bone tumors are also being found in male control rats in other studies using this strain and species.

The rats in this study are all dead; at completion of histopathologic examination, the results will be reported to define both the skeletal and soft tissue effects of mineral acid inhalation.

TABLE 1. Summary of Results on Rats Exposed to Acid Aerosols

Agent	Number of Rats	Amount Deposited, mg ^(b)	Median Survival Time, days	Number of Rats with Bone Tumors ^(c)
HNO ₃	62 ^(a)	0.1	785	0
	50	6.8	740	0
	50	2.3	760	2
	50	2.0	750	0
HCl	50	2.5	650 ^(d)	2
	50	1.3	715	1
	50	0.7	745	2
H ₂ SO ₄	50	26	710	0
	50	3.2	755	0
	50	0.7	730	2
<u>Controls</u>				
Treated	90 ^(e)		730	1
Nontreated	45		700	0

(a) Rats in this group were exposed for 30 min to 0.27 N nitric acid aerosols generated with a Lovelace nebulizer. Other rats were exposed repeatedly, as explained in the text, using a Retec nebulizer.

(b) Deposition was estimated from the aerosol particle size and assumed values for breathing parameters.

(c) Tumor diagnoses were based on radiography; histopathology is not completed.

(d) Nine rats drowned due to automatic watering device failure

(e) Treated controls were exposed to H₂O vapor using the repeated exposure regimen.

● Mobilization of Deposited Metals

This project seeks to develop methods of treatment for exposure to organic, metallic and organo-metallic compounds which may be used in research, process technology or products of the energy industry. Biological model systems, capable of response to these toxicants, are used to evaluate treatment regimes that may ameliorate effects of both chronic and acute exposures.

PERCUTANEOUS PENETRATION OF ^{14}C BENZO(A)PYRENE IN THE MOUSE

Investigators:

R. A. Gelman, C. Skinner and V. H. Smith

About 70% of a dermally applied dose of ^{14}C -benzo(a)pyrene was absorbed in 48 hr. Tissue concentrations peaked between 3 and 6 hr after application. Absorption was not linearly related to dose as the dose increased from $12.5 \mu\text{g}/\text{cm}^2$ to $125 \mu\text{g}/\text{cm}^2$. Excretion was principally via the feces.

Pulmonary, gastrointestinal, and dermal routes of absorption contribute to the body burden following exposure to airborne toxicants. Of the polynuclear aromatic hydrocarbons, benzo(a)pyrene (BaP) is notably potent in the induction of tumors.

Benzo(a)pyrene is produced by the pyrolysis of anthracene during combustion of fossil fuels. In the exposed animal, BaP is detoxified by hydroxylation and subsequent glucuronide or sulfate conjugation then excreted, principally by the hepatobiliary system.

Dermal penetration and distribution of BaP has not been well characterized. This study was designed to investigate the extent of penetration and tissue distribution following dermal application of BaP in mice. The effect of dose and time after application were also evaluated.

Swiss-Webster adult male mice, 40 g, were randomized prior to treatment. The nuchal area of each animal was shaved with electrical clippers 24 hr prior to application of BaP.

Isotopically labeled ^{14}C -7,10-benzo(a)pyrene was mixed with cold carrier BaP, then dissolved in acetone to give a final concentration of $14.8 \text{ mg}/\text{ml}$ in BaP (specific activity - $0.613 \mu\text{Ci}/\mu\text{mole}$ BaP). Fifteen μl of this solution

was applied to a 1.8-cm^2 shaved area of the animal's back to give a dose of $125 \mu\text{g}/\text{cm}^2$; for dose-response studies, 1/10 and 1/100 of this dose were given. Following BaP administration animals were placed in glass metabolism cages, and urine, feces, and expired air were collected. Tissue samples, excised application site, excreta, and trapped CO_2 were analyzed at 1, 2, 6, 24, 48, and 168 hr after administration. Samples were homogenized and extracted with chloroform-methanol (1:1;v/v), and the extract radioassayed by scintillation counting.

Recovery of radioactivity was greater than 75% of the dose administered. For purposes of normalization, data have been calculated based on the recovered ^{14}C label in each rat.

Disappearance of radioactivity from the application site was rapid; 6% in 1 hr and 40% in 24 hr, with 7% retained at the site for 1 wk (Table 2). Most of the BaP and its metabolites were excreted via the bile. Twenty percent of the radioactivity was found in the feces at 24 hr, 50% at 48 hr, and 80% at 1 wk. Excretion of radioactivity by the kidneys was approximately 10% that of the gut. Fecal and urinary excretion were log linear for 7 days, with a half-life of about 30 hr.

TABLE 2. Effect of Time on Distribution and Excretion of Radioactivity Following Application of ^{14}C -7,10 Benzo(a)pyrene to the Dorsal Skin of Mice *

Time After Application:	1 Hr	3 Hr	6 Hr ^(b)	24 Hr ^(b)	48 Hr	1 Wk
Tissue	Percentage of Total Radioactivity Recovered ^(a)					
Blood	0.03 $\pm$ 0.02	0.03 $\pm$ 0.02	0.07 $\pm$ 0.02	0.01 $\pm$ 0.01	0.005 $\pm$ 0.003	-- ^(c)
Epididymal Fat Pad	0.03 $\pm$ 0.02	0.07 $\pm$ 0.04	0.09 $\pm$ 0.07	0.10 $\pm$ 0.13	0.08 $\pm$ 0.08	0.05 $\pm$ 0.05
Kidneys	0.17 $\pm$ 0.29	0.13 $\pm$ 0.09	0.18 $\pm$ 0.10	0.26 $\pm$ 0.10	0.20 $\pm$ 0.10	0.01 $\pm$ 0.01
Liver	0.68 $\pm$ 0.64	0.75 $\pm$ 0.53	0.45 $\pm$ 0.18	0.30 $\pm$ 0.10	0.19 $\pm$ 0.13	0.04 $\pm$ 0.02
Carcass ^(f)	1.79 $\pm$ 0.64	3.96 $\pm$ 1.48	2.00 $\pm$ 0.63	2.73 $\pm$ 1.19	-- ^(e)	0.30 $\pm$ 0.08
Gut and Contents	3.65 $\pm$ 2.81	17.1 $\pm$ 5.1	20.9 $\pm$ 11.9	12.6 $\pm$ 3.2	-- ^(e)	0.14 $\pm$ 0.05
Cumulative Urine	-- ^(d)	-- ^(d)	1.12 $\pm$ 0.57	2.92 $\pm$ 0.97	5.98 $\pm$ 0.96	10.8 $\pm$ 1.7
Cumulative Feces	-- ^(d)	-- ^(d)	-- ^(d)	22.1 $\pm$ 5.7	54.8 $\pm$ 15.3	81.6 $\pm$ 6.2
Dose Site	93.6 $\pm$ 4.0	77.8 $\pm$ 5.0	75.2 $\pm$ 12.6	58.9 $\pm$ 7.1	28.5 $\pm$ 10.5	7.07 $\pm$ 6.15
% Recovery of ^{14}C ^(g)	76.7 $\pm$ 9.4	90.8 $\pm$ 5.7	89.8 $\pm$ 8.0	74.1 $\pm$ 0.3	75.2 $\pm$ 6.3	63.3 $\pm$ 6.6

* Dose: $^{125}\mu\text{g}/\text{cm}^2$ [$^{125}\mu\text{g}$ ^{14}C -7,10 benzo(a)pyrene in 15 μl acetone] was applied to a 1.8-cm² area. Data are means $\pm$

(a) standard deviation (n = 6)

(b) Data normalized to 100% of recovered ^{14}C

(c) n = 5 in 6 hr group and 4 in 24 hr group

(d) Not analyzed

(e) No sample available to analyze

(f) Gut and contents and carcass analyzed together (10.1 $\pm$ 7.4)

(g) Carcass values included remaining tissues

% applied ^{14}C recovered

Tissue concentrations of BaP and its metabolites were greatest within 6 hr following application (Table 2) and decreased rapidly. At 24 hr, only the kidneys and epididymal fat pad remained at the 6-hr levels.

The effect of the concentration of BaP on the percentage absorbed in 24 hr is shown in Table 3. Absorption, as determined by the amount of radioactivity recovered in the tissues and excreta, was greater at the highest dose (125 $\mu\text{g}/\text{cm}^2$). However, mass absorbed did not increase as a linear function of dose (Table 3).

Most of dermally absorbed BaP is rapidly metabolized and excreted. However, a small amount of BaP, or its metabolites may remain in the tissues, bound to DNA or other macromolecules, and eventually lead to tumor formation. Further studies will explore the fate and binding of various polyaromatic hydrocarbon following pulmonary administration.

TABLE 3. Effect of Various Dermal Dose Concentrations of ^{14}C -7,10 Benzo(a)pyrene on Tissue Distribution and Excretion of Radioactivity 24 hr Following Application to Dorsal Skin of Mice

Dose:	1.25 $\mu\text{g}/\text{cm}^2$	12.5 $\mu\text{g}/\text{cm}^2$	125 $\mu\text{g}/\text{cm}^2$
Tissue	Percentage of Total Radioactivity Recovered ^(a)		
Liver	0.88 $\pm$ 0.18	0.79 $\pm$ 0.16	0.30 $\pm$ 0.10
Lung	0.06 $\pm$ 0.05	0.04 $\pm$ 0.01	0.02 $\pm$ 0.001
Kidneys	0.79 $\pm$ 0.54	0.82 $\pm$ 0.15	0.26 $\pm$ 0.10
Epididymal Fat Pad	0.09 $\pm$ 0.05	0.15 $\pm$ 0.08	0.10 $\pm$ 0.13
Blood	0.21 $\pm$ 0.32	0.08 $\pm$ 0.05	0.01 $\pm$ 0.01
Carcass ^(b)	3.79 $\pm$ 2.61	4.40 $\pm$ 1.10	2.72 $\pm$ 1.19
Gut and Contents	51.9 $\pm$ 8.7	29.4 $\pm$ 9.1	12.6 $\pm$ 3.2
Urine	16.2 $\pm$ 4.5	10.8 $\pm$ 4.1	2.92 $\pm$ 0.97
Feces	8.28 $\pm$ 7.32	36.05 $\pm$ 8.23	22.1 $\pm$ 5.7
Dose Site	17.8 $\pm$ 4.7	17.4 $\pm$ 6.7	59.0 $\pm$ 7.1
% Recovery of ^{14}C ^(c)	70.4 $\pm$ 7.3	82.7 $\pm$ 1.3	74.1 $\pm$ 0.03

(a) Data normalized to 100% recovered ^{14}C , data are mean $\pm$

standard deviation (n = 6)

(b) Carcass values include remaining tissues

(c) % applied ^{14}C recovered

CYTOCHEMICAL COMPARISON OF METAL SULFATES AND CHLORIDES IN VERO CULTURES

Investigators:

M. E. Frazier, T. K. Andrews, Jr., and B. B. Thompson

Technical Assistance: J. E. Samuels

A clonal growth assay was used to determine the toxicity of metallic salts in vitro. Concentrations of metallic sulfates causing decreased plating efficiency of VERO cells are similar to those previously reported for metallic chlorides. Data from these assays provides the following ranking of metal toxicity: $V > Cr > Ru > Cd > Hg > Ni > Pt > Co > Pd > Zn > Cu > Mn > Pb > Fe \gg Mg > Ca$.

The toxicity of metallic salts for mammalian cells has been evaluated using VERO (monkey) cells as indicators. The assay provides a functional measure of cell viability during exposure of the cell to a specific concentration of metallic salt. Each cell is required to undergo several cell divisions in the presence of the test material and form a colony (10+ cells) before it is scored as viable. The test system also provides minimal cell-to-cell interaction (only 50 cells in a volume of 0.2 ml media) before colony formation. These isolated cells are more stringent in their nutritional requirements, and thus

more sensitive to stress, than high-density cultures of the same cells. Previous use of this assay (Annual Report, 1977, 1978) to evaluate toxicity of metallic compounds and chelating agents has shown that the results correlate well (0.98) with animal and human toxicity data.

Concentrations of Cu^{++} (tested as a sulfate salt) which caused a reduction in the plating efficiency of VERO cells are similar to those previously reported (Annual Report, 1978) for Cu^{++} when tested as a chloride salt (Figure 1). Similarly, concentrations of several other metallic ions

FIGURE 1. Toxicity of Copper Salts

(tested as sulfates) which caused a 50% decrease in plating efficiency were similar to those previously reported for the same metallic ions when tested as chloride salts (Table 4). While this observation is predictable, based on fundamental principals

TABLE 4. Comparative Cytotoxicity of Metallic Chlorides and Metallic Sulfates Measured with VERO Cells in the Clonal Growth Assay

Metal Ion	RPE ₅₀ ^(a) Dose In µg Metal/ml ^(b)	
	Chloride Salt	Sulfate Salt
Cd ⁺⁺	0.3	0.4
Zn ⁺⁺	6.9	5
Cu ⁺⁺	8.6	9
Mn ⁺⁺	14	10

- (a) RPE₅₀ = the concentration at which the plating efficiency of the exposed cell cultures is 50% that of control cultures.
(b) Metal concentrations were determined using X-ray fluorescence.

of solution chemistry, there has been disagreement about this point in the literature and a concern about which is the most important (environmentally and biologically) chemical form of the metals. Although solubility and thus, biological availability, obviously determine toxicity and other biological properties of a metal in a pollutant stream, information on long-term environmental fate of metals is not, in most cases, available at this time. If one assumes that certain metals in a given pollutant stream, over the course of time, will become soluble, then total specific metal concentration in the pollutant material may provide some estimate of potential health hazard.

In order to evaluate the relative toxicity of selected metals which might be important environmentally, dose-response curves similar to those shown in Figure 2 for palladium and platinum were generated for each metal chloride. The concentration which resulted in a relative plating efficiency that was 50% of control cultures (RPE₅₀) was then estimated from the dose-response curve. The data from this year's studies are combined with previously reported values (Table 5)

FIGURE 2. Toxicity of Platinum and Palladium

TABLE 5. In Vitro Cytotoxicity of Metals as Measured with VERO Cells in the Clonal Growth Assay

Test Agent ^(a)	RPE ₅₀ ^(b)	
	Dose µg Metal/ml ^(c)	
V	0.03	
Cr	0.06	
Ru	0.1	
Cd	0.3	
Hg	0.5	
Ni	1.1	
Pt	3.3	
Co	3.5	
Pd	4.8	
Zn	6.8	
Cu	8.6	
Mn	15	
Pb	37	
Fe	59	
Mg	>2000	
Ca	>3000	

- (a) All metals tested as chloride salts except Cr, which was tested as chromate
(b) RPE₅₀ = the concentration at which the plating efficiency to the exposed cultures is 50% that of control cultures.
(c) Metal concentrations were determined using X-ray fluorescence.

to provide a listing of the relative toxicity of these metals. Previous studies (Annual Report, 1978) have shown this system to be a sensitive and qualitatively reliable method of estimating the relative toxicity of these compounds for man and animals.

● Metal-Membrane Interactions

The ionic forms of most toxic metals penetrate cell membranes slowly, and it seems likely that their primary effects are exerted at the membrane level. Information on the interaction of metals with defined membrane functions can therefore be expected to aid in predicting potential effects of trace metals derived from fossil-fuel combustion. In its present stage, the research is attempting to define the role of membranes in regulating cellular synthetic activity and cell division and to study membrane alterations which accompany cell transformation to the neoplastic state.

AN EXTERNAL ATPASE OF A TUMORIGENIC MAMMALIAN CELL LINE

Investigators:

R. P. Schneider, T. K. Andrews and M. E. Frazier

Technical Assistance: L. M. Butcher

In order to test the correlation between the presence of external ATPase and tumorigenesis, nine mammalian cell lines were screened for both activities. In seven of these, the correlation was positive; in two, it was negative. The enzyme of VERO (tumorigenic) cells, when characterized biochemically, was found to be similar to that of avian leukemia cells with regard to orientation, substrate specificity, metal activation and inhibitor sensitivity.

Our previous work characterized an adenosine triphosphatase (ATPase) found on an avian leukemia virus (AMV). We have found that leukemia cells infected by and producing AMV contain an ATPase identical to that of the virus. The cellular enzyme has the unusual property of hydrolyzing the substrate (ATP) outside (rather than inside) the cells. Studies in another laboratory have shown that cultured tumorigenic liver cells possess a similar (external) activity. In order to examine further the correlation between tumorigenicity and exterior ATPase, we have screened a series of cultured cell lines for exterior ATPase activity.

Results of the screening tests are shown in Table 6. Of the 15 mammalian cell lines assayed for external ATPase, nine were tested for tumorigenicity in nude mice. Seven lines showed a positive correlation of ATPase activity and tumorigenicity; two had a negative correlation. The two that exhibited a negative correlation had low ATPase activity but did not cause tumors. Only two cell lines had high levels of ATPase; both were highly tumorigenic. Although the

presence of ATPase and tumorigenicity may be linked in some cell (or tumor) types, the correlation is not general. Similar ATPase activity has been found in some normal (non-cancerous) mammalian cells, e.g., polymorphonuclear leukocytes (PMN) and glial cells. The function of the external ATPase is unknown, and too few cell types have been examined to reveal a pattern of its occurrence. However, the presence of the enzyme may provide a means of identifying certain kinds of cancer cells, and is a potential tool for measuring transformation in some in vitro assays of carcinogenic activity.

We selected the external ATPase of VERO cells for characterization because it is a stable cell line, with high activity and a high degree of tumorigenesis. The specific activity of the ATPase is $84 \text{ nmoles/mg}^{-1}/\text{min}^{-1}$, about 20 times less than that of chicken leukemia cells, and six times more than that of normal polymorphonuclear leukocytes. The specific activity is constant as the cells grow from 20% of confluence on the flasks to a piled-up condition, i.e., it is independent of cell density and growth rate. Like the

TABLE 6. Occurrence of External ATPase and Tumorigenicity in Some Mammalian Cell Lines

Cell Line	Origin	External ATPase	Tumorigenicity, Nude Mice
P ₂ Kc	Pig Leukemia	+	+
P K ₁₅	Pig Kidney, Normal	-	NT (a)
P _C 2685	Pig Cornea, Normal	+	+
D Kid	Dog Kidney, Normal	+	NT
D 772 LT	Tumor, Dog Lung	++	-
NCTC MDCK	Dog Kidney, Normal	++	NT
D759 LT/	Tumor, Dog Lung	-	-
D1064 LT	Tumor, Dog Lung ^(b)	++++	+
HEC	Hamster Embryo, Normal	++	NT
HEC I	Hamster Embryo ^(c)	-	-
HEC II	Hamster Embryo ^(c)	-	-
HEC V	Hamster Embryo ^(c)	+	-
D1342 LT	Dog Lung Tumor	+	NT
D1006 HBT	Dog Bone Tumor	++	NT
VERO	Monkey Kidney, Normal	+++++	+

(a) Not Tested

(b) Thought to be metastasized bone tumor

(c) Chemically transformed in vitro

enzyme of chicken leukemia cells, the ATPase is activated by Ca²⁺ or Mg²⁺, is insensitive to most commonly used ATPase inhibitors, and has higher activity in intact than in broken cells.

All intracellular mammalian ATPases are inhibited by low concentrations of organic mercurials that react with sulfhydryl groups. The chicken leukemia cell ATPase is inhibited by low concentrations (<1 x 10⁻⁵M) of p-chloromercuribenzenesulfonate (PCMBs), but the inhibition is reversed by higher concentrations (1 x 10⁻⁴M). The activity of the VERO cell enzyme is stimulated by PCMBs (Figure 3) to a maximum of about 40% at a range of 0.4 x 10⁻⁴ to 2 x 10⁻⁴M. Although there was no detectable inhibition at lower concentrations, the stimulation at higher concentrations resembled the reversal of inhibition at higher concentrations with the avian cell ATPase.

The substrate specificity of the VERO ATPase is identical to that of the avian enzyme, i.e., all of the pyrimidine and

purine nucleotide triphosphates tested were hydrolyzed at the same rate (Table 7). Thus, both enzymes are more appropriately designated nucleotide triphosphatases (NTPase) than ATPase. The rate of hydrolysis of ADP is about one-third, and phosphate esters about one-eighth, that of ATP. Since hydrolysis of phosphate ester bonds by an ATPase is unusual, it is possible that an external phosphatase exists on the surface of VERO cells in addition to the NTPase.

This study has shown a rough correlation between tumorigenicity and the presence of an external NTPase in eight of ten cell lines tested. The enzyme of a tumorigenic mammalian cell line is similar to that found in avian cells with respect to orientation in the cell membrane, substrate specificity, metal requirements and sensitivity to sulfhydryl-active reagents. The enzyme may be useful for identifying certain kinds of cancer cells but it will be necessary to characterize it in a wider spectrum of cell types.

FIGURE 3. The Effect of p-Chloromercuribenzenesulfonic Acid Concentration on External ATPase of VERO Cells

TABLE 7. Substrate Specificity of the External NTPase of VERO Cells

Substrate (all 5 mM)	Relative Activity (ATP = 1)
Adenosine Triphosphate	1.0
Adenosine Diphosphate	0.39
Inosine Triphosphate	1.16
Uridine Triphosphate	1.13
Guanosine Triphosphate	1.00
Cytidine Triphosphate	1.20
Glucose-6-Phosphate	0.13
β -Glycerol Phosphate	0.13
Adenosine Monophosphate	0.13
p-Nitrophenyl Phosphate	0.21

PARTIAL PURIFICATION OF EXOCELLULAR ACID PROTEASES
INDUCED FROM NEUROSPORA CRASSA

Investigators:

W. G. Rhodes, L. D. Eirich, R. A. Lindberg and H. Drucker

A pepstatin-inhibitable acid protease and a metalloprotease of acid pH optima have been partially resolved from exocellular filtrate of N. crassa cultures induced for protease biosynthesis under conditions of nitrogen starvation, with protein as sole source of nitrogen.

As described earlier in this Annual Report, N. crassa produces exocellular acid proteases when grown under conditions in which protein is sole source of nitrogen, carbon, or sulfur. In this article we will discuss the partial purification of two of the acid proteases produced, and some of the properties of these enzymes.

The assay employed for detection of acid protease activity was a variant of the method used for detecting neutral and alkaline protease activities. It is based upon quantitative fluorimetric determination of amine groups liberated during proteolytic degradation of bovine serum albumin (BSA) at acid pH.

Exocellular filtrate from nitrogen-starved cells given gelatin as a source of nitrogen was concentrated 40-fold by ultrafiltration, and dialyzed against 0.01M sodium citrate buffer, pH 3.4. The dialysate was applied to a column of carboxymethylcellulose (CMC) equilibrated with the same buffer, and the column was eluted with a linear KCl gradient of 0.05 to 0.15M in citrate buffer after washing with 0.05M KCl in citrate buffer. Protein concentration was determined by optical density at 280 nm, and proteolytic activity was assayed at pH 5.2 (Figure 4). Two peaks of proteolytic activity were observed. The first peak of activity eluted from the column was found, by electrophoretic assay, to be pepstatin-inhibitable but not

FIGURE 4. Elution Profile of CMC Chromatography. Fractions 150-180 were pooled and contained the AcP activity. Fractions 195-230 were pooled as M2 protease.

inhibited by metal chelators. This peak corresponds to true acid protease (AcP) activity. The second peak of activity, assayed by electrophoresis, was not inhibited by pepstatin but was inhibited by metal chelators. Electrophoresis of this material suggested that it was M2 protease.

Pooled protease activity peaks corresponding to AcP and M2 from the CMC chromatography were chromatographed on Sephadex G-75 columns equilibrated with 0.01 M sodium citrate buffer, pH 3.4 (Figures 5A and 5B). Sephadex G-75 chromatography (Figure 5A) of the M2 peak from CMC chromatography demonstrated two peaks of protease activity, corresponding in electrophoretic assays to M2 and AcP. The molecular weight of M2, determined by this chromatography was approximately 18,000. The AcP peak from CMC chromatography when chromatographed on Sephadex G-75 showed one peak of proteolytic activity and two peaks of protein concentration (Figure 5B). The molecular weight of the AcP, based upon this chromatography, was approximately 66,000.

Material from the Sephadex G-75 chromatography corresponding to AcP and M2 were examined for activity as a function of pH (Figures 6A and B). The pH optima for AcP was approximately 3.5 (Figure 6A), similar

to that observed for other fungal, pepstatin-inhibitable acid proteases. The pH optima for M2 (Figure 6B) was found to be approximately 5, considerably higher than that for AcP and other fungal acid proteases.

The AcP and M2 activities from Sephadex G-75 chromatography were analyzed for sensitivity in inhibitors of protease activity. Proteolytic activity of AcP (Table 8) was inhibited almost totally by pepstatin and partially by parachloromercuribenzoate (PCMB), but not by metal chelators, phenylmethylsulfonylfluoride (PMSF) or mercaptoethanol. Proteolytic activity of M2 was inhibited only by metal chelators. These results suggest that AcP is a pepsin-like microbial protease, whereas M2 is a metalloprotease of low pH optima.

The AcP activity reported here is similar to that of other microbial acid proteases in sensitivity to inhibitors, and in pH optima. Its molecular weight is similar to that of *Aspergillus oryzae* acid protease (approximately 62,000), but greater than that of many other microbial acid proteases (approximate molecular weights, 30,000 to 35,000). To the best of our knowledge, no exocellular metalloproteases of acid pH optima from microbial origins have been reported in the literature.

FIGURE 5. Elution Profiles from G75 Filtrations. A. Gel Filtration of M2 pool from CMC chromatography. Fractions 81-89 were pooled as M2. B. Gel filtration of AcP pool from CMC chromatography. Fractions 50-60 were pooled as AcP.

TABLE 8. Effect of Inhibitors on Activity of *N. crassa* Low pH Optimum Proteases

Inhibitor	Inhibitor Concentration, mM	Remaining Activity, %	
		AcP	M-2
PCMB	1	65	93
2-Mer.	5	92	99
PMSF	2	85	91
OP	55	93	0
EDTA	30	104	0
Pepstatin	0.1	4	94

FIGURE 6. Effect of pH on Activity of *N. crassa* Proteases. Samples from G75 pools were assayed at various pH values with BSA as the substrate. A. AcP activity; B. M2 activity.

REGULATION IN NEUROSPORA CRASSA OF AN
INDUCED EXOCELLULAR ACID PROTEASE

Investigators:

R. A. Lindberg, W. G. Rhodes, L. D. Eirich and H. Drucker

We examined regulation of exocellular acid proteases induced from N. crassa grown on protein as sole source of C, N or S. Type of acid protease synthesized appeared to be a function of conditions of induction, with metalloproteases of acid pH optima produced under conditions of N- or C-starvation; and a true acid protease, inhibitable by pepstatin, produced under conditions of S-starvation. The data suggest that unique sequences of N. crassa's genome may be expressed as a function of induction, and that there may be different pathways for expression of exocellular protease genes, varying with exocellular milieu.

Previous work from our laboratory has shown that Neurospora crassa, grown under conditions where a protein is sole source of carbon (C), nitrogen (N) or sulfur (S), produces exocellular protease. The presence of protein substrate (inducer) and the absence of readily available sources of N, C or S are absolute requirements for this synthesis of exocellular enzyme. We found, using specific inhibitors, immunological detection, and electrophoretic methods, that under all three conditions of starvation (-N, -C, -S) two identical enzymes were produced. These are: 1) an alkaline protease, inhibited by phenylmethyl-sulfonyl fluoride (PMSF); and 2) a neutral protease, inhibited by metal chelators.

Analysis via pH optima and stability had suggested that N. crassa might also produce, under conditions of protease induction, acid proteases (pH optima, 3-5). This report verifies the induction of exocellular acid proteases from N. crassa and demonstrates differences in types of acid protease produced as a function of induction conditions.

An electrophoretic method, similar to that employed for visualizing exocellular neutral and alkaline protease, was used to detect acid protease. Electrophoresed cellulose acetate strips containing the proteases were laid on plates containing milk protein and incubated for 1 hr at 45°C. Zones of proteolytic activity were detected by staining the plates with buffalo black. Areas where milk protein was digested by protease appeared as clear zones in a blue, stained-protein background.

Cells of N. crassa were placed in media in which protein was sole source of C, N or S, and samples of exocellular filtrate were removed, as a function of induction times, for electrophoretic analyses as in the conditions described above (Figure 7A-C). Under all three conditions, alkaline and neutral protease were detected with kinetics of accumulation similar to those previously observed.

In the protease induction carried out under conditions where limiting nutritive was N (Figure 7A), two new bands of enzyme activity were observed: one near the origin, or with a slight negative charge; and the other possessing cationic electrophoretic mobility under the conditions of electrophoresis. Both these activities were inhibited by o-phenanthroline and EDTA, and thus appear to be metalloenzymes. We refer to these activities (Figure 7A) as M1 and M2. In terms of kinetics of induction and detection of these enzyme activities, M1 appears after approximately 1 hr of induction; M2, 30 min later. The M2 appears to increase in concentration throughout an 8-hr course of induction, whereas M1 reaches optimum levels at approximately 3-3.5 hr and decreases thereafter.

In the case in which protein is sole source of carbon, one band of enzyme activity, distinct from alkaline and neutral protease, is observed (Figure 7B). This activity has similar electrophoretic mobility to the M1 enzyme described above, and may also be inhibited by metal chelators. No M2 activity was observed. Kinetics of appearance for M1

FIGURE 7. Electrophoresis of Culture Filtrates Obtained During 8-hr Inductions of *N. crassa*. Symbols are defined as follows: AP, alkaline protease; NP, neutral protease; M1, metalloprotease 1; M2, metalloprotease 2; AcP, acid protease. Zones of increasing milk proteolysis are indicated by zones of increasing darkness. Dashed lines indicate zones in which clearing was barely visible. (A) Electrophoresis of filtrates obtained from nitrogen-derepressed cultures, (B) carbon-derepressed cultures, (C) sulfur-derepressed cultures.

enzyme were similar to those observed under conditions of nitrogen starvation. The enzyme is first detected after 2 hr of incubation, reaches an optimum in apparent concentration at 3-3.5 hr of induction, and decreases slowly through the remainder of the induction period.

It should be noted that in both the cases of N and C induction, the enzymes produced are not inhibited by the classic inhibitor of acid proteases, pepstatin, but rather by metal chelators. They therefore appear to be metalloproteases of low pH optima.

Under conditions of induction in which S is the limiting nutriment, two bands of protease activity, in addition to neutral and alkaline protease activity, were observed (Figure 7C). The band of activity near the origin, which was inhibited by metal chelators, appears to be similar to the M2 protease activity observed under conditions where nitrogen is limiting nutriment. The second band of protease activity, with electrophoretic mobility similar to M1 activity, was found to be inhibited by pepstatin but not by metal chelators. This activity therefore appears to be a true pepsin-like acid protease, distinct from the metalloenzyme activities produced under conditions where protein is sole source of N or C. Both this enzyme, which we refer to

as AcP, and M2 appear to have similar kinetics of detection. Both enzymes are first observed after 3 hr of induction and appear to accumulate throughout the induction period.

Direct enzyme measurement of exocellular filtrate as a function of time of induction was performed for the pepstatin-inhibitable acid protease produced under conditions where protein is sole source of S. Metal chelators and PMSF were added to the cell-free filtrate to inhibit neutral and alkaline protease and low-pH-optima metalloproteases (M1 and M2). Kinetics of accumulation (Figure 8) for the pepstatin-inhibitable enzyme were similar to those observed by electrophoresis. Acid protease first appeared at 2.5 hr and accumulated throughout the course of induction. When pepstatin was added in addition to metal chelator and PMSF, no protease activity was observed. Similar experiments were performed with exocellular filtrate from cells induced with protein as sole source of N or C. No pepstatin-inhibitable enzyme was produced under conditions of C-starvation, and only very low levels under conditions of N-starvation. This apparent difference, for the N case, between direct enzyme measurement and the results of electrophoresis, may be due to differences between the two methods in detection limits for the pepstatin-inhibitable acid protease.

FIGURE 8. Kinetics of Accumulation of Acid Protease in Sulfur-Derepressed Cultures. Assays were performed at pH 3.4.

The results of these experiments suggest that exocellular acid proteases of N. crassa, unlike the neutral and alkaline protease activities, are a function of conditions of induction. These data suggest that unique sections of Neurospora's genome may be

expressed as a function of conditions of induction, and that there may be different pathways for expression of exocellular protease genes, varying with the exocellular milieu.

FURTHER CHARACTERIZATION OF NEUROSPORA CRASSA EXOCELLULAR ALKALINE PROTEASE

Investigators:

L. D. Eirich, R. A. Lindberg and H. Drucker

Temperature optima, thermal stability, and pH optima were determined for the purified exocellular alkaline protease of N. crassa. Molecular weight was determined by hydrodynamic means and compared with molecular weight ascertained by other methods.

Previous reports have presented data describing the purification and some of the properties of the exocellular alkaline protease (AP) synthesized by N. crassa when grown on medium depleted of carbon, nitrogen, or sulfur, with a protein substrate as sole source of these nutrilites. This report describes results of further study of the properties of this enzyme.

The temperature optimum of AP was found to be 45° (Figure 9), and 50% loss of activity was found when the enzyme was incubated at 50° for 15 min (Figure 10). Thermal inactivation appears to be biphasic in nature. These results suggest that loss of activity up to 45° may be due to accelerated auto-digestion of the AP molecule, whereas activity loss at temperatures higher than 45° may be due to thermal denaturation.

FIGURE 9. Temperature Optimum for Alkaline Protease Activity. Pure alkaline protease was assayed; the incubation was carried out at several temperatures.

The effect of pH on AP activity (Figure 11) indicates a broad pH optimum in the pH range 6-10, whereas most microbial alkaline proteases have distinct pH optima in the range 9-10.5. The alkaline protease of Arthro-bacter, however, has a similar broad pH optimum.

Physical properties of the N. crassa AP are given in Table 9. Four methods of molecular weight measurement give essentially identical results (MW, approximately 30,500). Sedimentation coefficient ($S_{20,w}$) and partial specific volume ($\bar{v}$) are consonant with a molecule of this size. The diffusion constant given was determined by calculation from the Svedberg equation.

Results to date on the N. crassa AP suggest that it is similar to other alkaline proteases of microbial origin.

FIGURE 10. Thermal Stability of the Purified Protease. Samples were incubated for 15 min at indicated temperatures, and assayed for remaining activity.

FIGURE 11. Effect of pH on Activity of Alkaline Protease. Assays were carried out at indicated pH values, with casein as the substrate. The different buffers used overlapped and had no effect on activity.

TABLE 9. Summary of Physical Properties of Alkaline Protease

Property	Results
$S_{20, w}$	2.99 s
$D_{20, w}$	$8.61 \times 10^{-7} \text{ cm}^2/\text{s}$
$\bar{V}$	0.721 ml/g
$E_{280}^{1\text{cm}}$ 1mg/ml	1.10
Mol wt	
Sedimentation, e.g.	
Conventional	31,100
Meniscus Dep.	30,400
SDS Electrophoresis	30,500
Amino Acid Analysis	30,609

CELL SURFACE PROPERTIES IN RAT C₆ GLIAL CELLS AND SUBLINES

Investigators:

L. E. Anderson, J. E. Morris, L. S. Winn and W. R. Wiley

The relationship between density-dependent growth regulation and various cellular membrane properties was examined in a clonal glial cell culture, C₆, and two revertant sublines of C₆. Membrane proteins in both parental C₆ and in sublines show comparable electrophoretic patterns on polyacrylamide gels run in sodium dodecyl sulfate. Differences are seen in the ability of the cell types to bind the neurotransmitter, norepinephrine: The two "revertant" sublines bind from 12% to 25% less norepinephrine when compared to parental C₆ cells. In other studies, an in vitro cytotoxicity assay was developed for evaluating cell-mediated immunity in rats, using C₆ and subline cells as labeled target cells.

Examination of glial cells as a potential in vitro screening system for carcinogenic substances has necessitated an investigation of various cell surface properties. As reported previously (Annual Report, 1978), cell surface receptors for the plant lectin, concanavalin-A (Con-A), were examined in parental and subclone cells. The number of Con-A receptors were comparable between transformed and "revertant" cells. However, differences were seen between the two cell types in ability to bind sheep red blood cells following Con-A treatment. Further examination and comparison of cell surfaces and receptor binding properties has been performed.

The protein component of cell membranes was examined by electrophoresis on polyacrylamide gels. Glial cells, taken from plateau-phase growth, were sonicated, then centrifuged for 20 min at 500 x g. Pellets containing crude cell membranes were dissolved in 1.0% sodium dodecyl sulfate (5 min at 95°C) and subjected to electrophoresis on polyacrylamide gels (7.5% and 15% acrylamide). Electrophoretic patterns for membrane proteins from both parental transformed C₆ cells and subline cells were compared. No differences were observed among the three cell types.

The binding of a putative neurotransmitter, norepinephrine, has also been investigated. Glial cells were incubated with various concentrations of D,L-[7-³H(N)]-norepinephrine after 4 and 8 days of growth. As shown in Figure 12, at 4 days during exponential growth (and also at 8 days, when cells are entering a stationary phase of growth),

FIGURE 12. Binding of ³H-Norepinephrine by C₆ Glial Cells (840, open bar) and Sublines (14, solid bar; 6, hatched bar) after 4 and 8 Days of Growth. Cells were incubated with 10⁻⁶ M (6.82 μCi) ³H-norepinephrine for 10 min at 37°C.

differential binding of norepinephrine (10^{-6} M) occurs between parental and subclone cells. Neurotransmitter binding in glial cells (specifically, the differences observed between cell types), is under further examination as a probe of cell surface properties. This technique, which assays a difference between transformed and revertant cells, may serve as a valuable screen for potential carcinogens.

In other studies, the tumorigenicity of cells from C_6 and sublines was evaluated in vivo by injection of cells (10^7 cells/injection) into nude mice. Subcutaneous and intraperitoneal tumor growth were rapid and extensive in mice injected with parental C_6 cells. Revertant subcell lines showed only limited tumor growth after an extended period of time. The in vivo data complement the in vitro growth patterns reported earlier. In vivo results in the nude mouse also suggest some selectivity of lymphocyte cytotoxicity toward the target cells, C_6 and sublines. Efforts were made to determine if labeled glial cells could be used as the target cells in an in vitro assay of natural cell-mediated immunity.

Techniques were first established for labeling glial cells with 125 I-iododeoxyuridine (IUDR). After testing several different media, RPMI, with 5% fetal bovine serum added, was chosen as the preferred medium for growth and labeling of the glial cells. An increase in uptake and labeling of IUDR was promoted by inclusion of 10^{-6} M

fluorodeoxyuridine in the media. Various parameters of incubation were examined to determine the optimum labeling conditions (a time course of labeling is shown in Figure 13). Twenty-four hr was chosen as a convenient time period of incubation with effective labeling of cells. After examination of the data showing the response to IUDR concentration (Figure 14), 0.5 nCi of 125 I-IUDR were used in each incubation.

Parental and subline glial cells were examined as target cells of lymphocyte toxicity in the following manner. Four hr after glial cells (10^4 cells) were cultured, 10^{-6} M FUDR and 0.5 nCi 125 I-IUDR were added. The cells were incubated for 24 hr at 37°C , then effector cells from rat spleen or lymph nodes were added at concentrations of 0, 10^5 , 5×10^5 , 10^6 cells per 10^4 glial cells. Following incubation of this cell mixture for 24 or 48 hr, release of 125 I-IUDR provided an indicator of lymphocyte toxicity in vitro. Data from 24 hr incubation is shown in Table 10. Preliminary experiments indicate that some lysis of parental C_6 tumor cells and one subline may occur, whereas one subline of "revertant" cells appears to be resistant to attack. In light of these results, an explanation for the differential growth of C_6 and sublines in vivo cannot be explained solely on the basis of cell-mediated immunity. Investigation is continuing since C_6 glial cells and sublines do appear to serve as target cells in the assay of natural cell-mediated immunity.

FIGURE 13. Time Course of Glial Cell Labeling with 125 I-iododeoxyuridine; C_6 (Δ) and Sublines 14 (o) and 6 ($\bullet$).

FIGURE 14. Concentration Dependence of 125 I-iododeoxyuridine Incorporation in Glial Cells; C_6 (Δ) and Sublines 14 (o) and 6 ($\bullet$); incubated for 24 hr at 37°C .

TABLE 10. Glial Cells as Target Cells of Lymphocyte Toxicity. Percent surviving cells $\pm$ standard deviation following 24 hr incubation of 125 I-IUDR-labeled glial cells with rat spleen or lymph node effector cells (10^5 , 5×10^5 , 10^6 effector cells/ 10^4 target cells).

Target Cells, 10^4	Effector Cells					
	Spleen, 10^5	Lymph Node, 10^5	Spleen, 5×10^5	Lymph Node, 5×10^5	Spleen, 10^6	Lymph Node, 10^6
840	94 ± 7	91 ± 16	84 ± 11	70 ± 22	85 ± 11	75 ± 18
14	96 ± 13	95 ± 3	72 ± 2	77 ± 18	71 ± 8	71 ± 9
6	97 ± 3	100 ± 8	94 ± 5	116 ± 2	107 ± 2	116 ± 8

• Toxic Effects of Geothermal Effluents

The purpose of this project is to investigate the potential for toxic effects of exposure to effluents from geothermal power plants. We are currently examining the potential toxic effects of exposure to hydrogen sulfide and/or ammonia, utilizing in vivo and in vitro biological systems.

ACUTE AND SUBACUTE INHALATION TOXICOLOGY OF HYDROGEN SULFIDE AND AMMONIA IN RODENTS

Investigators:

R. A. Renne and K. E. McDonald

Technical Assistance:

B. R. Garrity, W. C. Cannon, K. T. Brasel and E. C. DeBattista

Rats and guinea pigs were exposed for 7 or 22 days to hydrogen sulfide, ammonia, or a combination of the two gases. Exposure for 7 days to 220 ppm H₂S, 250 ppm NH₃, or a combination of these two gases at these concentrations, induced a mild inflammation of the respiratory tract in guinea pigs. Evaluation of data from the 22-day study is in progress.

In FY 1978, a series of acute exposures of rats and guinea pigs to H₂S and NH₃ were begun. The experimental design for these studies was described previously (Annual Report, 1978).

The initial 7-day exposure to 100 ppm H₂S, 250 ppm NH₃, or a combination of these two gases at these concentrations produced no significant histopathologic lesions or clinicopathologic alterations in either species.

A subsequent 7-day exposure to 220 ppm H₂S, 250 ppm NH₃, or a combination, produced a mild increase in incidence of acute inflammatory lesions of the respiratory tract in guinea pigs. A mild interstitial pneumonitis was observed in 70% of guinea pigs exposed to H₂S + NH₃, compared with a 30% incidence in chamber-control and shelf-control groups, and a 40% incidence in groups exposed to either gas alone. A mild suppurative bron-

chiolitis was observed in 40% of H₂S + NH₃-exposed guinea pigs; mild acute suppurative tracheitis and laryngitis were observed in increased incidence in all exposed groups when compared with controls. A minimal to mild chronic nephritis was present in all groups, but the incidence of this lesion was higher in groups exposed to NH₃ alone (100%) or to H₂S + NH₃ (100%), when compared with controls (60%).

The increased incidence of respiratory tract lesions in exposed groups appears to be related to exposure to the inhaled gases; the significance of the increased incidence of chronic nephritis in exposed groups is not known.

In FY 1979, a 22-day exposure of rats and guinea pigs to 220 ppm H₂S, 250 ppm NH₃ or a combination of the two was carried out. Histopathologic evaluation of tissues from these animals is in progress.

REPRODUCTIVE TOXICITY TESTING FOR EFFECTS OF H₂S IN RATS

Investigators:

F. D. Andrew, R. A. Renne and W. C. Cannon

Technical Assistance:

R. Music, P. Lytz, K. Poston, B. R. Garrity and K. T. Brasel

Subacute inhalation exposure to 220 ppm H₂S did not induce dominant lethal effects during spermatogenesis in male rats or embryotoxicity in the progeny of female rats. Minor rib anomalies were found in some of the fetuses; it is unclear whether their etiology is related to the H₂S exposure or to the genetic background of the animal stock used.

As reported previously (Annual Report, 1978), a comprehensive literature search revealed scant information regarding the effect of H₂S on gametogenesis, reproductive function, or intrauterine development, except for several Russian articles. The results of studies by Borilyak et al. (1975), for example, suggested that long-term exposures (70-90 days) of rats to H₂S with CS₂ at low levels (10 mg/m³) might elevate pre- and postimplantation loss. Under their experimental conditions, more evidence of embryotoxicity was seen following exposure of female than male rats, although both sexes appeared to be affected.

Therefore, a dominant lethal study was initiated to evaluate the effects of H₂S on spermatogenesis. A group of ten 14-wk-old male Wistar rats (Hilltop Labs) was exposed to 220 ppm of H₂S for 3 hr per day for 7 days; a contemporaneous control group was exposed to filtered air only. Following the last exposure, each male was caged with two unexposed 6- to 9-wk-old virgin female Wistar rats (Hilltop Labs) each week for 10 consecutive weeks. Subsequently, four male rats of known fertility (positive controls) were administered 0.5 mg/kg triethylenemelamine (TEM) intraperitoneally. They were mated with two females per week for 4 wk to confirm the expected results. Females were killed 18 days after their initial cohabitation with a male, and their uteri were examined for the presence of living and dead fetuses. The parameters considered for evaluation per group included: 1) fertility; 2) corpora lutea; 3) total implants; and 4) dead implants (Table 11).

The data summarized in Table 11 indicate that H₂S exposure did not induce any dominant lethal effects. Fertility was high: $\geq 85\%$ or 90% for control and exposed groups, respectively. Preimplantation loss was low; the average number of corpora lutea was 13 to 15 in the exposed groups and 13 to 14 for the control group, while implants averaged 12 to 13 for both groups. Postimplantation loss was also low; a mean of no more than 1 resorption per litter was seen in all groups. In contrast, mean resorptions of 3 to 5 per litter were obtained from the TEM groups over the period tested.

A subsequent study to evaluate the effects of H₂S on prenatal development has been completed. Pregnant female Wistar rats (Charles River Lab) were exposed to H₂S (220 ppm; 3 hr/day, 5 days/wk), either throughout gestation [1 to 18 days of gestation (dg); 14 days total] or during a portion of major organogenesis (7 to 11 dg or 12 to 16 dg; 5 days total). Concurrent control groups were exposed to filtered air only for the same duration at identical gestational stages. All animals were sacrificed at 21 dg and were examined for evidence of prenatal mortality (dead implants), fetal malformations (external, visceral and skeletal) and growth retardation (mean litter weight), using standard teratological procedures.

Weight gain of pregnant animals was approximately the same for all exposure regimens (120 to 138 g) and there was no other evidence of maternal toxicity (Table 12). Fertility of the H₂S-exposed groups was 70 to 100%, which was generally equivalent to

TABLE 11. Dominant Lethal Study

Group	Parameters	Week No. (a)									
		1	2	3	4	5	6	7	8	9	10
H ₂ S-Exposed (b)	Mating Index (d)	100	94	89	100	94	94	94	100	94	94
	Corpora Lutea										
	Total No.	237	232	235	258	239	251	241	257	221	238
	Mean/dam	13.2	13.7	14.7	14.3	14.1	14.8	14.2	14.3	13.0	14.0
	Implants										
	Total No.	208	218	215	224	228	204	222	222	201	207
Air-Exposed Controls (c)	Mean/dam	11.6	12.8	13.4	12.4	13.4	12.0	13.1	12.3	11.8	12.8
	Resorptions										
	Total No.	8	16	10	13	12	7	12	18	15	7
	Mean/dam	0.4	0.9	0.6	0.7	0.7	0.4	0.7	1.0	0.9	0.4
	Mating Index (d)	95	85	90	95	95	85	89	100	100	100
	Corpora Lutea										
	Total No.	242	242	254	260	257	240	220	259	272	273
	Mean/dam	12.7	14.2	14.1	13.7	13.5	14.1	12.9	13.0	13.6	13.7
	Implants										
	Total No.	231	216	242	235	224	214	201	232	237	237
	Mean/dam	12.1	12.7	13.4	12.4	11.8	12.6	11.9	11.6	11.9	11.9
	Resorptions										
	Total No.	8	12	9	13	9	10	13	13	14	6
	Mean/dam	0.4	0.7	0.5	0.7	0.5	0.6	0.8	0.7	0.7	0.3

(a) Weeks of mating following exposure of males

(b) Males exposed by inhalation to 220 ppm H₂S in air 3hr/day for 7 days prior to mating

(c) Males exposed by inhalation to filtered air only for 3 hr/day for 7 days prior to mating

(d) Mating Index = $\frac{\text{Total number of females pregnant}}{\text{Total number of female mated}} \times 100$

TABLE 12. Developmental Toxicity Study

Days Of Gestation At Exposure	Exposure Atmosphere	Adult Weight Gain, g (a)	Number Pregnant (b)	No. Prenatal Deaths (c)	Malformed % (Total No.) (d)	Fetal Weight, g (e)
7 - 11	Air Sham	136 ± 7	3/5	1/26	6 ± 3 (2)	5.0 ± .4
	H ₂ S	129 ± 21	4/5	5/37	20 ± 11 (4)	5.0 ± .3
12 - 16	Air Sham	138 ± 18	5/5	2/59	7 ± 3 (4)	5.3 ± .2
	H ₂ S	133 ± 15	4/5	3/53	6 ± 4 (3)	5.2 ± .2
1 - 18	Cage Controls	132 ± 18	11/13	7/113	15 ± 3 (15)	5.0 ± .3
	Air Sham	125 ± 16	8/10	1/92	10 ± 8 (7)	5.3 ± .3
	H ₂ S	120 ± 18	7/10	3/83	23 ± 9 (22)	5.4 ± .4

(a) Difference in weight between zero dg and 21 dg; mean ± SD

(b) Number pregnant at sacrifice versus number exposed

(c) Total resorptions versus all implants

(d) Includes visceral and skeletal anomalies calculated as mean percent per litter ± SE

(e) Mean of litter means ± SD

FIGURE 7. Electrophoresis of Culture Filtrates Obtained During 8-hr Inductions of *N. crassa*. Symbols are defined as follows: AP, alkaline protease; NP, neutral protease; M1, metalloprotease 1; M2, metalloprotease 2; AcP, acid protease. Zones of increasing milk proteolysis are indicated by zones of increasing darkness. Dashed lines indicate zones in which clearing was barely visible. (A) Electrophoresis of filtrates obtained from nitrogen-derepressed cultures, (B) carbon-derepressed cultures, (C) sulfur-derepressed cultures.

that for the controls and well within the expected range. There was no evidence of exposure-induced embryotoxicity, since there was neither an increase in prenatal mortality nor a decrease in mean litter weight.

Essentially all of the malformations seen involved the skeleton, and most of these (93%) involved the ribs. The structural appearance of the rib deformities is best described as "knobby," "curved" or "wavy." They are all generally classified as minor skeletal anomalies, since they are probably not detrimental to the survival of the offspring. This unusual anomaly was found in all exposure groups, but not all the litters in a group (50 to 82%). The animals for this study were not from our usual source (Hilltop Labs), although they were derived from the same genetic stock. They may have a slightly different genetic complement; unfortunately, we lack background

data for this source. The relatively high incidence of defects (23%) in the 14-day, H₂S-exposed group suggests that this may be exposure-related, although the unexposed cage controls and the 7 to 11-dg H₂S groups also had elevated incidences (15 to 20%) compared to those (6 to 10%) for all of the other groups.

No external malformations were seen in any group. One air-exposed (control) fetus had a visceral malformation (umbilical hernia).

Additional studies should be conducted to determine: a) whether the rib defects are peculiar to the animal source previously used (and are thus of genetic etiology) by comparing the two sources, and b) the incidence of rib defects and other malformations using a higher exposure level or a longer daily exposure duration.

EFFECT OF IN VITRO EXPOSURE TO HYDROGEN SULFIDE ON CULTURED RABBIT ALVEOLAR MACROPHAGES

Investigator: A. V. Robinson

Technical Assistance: S. Strankman

Cultured rabbit alveolar macrophages were exposed to 50 ppm or 200 ppm of hydrogen sulfide (H_2S). Various exposure regimens (continuous or intermittent) and various lengths of exposure were investigated. Viability of macrophages was measured using trypan blue and neutral red stains, and phagocytic ability of the cells was determined by measuring phagocytosis of 2- μ m polyvinyltoluene beads. Exposure to H_2S inhibited phagocytosis of beads at the lowest concentration tested, 50 ppm for 20 hr. Exposure to 200 ppm for 48 hr resulted in a decrease in the number of cells containing one or more beads to as low as 2% of control values. Exposure to H_2S for 24 hr followed by 24-hr incubation without further exposure decreased the phagocytic ability of macrophages as much as did 48 hr of continuous exposure. Viability measurements were less-sensitive indicators of cellular damage; measurement of metabolic integrity using neutral red (NR) staining was more sensitive than measurement of viability by passive permeability using trypan blue (TB) stain.

Alveolar macrophages were obtained by lavage from the lungs of New Zealand White rabbits of either sexes. Macrophages were cultured, as previously described (Annual Report, 1978), in M-199 culture medium, containing 10% heat-inactivated rabbit serum. Usually, cells were cultured for 24 hr before initiation of H_2S exposure. Cells were exposed to 50 ppm or 200 ppm H_2S (Table 13), introduced in the airstream of a flow-through incubator (flow rate, 8.4 l/min). The incubator atmosphere contained 5% CO_2 , humidified by the presence of a pan of distilled H_2O in the bottom of the incubator. Concentration of H_2S was determined with a Bendix® gas sampler utilizing calibrated tubes.

Immediately after exposure (or, in some cases, 24 hr later), 2- μ m-diameter polyvinyltoluene (PVT) beads suspended in Hanks' salt solution containing 4% BSA were added to the cell culture to give a bead-to-cell ratio of 25-50 to one. Following a period of 0.75 to 1.5 hr in which the macrophages were allowed to phagocytize the PVT beads, unattached cells and beads were aspirated by vacuum and the adherent cells were washed free of beads by two saline washes which were also aspirated from the flask. The saline, and the aspirated beads and cells,

were then centrifuged at approximately 100 x g for 5 min, resulting in pelleting of the macrophages, with most of the PVT beads remaining in suspension. The supernatant was aspirated and the cell pellet was resuspended in 1.0 ml of saline. Twenty μ l of 1% NR in saline was then added to a 0.5-ml aliquot of the resuspended cells; this mixture was incubated for 10 to 15 min at 37°C, and the numbers of red-stained cells were counted immediately, using a hemocytometer. The remaining cells were assayed for viability by adding 0.5 ml of 0.4% TB to the cells, centrifuging at 350 x g for 5 min, resuspending in 0.5 ml saline and counting the number of blue-staining (dead) cells. The cells were then fixed by adding two volumes of McDowell-Trumps fixative to one volume of cell suspension. Fixed cells were assayed for phagocytic ability by counting the number of cell-associated beads; 150 to 300 cells from each flask were counted.

Cells remaining attached to the flask after phagocytosis and rinsing were detached by adding 5.0 ml of a pancreatic enzyme preparation (ENZAR T®), diluted 1/15 or 1/20 in Hanks' salts. The enzyme solution was incubated on the cells at 37°C for 0.75-1.5 hr; loosened cells were then aspirated, and the

TABLE 13. Effect of In Vitro H₂S Exposure on Phagocytic Ability and Viability of Cultured Rabbit Alveolar Macrophages

Exposure Level and Regimen	Relative Phagocytic Ability(a), %	% Viable Cells(b)	
		TB(c)	NR(d)
20 hr @ 50 ppm + 24 hr recovery	88 ± 14	88 ± 2	94 ± 3
24 hr @ 200 ppm	77 ± 8, 73 ± 16, 69 ± 11	90 ± 4	95 ± 2
24 hr @ 200 ppm + 24 hr recovery	15 ± 12, 7 ± 4	50 ± 20	37 ± 22
8 hr @ 200 ppm followed by 16 hr recovery and 8 hr @ 200 ppm (16 hr total exposure)	65 ± 9	--	--
8 hr @ 200 ppm followed by 16 hr recovery 8 hr @ 200 ppm followed by 16 hr recovery 8 hr @ 200 ppm (24 hr total exposure)	32 ± 7	--	--
48 hr @ 200 ppm	7 ± 2, 2 ± 7	23 ± 8	9 ± 6

(a) Relative phagocytic ability = $\frac{\% \text{ of exposed cells containing 1 or more latex beads} \times 100}{\% \text{ of control cells containing 1 or more latex beads}}$

Multiple values indicate separate experiments.

(b) Viability measured on cells in suspension, following detachment from flask (see text).

(c) Trypan blue

(d) Neutral red

flask was washed with 5 ml of M-199 media containing 10% rabbit serum. The aspirated cells and wash were pooled, and cells were pelleted by centrifugation at 350 x g for 5 min. Cells were resuspended, assayed for viability, and fixed as described above. Fixed cells were assayed for phagocytic ability as described above.

The relative phagocytic ability and viability of the cultured macrophages generally decreased as a function of exposure time (Table 13). Selection of exposure regimen and time of sampling relative to cessation of exposure were important determinants of toxicity. Comparing the phagocytic ability of cultured macrophages sampled immediately following cessation of exposure with those sampled 24 hr following cessation of exposure, it is apparent that the macrophages have lost a great deal of their ability to phagocytize in the 24 hr following exposure. Indeed, there is no significant difference in the decrease in phagocytic ability between cells exposed to 24 hr of H₂S and sampled 24 hr later, versus those receiving 48-hr continuous exposure. Similarly, there was little difference between cells exposed for 24 hr without recovery and the cells with two 8-hr exposures and a 16-hr recovery between exposures.

The above data suggest that the cellular lesion responsible for a decrease in phagocytic ability occurs early in the exposure to H₂S and the expression of the lesion (decreased phagocytic ability, decreased viability) is delayed. Comparison of the 50 ppm, 20-hr exposure, 24-hr recovery and the 200-ppm, 24-hr exposure, 24-hr recovery indicates that the response is also dose-dependent as well as time-dependent. The phagocytic ability of the cells exposed to 50 ppm was much greater than that of the 200-ppm-exposed cells under the same regimen of exposure and sampling. It is unlikely that 4 hr less exposure time at 50 ppm had any significant effect.

The viability data parallel the phagocytic ability; however, the former is a less-sensitive indicator of damage. The use of NR staining as a viability measurement (live cells stain red) appears to be a more accurate indicator of cytotoxicity than TB staining, particularly at the higher concentrations of hydrogen sulfide exposure. When viability was measured on suspended cells (Table 13) the TB and NR data were in fairly good agreement. When viability tests were performed on cells remaining adherent to flasks, it was found that TB staining indicated 90 to 100% viability, even on flasks containing cells which did not phagocytize or stain with NR.

These data indicate that H₂S causes a dose-related impairment of phagocytic ability and viability. It appears that the responsible lesion occurs early in the exposure period but is not fully expressed for another 24 hr. Identification of the exact kinetics of these effects is not possible, given the number of sample points; however, these data indicate

that doses as low as 50 ppm result in phagocytic impairment. We plan studies to investigate lesions induced by low doses (50 ppm and less), and to investigate the kinetics of the phagocytic impairment. It may also be possible to measure the influence of H₂S exposure on the bactericidal ability of the alveolar macrophage.

EFFECT OF IN VIVO EXPOSURE OF HYDROGEN SULFIDE ON FREE CELLS
OBTAINED FROM LUNGS OF RATS AND GUINEA PIGS

Investigators:

A. V. Robinson, K. E. McDonald, and R. A. Renne

Technical Assistance:

S. Strankman

Rats and guinea pigs were exposed to 200 ppm H₂S for 24 hr. Twenty-four hours following cessation of exposure, the lungs of these animals were lavaged and the number, type and viability of lavaged cells ascertained. The phagocytic capability of lavaged cells was assessed by measuring the uptake of 2- μ m polyvinyltoluene beads. The number of cells obtained increased by approximately 475% in exposed rats and about 21% in exposed guinea pigs compared with unexposed controls. Distribution of cell types in rats revealed a 1600% increase in PMNs, a 57% decrease in lymphocytes and a 50% decrease in macrophages. Corresponding values for the guinea pig revealed a 190% increase in PMNs, a 62% decrease in lymphocytes and a 16% decrease in macrophages. Phagocytic ability was decreased by 26% for rats and 6% for guinea pigs. Cellular viability as measured by trypan blue and neutral red staining was not different from controls; however, a 13% decrease in the viability of lavaged cells in exposed rats was measured by neutral red staining.

Rats and guinea pigs were exposed to 200 ppm H₂S continuously for 24 hr, then removed from the exposure chamber and caged for 24 hr. Free cells were lavaged from the lungs of these animals (and controls) by six saline lavages of 5 ml each. Free cells from all animals in a group were pooled and total number of cells, percent PMNs, percent lymphocytes and percent alveolar macrophages were determined. Results (Table 14) are indicative of an inflammatory response in the respiratory tract of H₂S-exposed animals. The number of free cells increased in both exposed rats and guinea pigs, particularly in the former. The number of PMNs increased in both rats and guinea pigs; again, rats had the most noticeable increase. The number of macrophages and lymphocytes decreased in H₂S-exposed animals. In all respects, rats exhibited a greater response to H₂S exposure than guinea pigs. The increase in free cell numbers and PMNs, and the decrease in macrophages and lymphocytes, is typical of the response seen in pulmonary injury induced by other irritant gases such as NO₂ and ozone.

Aliquots of the pooled cells were centrifuged at 1000 x g for 5 min and the pellet was resuspended in Hanks' salts containing 4% BSA. Polyvinyl toluene (PVT) beads (2 μ m in Hanks' + 4% BSA) were added to the cell suspension to give a final cell concentration of 2.5×10^6 ml and a bead-to-cell ratio of 48/1. Cells and beads were incubated at 37°C for 45 min with shaking. At the end of the incubation period, 0.5 ml of media containing cells and beads was added to 20 μ l of 1% neutral red and another 0.5-ml aliquot was added to 0.5 ml 0.4% trypan blue. Cells were centrifuged at 500 x g for 5 min, resuspended in 0.5 ml Hanks' + 4% BSA and immediately examined for viability. A separate aliquot of cells was fixed in McDowell-Trump's fixative, and phagocytic ability was assessed by light microscopic counting of the number of beads ingested per cell.

The data in Table 15 show a decrease in the number of cells containing >1 bead in exposed rats and guinea pigs. As previously noted, the rat was a much more sensitive

TABLE 14. Effect of 200 ppm H₂S Exposure in Vivo on the Number and Type of Free Cells Obtained by Pulmonary Lavage of Rats and Guinea Pigs

Species (Treatment)	Average Number of Cells Lavaged per Animal (a) x10 ⁶	% of Cells of Each Type		
		PMN	Lymphocytes	Macrophages
Rat (Control)	1.9	3	23	74
Rat (Exposed)	10.9	51	12	37
Guinea Pig (Control)	5.7	23	16	61
Guinea Pig (Exposed)	7.2	44	5	51

(a) Six animals per group; each animal was lavaged 6 times with saline

TABLE 15. Effect of In Vivo H₂S Exposure (200 ppm) on the Phagocytic Ability and Viability of Free Cells Obtained by Pulmonary Lavage

Species (Treatment)	% of Cells Containing ≥ 1 Bead	% of Cells Containing ≥ 7 Beads	% of Cells Viable	
			TB (a)	NR (b)
Rat (Control)	94	78	95	98
Rat (Exposed)	70	50	100	85
Guinea Pig (Control)	96	87	91	95
Guinea Pig (Exposed)	90	71	99	96

(a) Trypan Blue

(b) Neutral Red

indicator of damage than the guinea pig. Viability measurements using trypan blue (TB) show no effect, while neutral red (NR) measurements show an effect in the rat but none in the guinea pig. These data also demonstrate greater sensitivity of the NR staining method of viability measurement than the TB staining method.

The decrease in phagocytic ability of the free cell population was much less pronounced in vivo compared to the in vitro studies with rabbit alveolar macrophages (Robinson, this Annual Report). This is not surprising, since no attempt was made in the in vivo

study to measure only macrophages, and it is not possible to ascertain the percentage of different cell types present in the lavage fluid that accumulated in the lung during the 24-hr recovery period. Pulmonary lavage of animals immediately following exposure, and subsequent in vitro culture of the lavaged cells, would allow more accurate assessment of phagocytic impairment of specific cell types. As with the in vitro studies, measurement of bactericidal capabilities of free cells lavaged from exposed animals may provide a more sensitive measurement of injury.

Appendix

APPENDIX

DOSE-EFFECT STUDIES WITH INHALED PLUTONIUM IN BEAGLES

On the following pages data are presented for all dogs employed in current life-span dose effect studies with inhaled $^{239}\text{PuO}_2$, $^{238}\text{PuO}_2$, and ^{239}Pu nitrate. Information is presented on the estimated initial alveolar deposition, based on external thorax counts and on estimated lung weights ($0.011 \times$ body weight) at time of exposure. Information is also provided on the current interpretation of the most prominent clinical-pathological features associated with the death of animals. These data represent information presently available, and are presented as reference material for scientists who desire to follow in detail the progress of these experiments.

DOSE-EFFECT STUDIES WITH INHALED PU-239 OXIDE IN BEAGLES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/79	DEATH	
CONTROL	738	F	0	0.00	0.00					125.2*		
CONTROL	740	F	0	0.00	0.00					125.2*		
CONTROL	749	F	0	0.00	0.00					123.9*		
CONTROL	755	M	0	0.00	0.00					123.8*		
CONTROL	766	M	0	0.00	0.00					123.5*		
CONTROL	775	F	0	0.00	0.00					123.1*		
CONTROL	785	M	0	0.00	0.00					122.4*		
CONTROL	789	M	0	0.00	0.00					122.1*		
CONTROL	792	M	0	0.00	0.00				04/28/76		79.5*	Oral Tumor
CONTROL	800	F	0	0.00	0.00					119.3*		
CONTROL	801	M	0	0.00	0.00					119.3*		
CONTROL	811	F	0	0.00	0.00					118.2*		
CONTROL	846	M	0	0.00	0.00					117.4*		
CONTROL	861	M	0	0.00	0.00					117.0*		
CONTROL	868	F	0	0.00	0.00					115.7*		
CONTROL	872	F	0	0.00	0.00					115.6*		
CONTROL	878	M	0	0.00	0.00					113.7*		
CONTROL	882	M	0	0.00	0.00					113.5*		
CONTROL	885	F	0	0.00	0.00					112.9*		
CONTROL	903	F	0	0.00	0.00					110.6*		
CONTROL SACRIFICE	701	F	0	0.00	0.00				04/18/79		121.0*	Sacrificed
CONTROL SACRIFICE	703	M	0	0.00	0.00				03/24/77		96.2*	Sacrificed
CONTROL SACRIFICE	724	M	0	0.00	0.00				03/30/78		107.9*	Sacrificed
D-1 LOWEST	756	M	0	0.00	0.00	13.0	19.5	01/19/71		104.3		
D-1 LOWEST	762	M	0	0.00	0.00	11.5	19.3	01/19/71	01/24/77		72.2	Sacrificed
D-1 LOWEST	847	M	0	0.00	0.00	13.0	18.5	07/06/71		98.8		
D-1 LOWEST	958	M	0	0.00	0.00	13.0	18.2	07/06/71		98.8		
D-1 LOWEST	865	F	0	0.00	0.00	9.0	17.4	07/06/71		98.8		
D-1 LOWEST	879	M	0	0.00	0.00	14.5	17.9	10/07/71		95.8		
D-1 LOWEST	886	F	0	0.00	0.00	10.5	18.2	11/10/71		94.7		
D-1 LOWEST	907	F	0	0.00	0.00	11.5	15.9	11/10/71		94.7		
D-1 LOWEST	825	F	1	0.01	0.12	11.5	18.1	06/08/71		99.7		
D-1 LOWEST	849	F	1	0.01	0.10	10.0	21.3	10/07/71	10/26/72		12.6	Sacrificed
D-1 LOWEST	904	F	1	0.01	0.07	10.0	15.9	11/10/71		94.7		
D-1 LOWEST	832	F	2	0.02	0.24	8.5	16.5	04/26/71		101.2		
D-1 LOWEST	900	M	3	0.02	0.22	13.0	16.0	11/10/71		94.7		
D-1 LOWEST	870	F	4	0.03	0.32	12.0	16.9	07/06/71		98.8		
D-1 LOWEST	899	F	4	0.03	0.31	11.5	16.0	11/10/71		94.7		
D-1 LOWEST	867	M	5	0.04	0.41	11.5	17.4	07/06/71		98.8		
D-1 LOWEST	891	M	6	0.04	0.41	14.0	16.0	11/10/71		94.7		
D-1 LOWEST	850	F	5	0.05	0.59	8.5	21.3	10/07/71		95.8		
D-1 LOWEST	853	M	8	0.05	0.51	15.0	21.3	10/07/71		95.8		
D-1 LOWEST	875	M	8	0.05	0.54	14.0	16.8	07/06/71	05/21/78		82.5	Kidney:Malignant Lymphoma

* Indicates age in months since birth, all other ages are in months since exposure.

DOSE-EFFECT STUDIES WITH INHALED PU-239 OXIDE IN BEAGLES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/79	DEATH	
D-1 LOWEST	770	F	6	0.06	0.63	9.5	19.1	01/19/71		104.3		
D-1 LOWEST	788	M	8	0.06	0.62	13.0	18.7	02/09/71		103.7		
D-1 LOWEST	893	M	9	0.06	0.61	14.0	14.9	10/07/71		95.8		
D-1 LOWEST	807	F	8	0.07	0.73	11.0	14.6	02/09/71		103.7		
D-1 LOWEST	841	F	6	0.07	0.75	8.0	17.7	06/08/71		99.7		
D-1 LOWEST	908	M	9	0.07	0.77	11.0	15.9	11/10/71		94.7		
D-2 LOW	776	M	10	0.07	0.74	13.5	20.2	03/04/71		102.9		
D-2 LOW	767	M	10	0.08	0.83	12.0	18.2	12/21/70		105.3		
D-2 LOW	842	M	10	0.08	0.83	12.5	18.6	07/06/71		98.8		
D-2 LOW	920	M	11	0.08	0.92	12.0	16.0	06/08/72	07/07/72		1.0	Sacrificed
D-2 LOW	862	M	13	0.09	1.00	13.0	17.3	06/08/71		99.7		
D-2 LOW	871	M	13	0.09	0.96	13.5	16.9	07/06/71		98.8		
D-2 LOW	874	M	16	0.11	1.24	13.0	16.8	07/06/71		98.8		
D-2 LOW	754	M	22	0.15	1.69	13.0	19.5	01/19/71	01/10/78		83.7	Status Epilepticus
D-2 LOW	845	F	19	0.15	1.63	11.5	17.6	06/08/71		99.7		
D-2 LOW	748	F	14	0.16	1.75	8.0	19.5	01/19/71		104.3		
D-2 LOW	798	F	16	0.16	1.78	9.0	15.6	02/09/71	08/29/74		42.6	Sacrificed
D-2 LOW	826	F	19	0.16	1.81	10.5	19.1	07/06/71		98.8		
D-2 LOW	831	F	21	0.18	2.00	10.5	17.9	06/09/71		99.7		
D-2 LOW	881	F	19	0.19	2.09	9.0	17.7	10/07/71		95.8		
D-2 LOW	780	F	24	0.22	2.40	10.0	18.2	01/19/71		104.3		
D-2 LOW	859	M	35	0.22	2.41	14.5	13.2	07/06/71		98.8		
D-2 LOW	757	M	36	0.23	2.57	14.0	18.5	12/21/70		105.3		
D-2 LOW	876	F	19	0.23	2.51	7.5	17.9	10/07/71		95.8		
D-2 LOW	806	F	26	0.25	2.74	9.5	15.3	03/04/71		102.9		
D-2 LOW	813	F	32	0.28	3.05	10.5	15.1	03/04/71		102.9		
D-2 LOW	877	F	34	0.29	3.24	10.5	17.9	10/07/71		95.8		
D-2 LOW	769	F	28	0.32	3.50	8.0	18.2	12/21/70	06/23/78		90.1	Ovarian Tumor
D-2 LOW	802	M	40	0.33	3.64	11.0	18.1	04/26/71		101.2		
D-3 MED-LOW	781	F	48	0.38	4.17	11.5	17.3	12/21/70		105.3		
D-3 MED-LOW	771	F	44	0.40	4.40	10.0	19.2	01/20/71		104.3		
D-3 MED-LOW	782	M	62	0.42	4.59	13.5	19.0	02/10/71		103.6		
D-3 MED-LOW	786	M	62	0.42	4.59	13.5	19.5	03/04/71		102.9		
D-3 MED-LOW	752	M	62	0.43	4.77	13.0	18.6	12/21/70	02/22/79		93.1	Lung Tumor, Adrenal Tumor
D-3 MED-LOW	823	M	65	0.44	4.81	13.5	16.8	04/26/71		101.2		
D-3 MED-LOW	883	M	63	0.44	4.85	13.0	17.7	10/07/71		95.8		
D-3 MED-LOW	778	M	74	0.46	5.10	14.5	20.2	03/04/71	08/26/79		101.7	Processing
D-3 MED-LOW	838	M	56	0.46	5.09	11.0	17.8	06/08/71		99.7		
D-3 MED-LOW	795	F	54	0.52	5.68	9.5	15.0	01/20/71		104.3		
D-3 MED-LOW	815	M	68	0.52	5.67	12.0	16.8	04/26/71	05/22/73		24.9	Sacrificed
D-3 MED-LOW	851	F	53	0.54	5.89	9.0	21.3	10/07/71		95.8		
D-3 MED-LOW	918	M	74	0.58	6.43	11.5	16.0	06/08/72	07/06/72		0.9	Sacrificed
D-3 MED-LOW	834	F	67	0.63	7.44	9.0	17.8	06/08/71	07/05/79		96.9	Processing

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DOSE-EFFECT STUDIES WITH INHALED PU-239 OXIDE IN BEAGLES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/79	DEATH	
D-3 MED-LOW	797	F	85	0.70	7.73	11.0	16.4	03/04/71		102.9		
D-3 MED-LOW	848	F	75	0.72	7.94	9.5	21.3	10/07/71		95.8		
D-3 MED-LOW	827	F	89	0.81	8.90	10.0	16.7	04/26/71		101.2		
D-3 MED-LOW	697	M	140	0.85	9.33	15.0	19.5	10/30/70		107.0		
D-3 MED-LOW	750	M	118	0.93	10.26	11.5	19.6	01/20/71		104.3		
D-3 MED-LOW	884	M	123	1.12	12.30	10.0	17.8	10/08/71		95.7		
D-3 MED-LOW	844	F	135	1.23	13.50	10.0	17.6	06/08/71		99.7		
D-3 MED-LOW	905	F	127	1.36	14.94	8.5	15.9	11/10/71		94.7		
D-4 MEDIUM	866	M	200	1.35	14.81	13.5	17.4	07/06/71		98.8		
D-4 MEDIUM	809	F	157	1.36	14.95	10.5	15.3	03/04/71		102.9		
D-4 MEDIUM	764	F	158	1.37	15.05	10.5	18.2	12/21/70		105.3		
D-4 MEDIUM	835	F	163	1.48	16.30	10.0	16.4	04/26/71	06/25/78		86.0	Reticulum Cell Sarcoma
D-4 MEDIUM	839	F	189	1.49	16.43	11.5	16.3	04/26/71		101.2		
D-4 MEDIUM	814	F	140	1.50	16.47	8.5	15.1	03/04/71		102.9		
D-4 MEDIUM	336	M	256	1.66	18.29	14.0	17.8	06/08/71		99.7		
D-4 MEDIUM	819	F	163	1.74	19.18	8.5	18.2	06/08/71		99.7		
D-4 MEDIUM	888	M	274	1.78	19.57	14.0	17.1	10/08/71	07/02/79		92.8	Lung Tumor
D-4 MEDIUM	824	F	227	1.79	19.74	11.5	18.1	06/08/71		99.7		
D-4 MEDIUM	860	M	254	1.85	20.32	12.5	17.3	06/08/71		99.7		
D-4 MEDIUM	833	F	248	2.37	26.11	9.5	16.5	04/26/71		101.2		
D-4 MEDIUM	810	F	302	2.39	26.26	11.5	15.3	03/04/71		102.9		
D-4 MEDIUM	794	M	444	2.60	28.65	15.5	17.7	03/04/71		102.9		
D-4 MEDIUM	854	M	465	2.64	29.06	16.0	21.3	10/08/71		95.7		
D-4 MEDIUM	478	M	298	2.71	29.80	10.0	64.0	10/09/70	10/16/70		0.2	Sacrificed
D-4 MEDIUM	808	F	270	2.73	30.00	9.0	14.6	02/10/71		103.6		
D-4 MEDIUM	805	F	257	3.12	34.27	7.5	18.5	06/08/71		99.7		
D-4 MEDIUM	812	M	438	3.19	35.04	12.5	17.1	04/26/71		101.2		
D-4 MEDIUM	857	M	486	3.40	37.38	13.0	17.3	06/08/71		99.7		
D-4 MEDIUM	892	M	494	3.59	39.52	12.5	16.0	11/10/71		94.7		
D-4 MEDIUM	816	M	398	3.62	39.80	10.0	16.8	04/26/71	05/11/71		0.5	Sacrificed
D-4 MEDIUM	777	M	546	3.97	43.68	12.5	20.2	03/04/71		102.9		
D-4 MEDIUM	803	M	547	4.32	47.57	11.5	18.1	04/26/71	11/10/77		78.5	Interstitial Pneumonitis
D-5 MED-HIGH	787	M	651	4.73	52.08	12.5	19.5	03/04/71	02/08/79		95.2	Lung Tmr, Intestinal Tmr
D-5 MED-HIGH	840	F	703	4.92	54.08	13.0	17.7	06/08/71		99.7		
D-5 MED-HIGH	727	M	733	5.33	58.64	12.5	18.8	10/26/70	11/10/76		72.5	Lung Tumor
D-5 MED-HIGH	898	F	711	5.39	59.25	12.0	16.0	11/10/71		94.7		
D-5 MED-HIGH	759	M	809	6.13	67.42	12.0	18.3	12/21/70	06/02/75		53.4	Lung Tumor
D-5 MED-HIGH	864	F	801	6.62	72.82	11.0	17.4	07/07/71		98.8		
D-5 MED-HIGH	909	M	737	6.70	73.70	10.0	15.9	11/10/71		94.7		
D-5 MED-HIGH	734	M	914	6.92	76.17	12.0	19.2	11/10/70	04/01/71		4.7	Sacrificed
D-5 MED-HIGH	856	F	818	7.08	77.90	10.5	18.2	07/07/71	05/02/79		93.8	Lung Tumor
D-5 MED-HIGH	837	M	1283	8.04	88.48	14.5	18.8	07/07/71	07/21/77		72.5	Lung Tumor
D-5 MED-HIGH	863	F	980	8.48	93.33	10.5	17.4	07/07/71	10/21/77		75.5	Lung Tumor

* Indicates age in months since birth, all other ages are in months since exposure.

DOSE-EFFECT STUDIES WITH INHALED PU-239 OXIDE IN BEAGLES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/79	DEATH	
D-5 MED-HIGH	320	F	347	8.56	94.11	9.0	18.2	06/08/71	06/01/79	95.8		Lung Tumor
D-5 MED-HIGH	852	F	1187	9.38	103.22	11.5	21.3	10/08/71	02/22/78	76.5		Lung Tumor
D-5 MED-HIGH	880	F	840	9.55	105.00	8.0	17.8	10/08/71	12/04/78	85.9		Lung Tumor
D-5 MED-HIGH	309	F	1089	9.90	108.90	10.0	16.0	11/10/71	09/20/79	94.3		Lng Tmr, Osteoarthropathy
D-5 MED-HIGH	783	M	1394	10.14	111.52	12.5	18.9	02/09/71	12/03/75	57.8		Lung Tumor
D-5 MED-HIGH	304	M	1344	10.13	112.00	12.0	20.5	07/07/71	08/18/74	37.4		Lung Tumor, Rad. Pneum.
D-5 MED-HIGH	873	M	1767	10.71	117.80	15.0	16.8	07/07/71	09/03/76	61.9		Lung Tumor
D-5 MED-HIGH	760	M	1378	10.89	119.83	11.5	19.3	01/20/71	03/15/73	30.8		Radiation Pneumonitis
D-5 MED-HIGH	796	F	1318	11.41	125.52	10.5	15.6	02/09/71	09/17/75	55.2		Lng Tmr, Osteoarthropathy
D-5 MED-HIGH	761	M	1460	12.07	132.73	11.0	19.3	01/20/71	11/02/76	69.4		Lung Tumor
D-5 MED-HIGH	709	M	1726	12.55	138.08	12.5	19.6	11/10/70	03/31/71	4.6		Sacrificed
D-5 MED-HIGH	772	M	1896	14.99	164.87	11.5	13.8	02/09/71	06/26/75	52.5		Lng Tmr, Osteoarthropathy
D-5 MED-HIGH	702	F	1082	15.29	168.20	10.0	19.3	11/10/70	03/31/71	4.6		Sacrificed
D-5 MED-HIGH	739	F	1511	17.17	188.88	8.0	18.5	11/10/70	04/01/71	4.7		Sacrificed
D-6 HIGH	753	F	2448	23.43	257.68	9.5	18.5	12/21/70	10/02/76	69.4		Lung Tumor
D-6 HIGH	817	M	3164	23.97	263.67	12.0	19.2	07/07/71	03/26/73	20.6		Radiation Pneumonitis
D-6 HIGH	829	M	3515	24.58	270.38	13.0	19.1	07/07/71	09/13/73	26.3		Radiation Pneumonitis
D-6 HIGH	090	F	3101	31.32	344.56	9.0	16.0	11/10/71	06/13/74	31.1		Radiation Pneumonitis
D-6 HIGH	435	F	3840	33.25	365.71	10.5	75.5	11/05/70	11/12/70	0.2		Sacrificed
D-6 HIGH	913	M	4900	35.64	392.00	12.5	17.4	07/19/72	08/18/72	1.0		Sacrificed
D-6 HIGH	906	F	6632	63.46	698.11	9.5	15.9	11/10/71	11/22/72	12.4		Radiation Pneumonitis
D-6 HIGH	896	F	5515	56.85	735.33	7.5	16.0	11/10/71	02/12/73	15.1		Radiation Pneumonitis
D-6 HIGH	747	F	7476	97.09	1068.00	7.0	19.6	01/20/71	01/13/72	11.8		Radiation Pneumonitis
D-6 HIGH	910	M	14267	103.76	1141.36	12.5	15.9	11/10/71	10/12/72	11.1		Radiation Pneumonitis

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DOSE-EFFECT STUDIES WITH INHALED PU-238 OXIDE IN BEAGLES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/79	DEATH	
CONTROL	939	M	0	0.00	0.00					100.9*		
CONTROL	949	F	0	0.00	0.00					100.7*		
CONTROL	978	M	0	0.00	0.00					100.5*		
CONTROL	990	F	0	0.00	0.00				07/08/79		97.4*	Pyometra
CONTROL	996	F	0	0.00	0.00					100.0*		
CONTROL	1005	M	0	0.00	0.00					100.0*		
CONTROL	1007	F	0	0.00	0.00					99.9*		
CONTROL	1024	M	0	0.00	0.00					99.5*		
CONTROL	1038	M	0	0.00	0.00					97.4*		
CONTROL	1045	M	0	0.00	0.00					97.3*		
CONTROL	1054	F	0	0.00	0.00					97.1*		
CONTROL	1061	F	0	0.00	0.00					97.0*		
CONTROL	1093	M	0	0.00	0.00					93.3*		
CONTROL	1097	F	0	0.00	0.00					92.6*		
CONTROL	1112	M	0	0.00	0.00					92.4*		
CONTROL	1116	F	0	0.00	0.00					92.1*		
CONTROL	1186	F	0	0.00	0.00					85.5*		
CONTROL	1197	M	0	0.00	0.00					85.0*		
CONTROL	1209	M	0	0.00	0.00					84.7*		
CONTROL	1225	F	0	0.00	0.00					83.8*		
CONTROL SACRIFICE	966	M	0	0.00	0.00				04/30/77		71.6*	Sacrificed
CONTROL SACRIFICE	1011	F	0	0.00	0.00				06/01/78		83.9*	Sacrificed
CONTROL SACRIFICE	1013	F	0	0.00	0.00				05/29/79		95.3*	Sacrificed
CONTROL SACRIFICE	1087	M	0	0.00	0.00				12/14/76		60.0*	Sacrificed
CONTROL SACRIFICE	1118	M	0	0.00	0.00				01/13/76		47.5*	Sacrificed
CONTROL SACRIFICE	1223	M	0	0.00	0.00				05/15/75		31.9*	Sacrificed
CONTROL SACRIFICE	1227	M	0	0.00	0.00				12/01/76		49.9*	Sacrificed
CONTROL SACRIFICE	1228	M	0	0.00	0.00				10/31/78		72.9*	Sacrificed
D-1 LOWEST	993	M	0	0.00	0.00	10.5	19.6	01/18/73		80.4		
D-1 LOWEST	1003	M	0	0.00	0.00	14.0	19.6	01/18/73		80.4		
D-1 LOWEST	1023	F	0	0.00	0.00	12.5	19.2	01/18/73		80.4		
D-1 LOWEST	1039	M	0	0.00	0.00	11.0	17.0	01/18/73		80.4		
D-1 LOWEST	1044	F	0	0.00	0.00	11.5	17.0	01/18/73		80.4		
D-1 LOWEST	1055	M	0	0.00	0.00	13.0	16.8	01/18/73		80.4		
D-1 LOWEST	1063	M	0	0.00	0.00	14.5	16.7	01/18/73		80.4		
D-1 LOWEST	1105	F	0	0.00	0.00	10.0	16.4	05/31/73		76.0		
D-1 LOWEST	1194	F	0	0.00	0.00	10.5	19.8	04/18/74		65.4		
D-1 LOWEST	1215	M	2	0.00	0.00	15.5	19.3	04/18/74	04/26/77		36.3	Sacrificed
D-1 LOWEST	1230	M	0	0.00	0.00	12.5	18.4	04/18/74		65.4		
D-1 LOWEST	951	M	2	0.01	0.14	14.0	19.3	12/19/72		81.3		
D-1 LOWEST	1008	M	2	0.01	0.15	13.5	19.6	01/18/73		80.4		
D-1 LOWEST	1193	F	2	0.01	0.16	12.5	19.8	04/18/74		65.4		
D-1 LOWEST	959	M	3	0.02	0.22	13.5	19.2	12/19/72		81.3		

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DOSE-EFFECT STUDIES WITH INHALED PU-238 OXIDE IN BEAGLES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/79	DEATH	
D-1 LOWEST	1069	F	2	0.02	0.24	8.5	18.1	05/31/73		76.0		
D-1 LOWEST	1095	F	2	0.02	0.19	10.5	16.6	05/31/73		76.0		
D-1 LOWEST	921	F	3	0.03	0.31	10.0	19.5	11/30/72	12/27/72		0.9	Sacrificed
D-1 LOWEST	923	F	3	0.03	0.35	8.5	19.5	11/30/72	01/26/73		1.9	Sacrificed
D-1 LOWEST	989	F	3	0.03	0.32	9.5	18.8	12/19/72		81.3		
D-1 LOWEST	925	M	5	0.04	0.40	12.5	19.5	11/30/72	02/27/73		2.9	Sacrificed
D-1 LOWEST	1204	M	6	0.04	0.43	14.0	17.7	02/26/74		67.1		
D-1 LOWEST	970	F	6	0.05	0.55	11.0	19.2	12/19/72	01/04/77		48.5	Sacrificed
D-1 LOWEST	993	F	6	0.05	0.50	12.0	18.8	12/19/72		81.3		
D-1 LOWEST	1106	F	5	0.05	0.50	10.0	16.4	05/31/73		76.0		
D-2 LOW	1065	F	6	0.05	0.60	10.0	18.3	05/31/73		76.0		
D-2 LOW	1082	M	11	0.06	0.69	16.0	13.0	05/31/73		76.0		
D-2 LOW	1188	M	11	0.06	0.71	15.5	18.4	02/26/74		67.1		
D-2 LOW	1084	M	13	0.07	0.74	17.5	17.5	05/31/73		76.0		
D-2 LOW	1090	F	10	0.08	0.83	12.0	17.3	05/31/73		76.0		
D-2 LOW	1222	M	15	0.10	1.11	13.5	19.0	04/18/74		65.4		
D-2 LOW	971	F	13	0.11	1.24	10.5	19.2	12/19/72		81.3		
D-2 LOW	999	F	11	0.11	1.16	9.5	18.7	12/19/72		81.3		
D-2 LOW	1229	M	16	0.11	1.23	13.0	16.8	02/26/74		67.1		
D-2 LOW	1070	M	22	0.12	1.33	16.5	18.1	05/31/73		76.0		
D-2 LOW	1214	M	17	0.12	1.36	12.5	19.3	04/18/74	05/12/75		12.8	Sacrificed
D-2 LOW	955	M	17	0.14	1.55	11.0	19.2	12/19/72		81.3		
D-2 LOW	1033	M	17	0.14	1.55	11.0	19.1	02/22/73		79.2		
D-2 LOW	1036	F	16	0.14	1.52	10.5	18.2	02/22/73		79.2		
D-2 LOW	1216	M	23	0.16	1.77	13.0	19.3	04/18/74		65.4		
D-2 LOW	1060	F	22	0.18	2.00	11.0	17.8	02/22/73		79.2		
D-2 LOW	981	M	30	0.21	2.31	13.0	19.0	12/19/72		81.3		
D-2 LOW	1046	M	27	0.22	2.45	11.0	18.1	02/22/73		79.2		
D-2 LOW	1050	F	22	0.22	2.44	9.0	18.1	02/22/73		79.2		
D-2 LOW	1078	F	29	0.22	2.42	12.0	18.0	05/31/73		76.0		
D-2 LOW	1207	F	22	0.24	2.59	8.5	17.6	02/26/74		67.1		
D-2 LOW	1196	F	23	0.25	2.80	10.0	17.9	02/26/74		67.1		
D-2 LOW	1189	M	38	0.26	2.81	13.5	20.0	04/18/74	04/25/79		60.2	Sacrificed
D-2 LOW	930	M	38	0.27	2.92	13.0	19.2	11/30/72	12/28/72		0.9	Sacrificed
D-3 MED-LOW	1066	M	54	0.31	3.38	16.0	18.3	05/31/73		76.0		
D-3 MED-LOW	1089	F	41	0.31	3.42	12.0	17.3	05/31/73		76.0		
D-3 MED-LOW	972	F	40	0.33	3.64	11.0	19.2	12/19/72		81.3		
D-3 MED-LOW	1310	M	54	0.34	3.72	14.5	18.5	03/04/75	04/01/77		24.9	Sacrificed
D-3 MED-LOW	1312	M	58	0.34	3.74	15.5	18.5	03/04/75	03/26/79		48.7	Sacrificed
D-3 MED-LOW	1311	M	54	0.36	4.00	13.5	18.5	03/04/75	04/03/78		37.0	Sacrificed
D-3 MED-LOW	1219	F	46	0.40	4.38	10.5	19.0	04/18/74		65.4		
D-3 MED-LOW	1317	M	72	0.41	4.50	16.0	18.1	03/04/75	04/01/77		24.9	Sacrificed
D-3 MED-LOW	1158	M	73	0.43	4.71	15.5	17.7	11/06/73		70.8		

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DOSE-EFFECT STUDIES WITH INHALED PU-238 OXIDE IN BEAGLES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/79	DEATH	
D-3 MED-LOW	1165	M	76	0.43	4.75	16.0	17.3	11/06/73		70.8		
D-3 MED-LOW	1309	M	60	0.44	4.80	12.5	18.5	03/04/75		54.9		
D-3 MED-LOW	1318	M	67	0.45	4.96	13.5	18.1	03/04/75	03/06/76		12.2	Sacrificed
D-3 MED-LOW	929	F	41	0.50	5.47	7.5	19.2	11/30/72	01/25/73		1.8	Sacrificed
D-3 MED-LOW	1316	M	84	0.53	5.79	14.5	18.1	03/04/75		54.9		
D-3 MED-LOW	960	M	68	0.54	5.91	11.5	19.2	12/19/72		81.3		
D-3 MED-LOW	1072	M	98	0.54	5.94	16.5	18.1	05/31/73		76.0		
D-3 MED-LOW	1190	F	71	0.54	5.92	12.0	18.1	02/26/74		67.1		
D-3 MED-LOW	926	M	75	0.55	6.00	12.5	19.5	11/30/72	02/28/73		3.0	Sacrificed
D-3 MED-LOW	1315	M	90	0.55	6.00	15.0	18.1	03/04/75	03/31/77		24.9	Sacrificed
D-3 MED-LOW	982	M	76	0.58	6.33	12.0	19.0	12/19/72		81.3		
D-3 MED-LOW	1040	M	84	0.61	6.72	12.5	18.2	02/22/73		79.2		
D-3 MED-LOW	1059	F	71	0.65	7.10	10.0	17.8	02/22/73		79.2		
D-3 MED-LOW	1319	M	99	0.67	7.33	13.5	18.1	03/04/75	03/09/76		12.2	Sacrificed
D-3 MED-LOW	1108	F	84	0.69	7.64	11.0	16.4	05/31/73		76.0		
D-3 MED-LOW	1000	F	70	0.71	7.78	9.0	18.7	12/19/72		81.3		
D-3 MED-LOW	1056	M	97	0.71	7.76	12.5	17.9	02/22/73		79.2		
D-3 MED-LOW	1004	M	116	0.73	8.00	14.5	19.6	01/18/73		80.4		
D-3 MED-LOW	1026	M	116	0.78	8.59	13.5	19.2	01/18/73		80.4		
D-3 MED-LOW	1043	F	98	0.89	9.80	10.0	18.1	02/22/73		79.2		
D-3 MED-LOW	1031	F	76	0.92	10.13	7.5	19.1	02/22/73		79.2		
D-3 MED-LOW	1212	F	111	1.12	12.33	9.0	17.6	02/26/74		67.1		
D-4 MEDIUM	1176	M	129	0.87	9.56	13.5	16.6	11/06/73		70.8		
D-4 MEDIUM	1221	F	124	1.13	12.40	10.0	19.0	04/18/74		65.4		
D-4 MEDIUM	1195	M	228	1.38	15.20	15.0	18.1	02/26/74		67.1		
D-4 MEDIUM	1032	M	162	1.40	15.43	10.5	16.3	11/30/72	12/08/72		0.3	Sacrificed
D-4 MEDIUM	1053	F	140	1.42	15.58	9.5	17.9	02/22/73		79.2		
D-4 MEDIUM	997	M	203	1.60	17.65	11.5	19.6	01/18/73		80.4		
D-4 MEDIUM	991	F	194	1.76	19.40	10.0	18.8	12/19/72		81.3		
D-4 MEDIUM	1177	M	262	1.76	19.41	13.5	16.6	11/06/73		70.8		
D-4 MEDIUM	932	F	216	1.79	19.64	11.0	19.1	11/30/72	01/25/73		1.8	Sacrificed
D-4 MEDIUM	1103	F	260	1.89	20.80	12.5	16.5	05/31/73		76.0		
D-4 MEDIUM	973	F	271	2.24	24.64	11.0	19.2	12/19/72		81.3		
D-4 MEDIUM	931	F	289	2.39	26.27	11.0	19.1	11/30/72	12/28/72		0.9	Sacrificed
D-4 MEDIUM	1091	F	243	2.60	28.59	8.5	17.3	05/31/73		76.0		
D-4 MEDIUM	1114	M	430	2.70	29.66	14.5	16.4	05/31/73		76.0		
D-4 MEDIUM	1062	M	435	2.93	32.22	13.5	17.8	02/22/73		79.2		
D-4 MEDIUM	934	M	454	3.06	33.63	13.5	19.1	11/30/72	03/01/73		3.0	Sacrificed
D-4 MEDIUM	1081	M	541	3.07	33.81	16.0	18.0	05/31/73		76.0		
D-4 MEDIUM	1030	F	340	3.25	35.79	9.5	19.1	02/22/73		79.2		
D-4 MEDIUM	952	F	365	3.49	38.42	9.5	19.2	12/19/72		81.3		
D-4 MEDIUM	1198	M	539	3.50	38.50	14.0	17.9	02/26/74		67.1		
D-4 MEDIUM	1166	M	673	4.08	44.87	15.0	17.3	11/06/73		70.8		

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DOSE-EFFECT STUDIES WITH INHALED PU-238 OXIDE IN BEAGLES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/79	DEATH	
D-4 MEDIUM	1220	F	518	4.28	47.09	11.0	19.0	04/18/74		65.4		
D-4 MEDIUM	992	F	555	4.39	48.26	11.5	18.8	12/19/72		81.3		
D-4 MEDIUM	983	M	617	4.67	51.42	12.0	19.0	12/19/72		81.3		
D-5 MED-HIGH	1191	F	591	4.48	49.25	12.0	19.8	04/18/74	03/21/77		35.1	Interstitial Pneumonitis
D-5 MED-HIGH	1157	M	700	4.71	51.85	13.5	17.7	11/06/73		70.8		
D-5 MED-HIGH	1035	F	571	5.46	60.11	9.5	18.2	02/22/73		79.2		
D-5 MED-HIGH	1192	F	754	6.23	68.55	11.0	18.1	02/26/74		67.1		
D-5 MED-HIGH	1140	M	1014	6.58	72.43	14.0	18.2	11/06/73		70.8		
D-5 MED-HIGH	1071	M	1269	6.79	74.65	17.0	18.1	05/31/73		76.0		
D-5 MED-HIGH	1173	M	1023	7.75	85.25	12.0	17.3	11/06/73		70.8		
D-5 MED-HIGH	1178	M	1125	8.52	93.75	12.0	16.6	11/06/73		70.8		
D-5 MED-HIGH	1047	M	900	8.61	94.74	9.5	18.1	02/22/73		79.2		
D-5 MED-HIGH	1109	F	1119	8.85	97.30	11.5	16.4	05/31/73		76.0		
D-5 MED-HIGH	1160	F	1344	10.18	112.00	12.0	17.3	11/06/73		70.8		
D-5 MED-HIGH	1211	M	1764	11.06	121.66	14.5	17.6	02/26/74		67.1		
D-5 MED-HIGH	1096	F	1476	12.20	134.18	11.0	16.6	05/31/73	05/08/78		59.2	Addison's Disease
D-5 MED-HIGH	1218	F	1710	12.95	142.50	12.0	17.3	02/26/74		67.1		
D-5 MED-HIGH	1092	M	1848	13.44	147.84	12.5	17.3	05/31/73	10/23/78		64.8	Bone Tumor
D-5 MED-HIGH	1027	M	2148	13.95	153.43	14.0	19.2	01/18/73	12/01/78		70.4	Bone Tumor, Lung Tumor
D-5 MED-HIGH	1115	F	1885	14.90	163.91	11.5	16.1	05/31/73	07/11/78		61.3	Bone Tumor
D-5 MED-HIGH	974	F	1718	15.62	171.80	10.0	20.2	01/18/73	05/24/78		64.1	Bone Tumor
D-5 MED-HIGH	1079	M	2620	15.80	174.67	15.0	18.0	05/31/73	02/12/78		56.4	Addison's, G.I. Tumor
D-5 MED-HIGH	1058	F	1907	16.51	181.62	10.5	17.8	02/22/73		79.2		
D-6 HIGH	1002	M	2907	18.88	207.64	14.0	19.6	01/18/73		80.4		
D-6 HIGH	1057	M	3116	20.98	230.81	13.5	17.9	02/22/73	03/07/79		72.4	Bone Tumor
D-6 HIGH	1009	M	3630	26.40	290.40	12.5	19.6	01/18/73	04/01/78		62.4	Lung Tmr, Osteoarthropathy
D-6 HIGH	1042	F	2959	28.32	311.47	9.5	18.1	02/22/73	11/10/78		68.6	Bone Tumor, Lung Tumor
D-6 HIGH	994	F	3453	31.39	345.30	10.0	19.6	01/18/73	07/04/76		41.5	Addison's Disease
D-6 HIGH	1006	F	3810	31.49	346.36	11.0	19.6	01/18/73	01/18/79		72.0	Bone Tumor, Lung Tumor
D-6 HIGH	975	F	3968	36.07	396.80	10.0	20.2	01/18/73	07/25/78		66.2	Bone Tumor, Lung Tumor
D-6 HIGH	1037	M	4854	44.13	485.40	10.0	18.2	02/22/73	11/21/78		68.9	Bone Tumor
D-6 HIGH	1143	M	7691	53.78	591.62	13.0	18.2	11/06/73	12/05/77		49.0	Bone Tumor, Lung Tumor
D-6 HIGH	1025	M	8479	57.10	628.07	13.5	19.2	01/18/73	03/17/77		49.9	Lung Tumor
D-6 HIGH	1064	M	9453	63.66	700.22	13.5	16.7	01/18/73	04/14/77		50.8	Bone Tumor, Lung Tumor
D-6 HIGH	1162	F	6959	70.29	773.22	9.0	17.3	11/06/73	12/19/78		61.4	Bone Tumor, Addison's
D-6 HIGH	1175	F	6201	75.16	826.80	7.5	16.6	11/06/73	02/24/78		51.6	Lung Tumor

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LOW LEVEL PU-239 NITRATE INHALATION STUDIES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/79	DEATH	
CONTROL	1356	M	0	0.00	0.00						64.7*	
CONTROL	1365	M	0	0.00	0.00						64.6*	
CONTROL	1376	F	0	0.00	0.00						63.4*	
CONTROL	1388	M	0	0.00	0.00						63.3*	
CONTROL	1393	M	0	0.00	0.00						63.3*	
CONTROL	1405	M	0	0.00	0.00						62.9*	
CONTROL	1409	M	0	0.00	0.00						62.8*	
CONTROL	1418	M	0	0.00	0.00						62.5*	
CONTROL	1425	M	0	0.00	0.00						62.5*	
CONTROL	1450	F	0	0.00	0.00						62.3*	
CONTROL	1455	F	0	0.00	0.00						61.8*	
CONTROL	1483	F	0	0.00	0.00						60.9*	
CONTROL	1509	M	0	0.00	0.00						60.1*	
CONTROL	1516	F	0	0.00	0.00						59.8*	
CONTROL	1525	M	0	0.00	0.00						59.6*	
CONTROL	1526	M	0	0.00	0.00						59.6*	
CONTROL	1528	F	0	0.00	0.00						59.0*	
CONTROL	1543	M	0	0.00	0.00						58.9*	
CONTROL	1563	F	0	0.00	0.00						48.8*	
CONTROL	1572	F	0	0.00	0.00						48.7*	
CONTROL	1577	M	0	0.00	0.00						48.7*	
CONTROL	1584	F	0	0.00	0.00						48.6*	
CONTROL	1594	F	0	0.00	0.00						48.6*	
CONTROL	1608	M	0	0.00	0.00						48.3*	
CONTROL	1633	F	0	0.00	0.00						41.6*	
CONTROL	1638	F	0	0.00	0.00						41.3*	
VEHICLE	1361	M	0	0.00	0.00	15.5	21.0	02/13/76			43.5	
VEHICLE	1381	F	0	0.00	0.00	8.5	19.8	02/13/76			43.5	
VEHICLE	1392	M	0	0.00	0.00	13.0	22.0	04/22/76			41.3	
VEHICLE	1406	M	0	0.00	0.00	13.5	21.6	04/22/76			41.3	
VEHICLE	1412	F	0	0.00	0.00	9.0	19.0	02/13/76			43.5	
VEHICLE	1421	M	0	0.00	0.00	13.0	23.3	06/23/76			39.2	
VEHICLE	1457	F	0	0.00	0.00	12.0	20.6	04/22/76			41.3	
VEHICLE	1491	F	0	0.00	0.00	8.5	21.6	06/23/76			39.2	
VEHICLE	1504	F	0	0.00	0.00	10.0	20.9	06/23/76			39.2	
VEHICLE	1514	M	0	0.00	0.00	14.0	20.9	06/23/76			39.2	
VEHICLE	1524	M	0	0.00	0.00	12.0	21.5	07/27/76			38.1	
VEHICLE	1531	F	0	0.00	0.00	9.0	20.9	07/27/76			38.1	
VEHICLE	1542	M	0	0.00	0.00	12.0	20.8	07/27/76			38.1	
VEHICLE	1566	M	0	0.00	0.00	14.0	18.3	03/15/77			30.5	
VEHICLE	1578	M	0	0.00	0.00	10.5	18.2	03/15/77			30.5	
VEHICLE	1593	F	0	0.00	0.00	11.0	18.0	03/15/77			30.5	
VEHICLE	1601	F	0	0.00	0.00	8.5	18.0	03/15/77			30.5	

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LOW LEVEL PU-239 NITRATE INHALATION STUDIES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/79	DEATH	
VEHICLE	1620	M	0	0.00	0.00	13.0	21.1	12/01/77		21.9		
VEHICLE	1634	F	0	0.00	0.00	10.5	19.6	12/01/77		21.9		
VEHICLE	1651	F	0	0.00	0.00	11.0	19.2	12/01/77		21.9		
D-1 LOWEST	1416	M	0	0.00	0.00	12.0	22.1	05/20/76		40.3		
D-1 LOWEST	1458	F	0	0.00	0.00	10.5	21.5	05/20/76		40.3		
D-1 LOWEST	1489	F	0	0.00	0.00	8.5	20.5	05/20/76		40.3		
D-1 LOWEST	1501	M	0	0.00	0.00	14.0	20.4	05/20/76		40.3		
D-1 LOWEST	1515	M	0	0.00	0.00	13.5	19.8	05/20/76		40.3		
D-1 LOWEST	1573	M	0	0.00	0.00	11.5	19.4	04/19/77		29.4		
D-1 LOWEST	1581	M	0	0.00	0.00	16.5	19.3	04/19/77		29.4		
D-1 LOWEST	1596	M	0	0.00	0.00	14.0	19.2	04/19/77		29.4		
D-1 LOWEST	1600	F	1	0.01	0.11	11.0	19.2	04/19/77		29.4		
D-1 LOWEST	1603	M	2	0.01	0.12	14.0	19.2	04/19/77		29.4		
D-1 LOWEST	1339	F	2	0.02	0.22	9.0	17.5	10/16/75	11/13/75	0.9	Sacrificed	
D-1 LOWEST	1519	M	2	0.02	0.18	12.5	19.5	05/20/76		40.3		
D-1 LOWEST	1570	F	2	0.02	0.17	10.5	19.4	04/19/77		29.4		
D-1 LOWEST	1465	F	4	0.03	0.33	12.5	21.0	05/20/76		40.3		
D-1 LOWEST	1470	F	3	0.03	0.29	10.5	21.0	05/20/76		40.3		
D-1 LOWEST	1507	M	4	0.03	0.32	14.0	19.8	05/20/76		40.3		
D-1 LOWEST	1592	F	4	0.03	0.29	13.5	19.2	04/19/77		29.4		
D-1 LOWEST	1607	M	5	0.03	0.35	13.0	19.0	04/19/77		29.4		
D-1 LOWEST	1335	M	5	0.04	0.42	11.5	18.0	10/16/75	11/13/75	0.9	Sacrificed	
D-1 LOWEST	1487	F	6	0.04	0.46	13.0	20.5	05/20/76		40.3		
D-1 LOWEST	1583	F	4	0.04	0.40	9.5	19.2	04/19/77		29.4		
D-1 LOWEST	1351	M	7	0.06	0.61	11.0	17.2	10/16/75	11/13/75	0.9	Sacrificed	
D-1 LOWEST	1565	F	8	0.06	0.67	11.5	19.4	04/19/77		29.4		
D-2 LOW	1513	M	0	0.00	0.00	11.5	19.8	05/20/76		40.3		
D-2 LOW	1520	M	1	0.01	0.12	10.5	19.5	05/20/76		40.3		
D-2 LOW	1415	M	2	0.02	0.20	11.5	22.2	05/20/76		40.3		
D-2 LOW	1575	M	3	0.02	0.19	14.0	19.4	04/19/77		29.4		
D-2 LOW	1466	F	5	0.03	0.37	14.0	21.0	05/20/76		40.3		
D-2 LOW	1606	F	5	0.04	0.42	12.5	19.0	04/19/77		29.4		
D-2 LOW	1579	M	8	0.05	0.59	14.0	19.3	04/19/77		29.4		
D-2 LOW	1590	F	6	0.05	0.51	12.0	19.2	04/19/77		29.4		
D-2 LOW	1585	F	8	0.06	0.68	12.0	19.2	04/19/77		29.4		
D-2 LOW	1580	F	9	0.07	0.82	11.0	19.3	04/19/77		29.4		
D-2 LOW	1591	M	11	0.07	0.76	15.0	19.2	04/19/77		29.4		
D-2 LOW	1417	M	11	0.08	0.89	12.0	22.1	05/20/76		40.3		
D-2 LOW	1423	M	10	0.08	0.87	11.0	22.1	05/20/76		40.3		
D-2 LOW	1567	M	10	0.08	0.83	12.0	19.4	04/19/77		29.4		
D-2 LOW	1472	F	10	0.09	1.01	10.0	21.0	05/20/76		40.3		
D-2 LOW	1503	F	9	0.09	0.97	9.0	19.8	05/20/76		40.3		
D-2 LOW	1602	M	15	0.09	1.03	14.5	19.2	04/19/77		29.4		

* Indicates age in months since birth, all other ages are in months since exposure.

LOW LEVEL PU-239 NITRATE INHALATION STUDIES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/79	DEATH	
D-2 LOW	1484	F	11	0.10	1.08	10.0	20.5	05/20/76		40.3		
D-2 LOW	1599	F	10	0.10	1.14	9.0	19.2	04/19/77		29.4		
D-2 LOW	1490	F	16	0.15	1.65	9.5	20.5	05/20/76		40.3		
D-3 MED-LOW	1336	M	21	0.14	1.52	13.5	18.0	10/16/75	11/13/75		0.9	Sacrificed
D-3 MED-LOW	1341	F	19	0.16	1.78	10.5	17.2	10/16/75	11/13/75		0.9	Sacrificed
D-3 MED-LOW	1605	F	25	0.20	2.19	11.5	17.8	03/15/77		30.5		
D-3 MED-LOW	1386	M	34	0.21	2.36	14.5	22.0	04/20/76		41.3		
D-3 MED-LOW	1389	M	27	0.23	2.54	10.5	21.9	04/20/76	05/04/76		0.5	Sacrificed
D-3 MED-LOW	1413	F	29	0.24	2.68	11.0	18.2	01/20/78		44.3		
D-3 MED-LOW	1445	F	34	0.24	2.60	13.0	21.0	04/20/76	05/05/76		0.5	Sacrificed
D-3 MED-LOW	1568	M	46	0.29	3.17	14.5	18.3	03/15/77		30.5		
D-3 MED-LOW	1390	M	43	0.30	3.29	13.0	21.9	04/20/76	05/04/76		0.5	Sacrificed
D-3 MED-LOW	1391	M	54	0.30	3.26	16.5	21.9	04/20/76		41.3		
D-3 MED-LOW	1507	M	53	0.31	3.40	15.5	18.1	03/15/77		30.5		
D-3 MED-LOW	1595	M	50	0.31	3.45	14.5	18.0	03/15/77		30.5		
D-3 MED-LOW	1359	M	50	0.32	3.57	14.0	20.2	01/20/76	01/23/76		0.1	Sacrificed
D-3 MED-LOW	1540	M	54	0.32	3.51	15.5	20.7	07/22/76		38.3		
D-3 MED-LOW	1589	F	41	0.34	3.75	11.0	18.0	03/15/77		30.5		
D-3 MED-LOW	1588	M	50	0.36	3.98	12.5	18.1	03/15/77	03/22/78		12.2	Sacrificed
D-3 MED-LOW	1529	F	43	0.37	4.00	10.5	20.8	07/22/76	10/19/76		2.9	Sacrificed
D-3 MED-LOW	1344	F	41	0.38	4.14	10.0	17.2	10/16/75	11/14/75		1.0	Sacrificed
D-3 MED-LOW	1574	M	46	0.38	4.21	11.0	18.2	03/15/77		30.5		
D-3 MED-LOW	1375	F	50	0.40	4.35	11.5	19.1	01/20/76	01/23/76		0.1	Sacrificed
D-3 MED-LOW	1564	F	40	0.40	4.44	9.0	18.3	03/15/77	03/20/78		12.2	Sacrificed
D-3 MED-LOW	1444	F	49	0.41	4.50	11.0	21.0	04/20/76		41.3		
D-3 MED-LOW	1439	F	53	0.42	4.61	11.5	21.0	04/20/76		41.3		
D-3 MED-LOW	1523	F	55	0.42	4.60	12.0	21.3	07/22/76		38.3		
D-3 MED-LOW	1539	M	65	0.45	4.99	13.0	20.7	07/22/76	10/20/76		3.0	Sacrificed
D-3 MED-LOW	1380	M	63	0.46	5.06	12.5	19.1	01/20/76		44.3		
D-3 MED-LOW	1407	F	50	0.51	5.56	9.0	18.5	01/20/76	01/23/76		0.1	Sacrificed
D-3 MED-LOW	1569	F	58	0.53	5.82	10.0	18.2	03/15/77		30.5		
D-3 MED-LOW	1576	M	70	0.53	5.86	12.0	18.2	03/15/77		30.5		
D-3 MED-LOW	1582	F	57	0.54	5.96	9.5	18.1	03/15/77		30.5		
D-3 MED-LOW	1571	F	68	0.57	6.22	11.0	18.2	03/15/77	03/21/78		12.2	Sacrificed
D-3 MED-LOW	1427	F	68	0.62	6.81	10.0	21.1	04/20/76		41.3		
D-3 MED-LOW	1522	F	78	0.71	7.78	10.0	21.3	07/22/76	10/18/76		2.9	Sacrificed
D-3 MED-LOW	1363	M	85	0.74	8.09	10.5	20.2	01/20/76		44.3		
D-3 MED-LOW	1604	M	85	0.74	8.10	10.5	18.0	03/15/77		30.5		
D-3 MED-LOW	1530	F	72	0.76	8.41	8.5	20.8	07/22/76		38.3		
D-3 MED-LOW	1456	F	61	0.79	8.68	7.0	20.5	04/20/76		41.3		
D-3 MED-LOW	1598	F	93	1.06	11.65	8.0	18.0	03/15/77		30.5		
D-3 MED-LOW	1422	F	99	1.12	12.35	8.0	18.1	01/20/76		44.3		
D-4 MEDIUM	1637	M	192	1.45	15.99	12.0	18.9	11/07/77		22.7		

* Indicates age in months since birth, all other ages are in months since exposure.

LOW LEVEL PU-239 NITRATE INHALATION STUDIES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/79	DEATH	
D-4 MEDIUM	1404	M	260	1.48	16.25	16.0	21.5	04/20/76			41.3	
D-4 MEDIUM	1521	F	205	1.49	16.37	12.5	21.3	07/22/76			38.3	
D-4 MEDIUM	1656	M	211	1.54	16.90	12.5	18.4	11/07/77			22.7	
D-4 MEDIUM	1379	M	278	1.74	19.16	14.5	19.1	01/20/76			44.3	
D-4 MEDIUM	1362	M	267	1.87	26.54	13.0	20.2	01/20/76			44.3	
D-4 MEDIUM	1645	F	257	2.03	22.37	11.5	18.5	11/07/77			22.7	
D-4 MEDIUM	1639	F	248	2.05	22.57	11.0	18.5	11/07/77			22.7	
D-4 MEDIUM	1647	M	294	2.05	22.58	13.0	18.5	11/07/77			22.7	
D-4 MEDIUM	1640	M	307	2.06	22.71	13.5	19.0	11/22/77			22.2	
D-4 MEDIUM	1534	M	295	2.14	23.57	12.5	20.8	07/22/76			38.3	
D-4 MEDIUM	1414	F	233	2.35	25.86	9.0	18.2	01/20/76			44.3	
D-4 MEDIUM	1618	F	277	2.40	26.36	10.5	20.3	11/07/77			22.7	
D-4 MEDIUM	1385	M	373	2.42	26.63	14.0	19.0	01/20/76			44.3	
D-4 MEDIUM	1408	F	331	2.62	28.77	11.5	18.5	01/20/76			44.3	
D-4 MEDIUM	1428	F	378	3.12	34.36	11.0	21.1	04/20/76			41.3	
D-4 MEDIUM	1535	F	345	3.13	34.48	10.0	20.7	07/22/76			38.3	
D-4 MEDIUM	1446	F	354	3.22	35.40	10.0	21.0	04/20/76			41.3	
D-4 MEDIUM	1364	M	463	3.24	35.65	13.0	20.2	01/20/76			44.3	
D-4 MEDIUM	1387	F	345	4.43	49.30	7.0	19.0	01/20/76			44.3	
D-5 MED-HIGH	1329	F	363	3.30	36.27	10.0	18.0	10/16/75	11/14/75	1.0	Sacrificed	
D-5 MED-HIGH	1346	M	656	4.42	48.59	13.5	17.2	10/16/75	11/14/75	1.0	Sacrificed	
D-5 MED-HIGH	1648	M	811	5.90	64.90	12.5	18.5	11/07/77			22.7	
D-5 MED-HIGH	1659	F	990	6.93	76.18	13.0	18.3	11/07/77			22.7	
D-5 MED-HIGH	1347	F	688	6.95	76.47	9.0	17.2	10/16/75	11/14/75	1.0	Sacrificed	
D-5 MED-HIGH	1636	M	1212	8.48	93.25	13.0	18.9	11/07/77			22.7	
D-5 MED-HIGH	1621	M	1334	8.66	95.26	14.0	20.3	11/07/77			22.7	
D-5 MED-HIGH	1646	F	1061	8.77	96.45	11.0	18.5	11/07/77			22.7	
D-5 MED-HIGH	1429	M	1376	9.62	105.85	13.0	23.2	06/23/76			39.2	
D-5 MED-HIGH	1641	M	1275	9.66	106.24	12.0	18.5	11/07/77			22.7	
D-5 MED-HIGH	1660	M	1518	10.22	112.41	13.5	18.3	11/07/77			22.7	
D-5 MED-HIGH	1508	M	1716	10.76	118.37	14.5	20.9	06/23/76			39.2	
D-5 MED-HIGH	1655	M	1094	11.05	121.56	9.0	18.4	11/07/77			22.7	
D-5 MED-HIGH	1652	F	1320	12.00	131.95	10.0	18.4	11/07/77			22.7	
D-5 MED-HIGH	1619	F	1490	12.32	135.50	11.0	20.3	11/07/77			22.7	
D-5 MED-HIGH	1512	M	2411	14.61	160.71	15.0	20.9	06/23/76			39.2	
D-5 MED-HIGH	1419	M	1559	14.92	164.11	9.5	23.3	06/23/76			39.2	
D-5 MED-HIGH	1498	F	2013	16.68	183.45	11.0	21.5	06/23/76			39.2	
D-5 MED-HIGH	1502	F	3008	20.25	222.80	13.5	20.9	06/23/76			39.2	
D-5 MED-HIGH	1485	F	2330	21.18	233.00	10.0	21.7	06/23/76			39.2	
D-5 MED-HIGH	1471	F	2508	21.71	238.82	10.5	22.1	06/23/76	05/01/79	34.2	Radiation Pneumonitis	
D-5 MED-HIGH	1492	F	2473	24.98	274.82	9.0	21.6	06/23/76			39.2	
D-5 MED-HIGH	1459	F	2645	26.72	293.89	9.0	22.6	06/23/76			39.2	
D-6 HIGH	1518	M	3565	29.46	324.09	11.0	20.6	06/23/76			39.2	

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LOW LEVEL PU-239 NITRATE INHALATION STUDIES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/79	DEATH	
D-6 HIGH	1420	M	3840	30.36	333.91	11.5	23.3	06/23/76	07/12/78		24.6	Radiation Pneumonitis
D-6 HIGH	1517	F	5185	49.62	545.79	9.5	20.6	06/23/76	11/02/77		16.3	Radiation Pneumonitis
D-6 HIGH	1510	F	6969	55.09	606.02	11.5	20.9	06/23/76	11/09/77		16.6	Radiation Pneumonitis
D-6 HIGH	1424	M	7681	69.83	768.12	10.0	23.2	06/23/76	08/31/77		14.3	Radiation Pneumonitis

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LOW LEVEL PU-238 NITRATE INHALATION STUDIES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
			NCI	NCI/G LUNG	NCI/ KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/79	DEATH	
CONTROL	1548	M	0	0.00	0.00				12/27/77			31.3* Sacrificed
CONTROL	1555	M	0	0.00	0.00				12/20/77			30.9* Sacrificed
D-3 MED-LOW	1552	M	49	0.30	3.27	15.0	18.5	12/07/76	01/05/77			1.0 Sacrificed
D-3 MED-LOW	1553	F	44	0.35	3.83	11.5	18.5	12/07/76	01/04/77			0.9 Sacrificed
D-3 MED-LOW	1557	F	42	0.35	3.82	11.0	18.4	12/07/76	03/22/77			3.4 Sacrificed
D-3 MED-LOW	1550	F	57	0.40	4.33	13.0	18.5	12/07/76	12/14/77			12.2 Sacrificed
D-3 MED-LOW	1545	M	59	0.41	4.54	13.0	18.7	12/07/76	01/05/77			1.0 Sacrificed
D-3 MED-LOW	1546	M	70	0.41	4.52	15.5	18.7	12/07/76	03/22/77			3.4 Sacrificed
D-3 MED-LOW	1549	F	62	0.45	4.96	12.5	18.5	12/07/76	12/10/76			0.1 Sacrificed
D-3 MED-LOW	1554	M	73	0.51	5.62	13.0	18.5	12/07/76	12/10/76			0.1 Sacrificed
D-3 MED-LOW	1551	M	35	0.53	5.86	14.5	18.6	12/07/76	03/22/77			3.4 Sacrificed
D-3 MED-LOW	1547	M	75	0.57	6.25	12.0	18.7	12/07/76	12/15/77			12.3 Sacrificed
D-3 MED-LOW	1558	F	72	0.60	6.55	11.0	18.4	12/07/76	12/16/77			12.3 Sacrificed
D-3 MED-LOW	1544	F	107	0.88	9.73	11.0	18.7	12/07/76	12/10/76			0.1 Sacrificed

* Indicates age in months since birth, all other ages are in months since exposure.

Publications
and
Presentations

PUBLICATIONS

Reprints may be requested from the author at the address shown below^(a), except for those articles marked with an asterisk (*), which are no longer available.

Allen, M. D., J. K. Briant and O. R. Moss. 1979. Comparison of the aerodynamic size distribution of chain-like aggregates measured with a cascade impactor and a spiral centrifuge. Am. Ind. Hyg. Assoc. J. 40: 474-481.

Allen, M. D., O. R. Moss and J. K. Briant. 1978. Dynamic shape factors for LMFBR mixed-oxide fuel aggregates. J. Aerosol Sci. 10: 43-48.

Andrew, F. D. and D. D. Mahlum. 1979. Developmental toxicity of solvent refined coal-related hydrocarbons. Tox. Appl. Pharmacol. 48(Part 2): A-27.

Andrew, F. D. and M. R. Sikov. 1979. Studies on the transplacental carcinogenicity of ^{239}Pu plutonium in rats. Teratology 19(2): 17A.

Andrews, T. K. and M. E. Frazier. 1979. Characterization of cell cultures derived from ^{239}Pu -induced tumors in beagles. In Vitro 15: 196.

Bair, W. J. 1979. ICRP Publication 31, "Report of ICRP Task Group on Biological Effects of Inhaled Radionuclides."

Bair, W. J., J. W. Healy and B. W. Wachholz. 1979. The Enewetak Atoll Today. (R. W. Baalman, Ed.) Written for the U. S. Department of Energy by Pacific Northwest Laboratory, Richland, WA 99352.

Bair, W. J. 1979. Comparison of acute mortality in baboons and dogs after inhalation of $^{239}\text{PuO}_2$, p. 903-912. In: Proceedings of the 6th International Congress of Radiation Research (F. Okado, M. Imamura, T. Terashima, and H. Yamaguchi, Eds.), Dept. of Radiation Biophysics, Faculty of Medicine, University of Tokyo, Japan.

Ballou, J. E., G. E. Dagle, R. A. Gies and L. G. Smith. 1979. Late effects of inhaled $^{253}\text{Es}(\text{NO}_3)_3$ in the rat. Health Phys. 37: 301.

Burton, F. G., R. E. Schirmer, D. D. Mahlum and F. D. Andrew. 1979. Disposition of process solvent from solvent refined coal in tissues of the rat after oral dosing. Toxicol. Appl. Pharmacol. 48: A27.

*Causey, S. C. and F. P. Hungate. 1979. Studies of mutagenic effects of magnetic fields in bacteria, p. 487. In: Bioelectromagnetic Symposium June 18-22, 1979, Seattle, WA, sponsored by USNC/URSI. Abstracts only available from USNC/URSI, National Academy of Sciences, Washington, D.C. 20418. (Abstract)

Craig, D. K., J. F. Park, G. J. Powers and D. L. Catt. 1979. The disposition of americium-241 oxide following inhalation by beagles. Radiat. Res. 78: 455-473.

Cross, F. T. 1978. Study of the Combined Effects of Smoking and Inhalation of Uranium Ore Dust, Radon Daughters and Diesel Oil Exhaust Fumes in Hamsters and Dogs, PNL-2744, Battelle, Pacific Northwest Laboratory, Richland, WA.

Dagle, G. E., G. M. Zwicker and R. A. Renne. 1979. Morphology of spontaneous brain tumors in the rat. Vet. Pathol. 16: 318-324.

Dagle, G. E., G. M. Zwicker, R. R. Adee and J. F. Park. 1979. Cytoplasmic inclusions in urinary bladder epithelium of dogs. Vet. Pathol. 16: 258-259.

Decker, J. R., W. T. Kaune and M. F. Gillis. 1979. Animal housing design considerations in the dc electric field bioeffects research, pp. 5.1-5.16. In: Proceedings of the Workshop on Electrical and Biological Effects Related to HVDC Transmission (T. D. Bracken, ed.), Battelle, Pacific Northwest Laboratory, PNL-3121, Richland, WA.

Decker, J. R., J. F. Park and D. K. Craig. 1979. A method for measuring respiratory volume parameters of large animals during exposure to aerosols. Am. Ind. Hyg. Assoc. J. 40: 567-577.

Felix, W. D., H. Drucker, C. W. Gehrs, B. C. Almaula and J. Nardella. 1978. DOE environmental development program for coal gasification, pp. 381-385. In: Proceedings of Tenth Synthetic Pipeline Gas Symposium, Gas Research Institute, Chicago.

Frazier, M. E., T. K. Andrews, B. B. Thompson, M. R. Petersen and M. J. Hooper. 1979. Use of mammalian cell culture techniques for determining the toxic and carcinogenic potential of complex organic mixtures. Toxicol. Appl. Pharmacol. 48: A26.

^(a) Battelle, Pacific Northwest Laboratory
P.O. Box 999, Richland, WA 99352

- Free, M. J., R. A. Jaffe and R. A. Pelroy. 1977. Entry of circulating mutagens into the rete testis fluid of rats, pp. 105-114. In: Developmental Toxicology of Energy-Related Pollutants, Proceedings of the 17th Hanford Symposium, Richland, WA, (D. D. Mahlum, M. R. Sikov, P. L. Hackett, F. D. Andrew, Eds.) Oct 17-19, 1977 (CONF-771017). NTIS, Springfield, VA.
- *Gelman, R. A. and V. H. Smith. 1979. A sensitive assay for diethylenetriamine-pentaacetic acid in urine using paper chromatography. Fed. Proc. 38: 543. (Abstract)
- Graham, T. M. and J. E. Morris. 1978. Primary immune response in dogs exposed to $^{239}\text{PuO}_2$. Health Phys. 35: 888.
- *Hackett, P. L., J. O. Hess and M. R. Sikov. 1979. Cross-placental transfer and distribution of inhaled or ingested lead nitrate in rats. Teratology 19: 29A. (Abstract)
- Hackett, P. L., J. O. Hess and M. R. Sikov. 1977. Lead distribution and effects during development in the rat, pp. 505-519. In: Developmental Toxicology of Energy-Related Pollutants, Proceedings of the 17th Hanford Symposium, Richland, WA, (D. D. Mahlum, M. R. Sikov, P. L. Hackett, F. D. Andrew, Eds.) Oct 17-19, 1977 (CONF-771017). NTIS, Springfield, VA.
- Hadley, J. G., A. W. Conklin and C. L. Sanders. 1979. Systemic toxicity of inhaled cadmium oxide. Toxicol. Lett. 4: 107-111.
- *Hilton, D. I. and R. D. Phillips. 1979. Cardiovascular response of rats exposed to 60-Hz electric fields. Physiologist, 22(4): 56.
- *Hilton, D. I. and R. D. Phillips. 1979. Cardiovascular response of rats exposed to 60-Hz electric fields, p. 344. In: Proceedings of the Bioelectromagnetic Symposium, June 18-22, 1979, Seattle, WA, sponsored by USNC/URSI. Abstracts only available from USNC/URSI, National Academy of Sciences, Washington, D.C. 20418. (Abstract)
- Hilton, D. I., R. D. Phillips, M. J. Free, L. L. Lang, J. H. Chandon and W. T. Kaune. 1978. Physiologic response of rats to cold stress after exposure to 60-Hz electric fields. Health Phys. 35: 891.
- *Hjeresen, D. L. 1979. Effect of chronic exposure to an electric field on subsequent discrimination of electric fields, p. 483. In: Proceedings of the Bioelectromagnetic Symposium, June 18-22, 1979, Seattle, WA, sponsored by USNC/URSI. Abstracts only available from USNC/URSI, National Academy of Sciences, Washington, D.C. 20418. (Abstract)
- Kaune, W. T., J. R. Decker, R. D. Phillips and M. F. Gillis. 1978. Design of exposure for ELF electric field bioeffects. Health Phys. 35: 890.
- Kaune, W. T. and R. D. Phillips. 1979. Comparison of the coupling of grounded humans, swine and rats to vertical, 60-Hz electric fields. Physiologist 22(4): 56.
- *Killand, B. W. and S. M. Loscutoff. 1979. Measurement of esophageal pressure as an indirect measure of pleural pressure in awake guinea pigs. Fed. Proc. 38: 5806. (Abstract)
- McClanahan, B. J., W. T. Kaune and R. D. Phillips. 1978. Bone growth in electric fields. Health Phys. 35: 891.
- McDonald, K. E., J. V. Dilley, C. L. Sanders and J. A. Mahaffey. 1979. The influence of DTPA on the metabolism of inhaled $^{239}\text{PuF}_4$ in beagles. Health Phys. 36: 632-634.
- Mahlum, D. D. and F. D. Andrew. 1979. Acute and subchronic toxicity of solvent refined coal-related hydrocarbons. Toxicol. Appl. Pharmacol. 48: A25.
- Mahlum, D. D. 1977. Developmental toxicology of organic pollutants, p. 115-139. In: Developmental Toxicology of Energy-Related Pollutants, Proceedings of the 17th Hanford Symposium, Richland, WA, (D. D. Mahlum, M. R. Sikov, P. L. Hackett, F. D. Andrew, Eds.) Oct 17-19, 1977 (CONF-771017). NTIS, Springfield, VA.
- *Montgomery, L. D., L. G. Smith and M. R. Sikov. 1979. Effects of exposure to 60-Hz electric fields on growth and development in the rat, p. 396. In: Proceedings of the Bioelectromagnetic Symposium, June 18-22, 1979, Seattle, WA, Sponsored by USNC/URSI. Abstracts only available from USNC/URSI, National Academy of Sciences, Washington, D.C. 20418. (Abstract)

- Morris, J. E. 1978. The effects of electric fields on the immune system of exposed mice. Health Phys. 35: 890-891.
- Palmer, R. F., B. V. Andersen and L. E. Jenkins. 1978. A hold-up system to remove radon from inhalation study effluents. Health Phys. 35: 901.
- *Park, J. F., W. J. Bair, D. L. Catt, D. K. Craig, G. E. Dagle, R. A. Renne and H. A. Ragan. 1978. Late effects of inhaled plutonium oxide in dogs. Health Phys. 35: 888-889. (Abstract)
- *Pelroy, R. A. and M. R. Peterson. 1979. Comparative mutagenesis of solvent refined coal and shale oil samples from pilot plant operations. Toxicol. Appl. Pharmacol. 48: A26. (Abstract)
- *Phillips, R. D., J. H. Chandon, D. I. Hilton and R. L. Sheldon. 1979. Growth of rats and mice exposed to 60-Hz electric fields, p. 395. In: Proceedings of the Bioelectromagnetic Symposium, June 18-22, 1979. Seattle, WA, sponsored by USNC/URSI. Abstracts only available from USNC/URSI, National Academy of Sciences, Washington, D.C. 20418. (Abstract)
- Renne, R. A., A. J. Gandolfi, J. E. Lund, L. G. Smith, K. E. McDonald and C. A. Shields. 1979. Morphologic and biochemical effects of intratracheally administered oil shale in rats. J. Env. Path. and Toxicol. 3(2): ____.
- Rhoads, K. 1979. Dosimetry of Inhaled Plutonium-239 Dioxide in Rodent Lung - A Morphometric Study, PNL-3089. Pacific Northwest Laboratory. NTIS, Springfield, VA.
- Robinson, A. V. 1979. A rapid column chromatographic method for the isolation of catechol-type siderophores. Analyt. Biochem. 95: 364-370.
- Robinson, A. V. and R. P. Schneider. 1978. In vitro studies of actinides and alveolar macrophages. Health Phys. 35: 913-914.
- *Sanders, C. L., A. W. Conklin and J. G. Hadley. 1979. Mixed chelation therapy for removal of ^{109}Cd and ^{239}Pu . Pharmacologist 21: 210.
- Sanders, C. L. and J. A. Mahaffey. 1979. Carcinogenicity of inhaled air-oxidized $^{239}\text{PuO}_2$ in rats. Int. J. Radiat. Biol. 35: 95-98.
- Sanders, C. L., J. G. Hadley, A. Conklin and R. R. Adey. 1978. Antagonism of cadmium-induced pulmonary toxicity by WR-2721. Toxicol. Lett. 2: 323-328.
- Schneider, R. P. and G. S. Beaudreau. 1979. Characterization of the adenosine triphosphatase of avian myeloblastosis virus. Arch. Biochem. Biophys. 196: 475-483.
- Sikov, M. R., G. M. Zwicker, J. O. Hess and D. D. Mahlum. 1977. Late effects of perinatally administered plutonium, pp. 361-374. In: Developmental Toxicology of Energy-Related Pollutants, Proceedings of the 17th Hanford Symposium, Richland, WA, (D. D. Mahlum, M. R. Sikov, P. L. Hackett, F. D. Andrew, Eds.) Oct 17-19, 1977 (CONF-771017). NTIS, Springfield, VA.
- Sikov, M. R. and L. D. Montgomery. 1978. Developmental toxicology studies with 60-Hz electric fields. Health Phys. 35: 890.
- Sikov, M. R., F. D. Andrew, D. H. Willard and J. E. Ballou. 1978. Deposition of ^{85}Kr in gravid rats. Health Phys. 35: 896.
- Smith, V. H., R. A. Gelman, G. E. Dagle and H. A. Ragan. 1978. Studies of inhaled Ca-DTPA to the rat lung. Health Phys. 35: 913.
- Sullivan, M. F., J. L. Ryan, L. S. Gorham and K. M. McFadden. 1979. The influence of oxidation state on the absorption of Pu from the GI tract. Rad. Res. 80: 116-121.
- Sullivan, M. F. and V. H. Smith. 1979. Plutonium removal by continuous chelate administration. Pharmacologist 21: 209. (Abstract)
- Wehner, A. P., B. O. Stuart and C. L. Sanders. 1979. Inhalation Studies with Syrian Golden Hamsters, pp. 177-198. In: Progress in Experimental Tumor Research, Vol. 24 (F. Holmberger, ed.), S. Karger, Basel.
- Wildung, R. E., T. R. Garland and H. Drucker. 1978. Nickel complexes with microbial metabolites--Mobility and speciation in soils. In: Proceedings of Symposium on Chemical Modeling - Speciation, Sorption, Solubility and Kinetics in Aqueous Systems. American Chemical Society, Miami, FL, September 10-15, 1978.

Zwicker, G. M., M. D. Allen and D. L. Stevens.
1979. Toxicity of aerosols of sodium
reaction products. J. Environ. Pathol.
Toxicol. 2: 1139-1150.

PRESENTATIONS

Andrew, F. D. Developmental Toxicology of SRC Materials. Seminar, Biology Department, Pacific Northwest Laboratory, Richland, WA, March 27, 1979.

Andrew, F. D. and D. D. Mahlum. Developmental Toxicity of Solvent Refined Coal-Related Hydrocarbons. Annual Meeting of the Society of Toxicology, New Orleans, LA, March 11-15, 1979.

Andrew, F. D. and M. R. Sikov. Studies on the Transplacental Carcinogenicity of ^{239}Pu Plutonium in Rats. Teratology Society Meeting, Cedar, MI, June 10-14, 1979.

Andrews, T. K. and M. E. Frazier. Characterization of Cell Cultures Derived from ^{239}Pu -Induced Tumors in Beagles. 30th Annual Meeting of the Tissue Culture Association, Seattle, WA, June 11-14, 1979.

Bair, W. J. Review of Report of the International Commission of Radiological Protection Committee 2: Limits for Intakes of Radionuclides by Workers, Conference on Radioecology, Deutsches Atomforum e.V., Bonn, Germany, November 2, 1979.

Bair, W. J. Metabolism and Biological Effects of Alpha-Emitting Radionuclides, 6th International Congress of Radiation Research, Tokyo, Japan, May 13-19, 1979.

Bair, W. J. Comparison of Acute Mortality in Baboons and Dogs After Inhalation of $^{239}\text{PuO}_2$, 6th International Congress of Radiation Research, Tokyo, Japan, May 13-19, 1979.

Ballou, J. E. Uranium in Rodents. Workshop on Measurement and Interpretation of Actinide Accumulation by Man, Snowbird, UT, October 14-18, 1979.

Ballou, J. E., R. A. Gies and A. C. Case. Early Disposition of Inhaled $^{233}\text{UO}_2(\text{NO}_3)_2$ and $^{232}\text{UO}_2(\text{NO}_3)_2$ in the Rat. Health Physics Society Annual Meeting, Philadelphia, PA, July 8-13, 1979.

Burton, F. G., R. E. Schirmer and D. D. Mahlum. Disposition of Process Solvent from Solvent Refined Coal in Tissues of the Rat after Oral Dosing. Annual Meeting of the Society of Toxicology, New Orleans, LA, March 11-15, 1979.

Busch, R. H. and M. T. Karagianes. Biological Effects of Chronic Inhalation of Coal Mine Dust and/or Diesel Engine Exhaust in Rodents. American College of Veterinary Pathologists Meeting, Denver, CO, December 5-8, 1979.

Cannon, W. C., O. R. Moss, J. R. Laidler and B. R. Garrity. Increasing the Range of a Particulate Mass Concentration Meter. American Industrial Hygiene Association Conference, Chicago, IL, May 27 - June 1, 1979.

Causey, S. C. and F. P. Hungate. Studies of Mutagenic Effects of Magnetic Fields in Bacteria. Bioelectromagnetics Symposium and International IEEE/APS Symposium and National Radio Science Meeting, Seattle, WA, June 18-22, 1979.

Cochrane, S. and E. L. Wierman. Breeding Procedures to Insure Conception in a Closed Dog Colony. AALAS District 8 Meeting, Salt Lake City, UT, Feb. 28 - March 2, 1979.

Conklin, A. Translocation of Intratracheally Instilled $^{48}\text{V}_2\text{O}_5$ in Rats. Northwest Section of the Society for Experimental Biology and Medicine Meeting, Richland, WA, October 20, 1979.

Dagle, G. E. and C. L. Sanders. The Hazards of Cigarette Smoking. Richland Rotary Club, Richland, WA, January 30, 1979.

Dagle, G. E., J. F. McShane and A. P. Wehner. Relationship of Chronic Nephropathy to Body Weight in Laboratory Rats. American College of Veterinary Pathologists Meeting, Denver, CO, December 4-8, 1979.

Dagle, G. E., J. F. Park, H. A. Ragan and J. E. Morris. Toxicity of Inhaled Plutonium in Dogs. IAEA International Symposium on Biological Implications of Radionuclides Released from Nuclear Industries, Vienna, Austria, March 26-30, 1979.

Dagle, G. E., J. F. Park, C. L. Sanders and J. A. Mahaffey. Pulmonary Carcinogenesis with Inhaled Plutonium in Dogs and Rats. 19th Annual Hanford Life Sciences Symposium, "Pulmonary Toxicology of Respirable Particles," Richland, WA, October 22-24, 1979.

Dragoo, K. M., C. L. Sanders and K. E. McDonald. Bone and Lung Tumor Transplantation Techniques in Hamsters and Nude Mice. AALAS District 8 Meeting, Salt Lake City, UT, Feb. 28 - March 2, 1979.

Filipy, R. E., F. J. Borst, F. T. Cross, J. F. Park, O. R. Moss, R. L. Roswell and D. L. Stevens. A Mathematical Model for Predicting the Probability of Acute Mortality in a Human Population Exposed to Accidentally Released Airborne Radionuclides. Nuclear Regulatory Commission, Washington, D.C., June 19, 1979.

Filipy, R. E. and W. L. Nicholson. Automated Pulmonary Morphometry: A Status Report. 5th International Congress for Sterology, Salzburg, Austria, September 3-8, 1979.

Frazier, M. E. Cellular Toxicity and Transformation of SRC Materials. Biology Department Seminar, Pacific Northwest Laboratory, Richland, WA, April 24, 1979.

Frazier, M. E., T. K. Andrews, B. B. Thompson, M. R. Peterson and M. J. Hooper. Use of Mammalian Cell Culture Techniques for Determining the Toxic and Carcinogenic Potential of Complex Organic Mixtures. Annual Meeting of the Society of Toxicology, New Orleans, LA, March 11-15, 1979.

Frazier, M. E., J. R. Decker, W. T. Kaune, D. D. Mahlum, M. R. Sikov and J. A. Strand. Biological Effects of Magnetic Fields: A Progress Report. Health Implications of the New Energy Technologies Meeting, Park City, UT, April 4-7, 1979.

Gelman, R. A. and V. H. Smith. A Sensitive Assay for Diethylenetriaminepentaacetic Acid in Urine Using Paper Chromatography. 63rd Annual Meeting of the Federation of American Societies for Experimental Biology, Dallas, TX, April 2-5, 1979.

Hackett, P. L., J. O. Hess and M. R. Sikov. Cross-Placental Transfer and Distribution of Inhaled or Ingested Lead Nitrate in Rats. Teratology Society Meeting, Cedar, MI, June 10-14, 1979.

Hadley, J. G. and R. R. Adey. Particle Uptake by Alveolar Macrophages Through Agranulocyte Mediated Phagocytosis. 19th Annual Hanford Life Sciences Symposium, "Pulmonary Toxicology of Respirable Particles," Richland, WA, October 22-24, 1979.

Hilton, D. I. and R. D. Phillips. Cardiovascular Response in Rats Exposed to 60-Hz Electric Fields. Bioelectromagnetic Symposium, International IEEE/APS Symposium and National Radio Science Meeting, Seattle, WA, June 18-22, 1979.

Hilton, D. I. and R. D. Phillips. Cardiovascular Response of Rats Exposed to 60-Hz Electric Fields. 30th Annual Fall Meeting of the American Physiological Society, New Orleans, LA, October 15-19, 1979.

Hjeresen, D. L. Effect of Chronic Exposure to an Electric Field on Subsequent Discrimination of Electric Fields. Bioelectromagnetics Symposium and International IEEE/APS Symposium and National Radio Science Meeting, Seattle, WA, June 18-22, 1979.

Horstman, V. G. LSL-I Animals in Biomedical Research. Hanford Patrol Training Academy, Richland, WA, November 8, 1979.

Horstman, V. G. Animals in Biomedical Research. Kamiakin High School Summer Students, Kennewick, WA, July 10, 1979.

Horstman, V. G. Animals in Biomedical Research. Lions Club Meeting, Kennewick, WA, June 20, 1979.

Horstman, V. G. Animals in Biomedical Research. Women's Energy Conservation Group, Richland, WA, May 31, 1979.

Horstman, V. G. Animals in Biomedical Research. Kiwanis Farm City Day, Richland, WA, January 17, 1979.

Horstman, V. G. Animals in Biomedical Research. Cashmere Junior and Senior High School students, Hanford Science Center, Richland, WA, April 18, 1979.

Hungate, F. P. Development of a Portable Blood Irradiator. Nuclear, Chemical and Mechanical Engineers' Seminar, Joint Center for Graduate Study, Richland, WA, January, 1979.

Hungate, F. P. and S. C. Causey. Mutagenic Effects of Large Electric Fields. International Congress of Radiation Research Society, Tokyo, Japan, May 13-19, 1979.

Kaune, W. T. Biological Effects of 60-Hz Electric Fields: An Example of Environmental Research. Northwest Section of the Society for Experimental Biology and Medicine Meeting, Richland, WA, October 20, 1979.

Kaune, W. T. and R. D. Phillips. Comparison of the coupling of grounded humans, swine, and rats to vertical, 60-Hz electric fields. Bioelectromagnetic Symposium, International IEEE/APS Symposium and National Radio Science Meeting, Seattle, WA, June 18-22, 1979.

Kelman, B. J. Cadmium Movements Between Mother and Fetus. Northwest Section of the Society for Experimental Biology and Medicine Meeting, Richland, WA, October 20, 1979.

Kelman, B. J. Transplacental Measurements and Fetal Metabolism of Toxic Metals. Seminar, Biology Department, Pacific Northwest Laboratory, Richland, WA, April, 1979.

Killand, B. W. and S. M. Loscutoff. Measurement of Esophageal Pressure as an Indirect Measure of Pleural Pressure in Awake Guinea Pigs. American Physiological Society Meeting, Dallas, TX, April 6-10, 1979.

Kinsley, J. T. and E. L. Wierman. Prenatal and Postnatal Care of Beagle Dams and Pups at Battelle Pacific Northwest Laboratory. AALAS District 8 Meeting, Salt Lake City, UT, Feb. 28 - March 2, 1979.

Laszewski, B. The Effects of an Electric Field on Rat Superior Cervical Sympathetic Ganglia. Northwest Section of the Society for Experimental Biology and Medicine Meeting, Richland, WA, October 20, 1979.

Mahlum, D. D. Toxicologic Studies with Solvent Refined Coal Materials. American Association for the Advancement of Science, Pacific Division Meeting, University of Idaho, Moscow, ID, June 3-7, 1979.

Mahlum, D. D., J. O. Hess and M. R. Sikov. The Late Effects of Plutonium Administered During Pregnancy or Lactation. 6th International Congress of Radiation Research, Hiroshima, Japan, May 21-22, 1979.

Mahlum, D. D., M. R. Sikov, G. M. Zwicker and J. O. Hess. Age and Carcinogenesis of ^{239}Pu . IAEA International Symposium on Biological Implications of Radionuclides Released from Nuclear Industries, Vienna, Austria, March 26-30, 1979.

Mahlum, D. D. and F. D. Andrew. Acute and Subchronic Toxicity of Solvent Refined Coal-Related Hydrocarbons. Annual Meeting of the Society of Toxicology, New Orleans, LA, March 11-15, 1979.

Mallavia, L. P., M. J. Hooper and M. E. Frazier. Characterization of the Reverse Transcriptase from a Porcine Retrovirus. 79th Annual Meeting of the American Society for Microbiology, Los Angeles, CA, May 4-8, 1979.

McShane, J. F. G. E. Dagle and J. F. Park. Pulmonary Distribution of Inhaled $^{239}\text{PuO}_2$ in Dogs. 19th Annual Hanford Life Sciences Symposium, "Pulmonary Toxicology of Respirable Particles," Richland, WA, October 22-24, 1979.

Montgomery, L. D., L. G. Smith and M. R. Sikov. Effects of Exposure to 60-Hz Electric Fields on Growth and Development in the Rat. Bioelectromagnetics Symposium and International IEEE/APS Symposium and National Radio Science Meeting, Seattle, WA, June 18-22, 1979.

Moss, O. R. Use of a 35-mm Camera as a Nephelometer in Field Testing a Unique Animal Exposure Chamber. American Industrial Hygiene Conference, Chicago, IL, May 27 - June 1, 1979.

Moss, O. R. and G. M. Kanapilly. Dissolution of Inhaled Aerosols. Division of Environmental Chemistry, American Chemical Society Meeting, Honolulu, HI, April 2-3, 1979.

Park, J. F. Metabolic Model of Plutonium in Man and Experimental Animals for Radiation Protection Purposes. Workshop on "Measurement and Interpretation of Actinides in Man," Snowbird, UT, October 14-17, 1979.

Pelroy, R. A. Mutagenicity Testing of Materials from Solvent Refined Coal Processes. ASTM Symposium, Pittsburgh, PA, June 4-5, 1979.

- Pelroy, R. A. Comparative Mutagenesis of Solvent Refined Coal and Shale Oil Samples from Pilot Plant Operations. American Association for the Advancement of Science, Pacific Division Meeting, University of Idaho, Moscow, ID, June 3-7, 1979.
- Pelroy, R. A. Comparative Mutagenesis of Solvent Refined Coal and Shale Oil Samples from Pilot Plant Operations. EPA/ORNL Meeting on Disposition of Synthetic Fuel Repositories, Research Triangle Park, NC, April 26, 1979.
- Pelroy, R. A. Mutagenesis of SRC-Related Materials. Seminar, Biology Department, Battelle, Pacific Northwest Laboratory, March 6, 1979.
- Pelroy, R. A. and M. R. Peterson. Comparative Mutagenesis of Solvent Refined Coal and Shale Oil Samples from Pilot Plant Operations. Annual Meeting of the Society of Toxicology, New Orleans, LA, March 11-15, 1979.
- Pelroy, R. A. and B. W. Wilson. Use of In Vitro Bioassay for Various Complex Materials. 19th Annual Hanford Life Sciences Symposium, "Pulmonary Toxicology of Respirable Particles," Richland, WA, October 22-24, 1979.
- Peterson, W. B. and K. E. McDonald. Establishment, Maintenance and Breeding of a Nude (Thymus-Deficient) Mouse Colony. AALAS District 8 Meeting, Salt Lake City, UT, Feb. 28 - March 2, 1979.
- Phillips, R. D. and W. T. Kaune. Biological Effects of Electric Fields on Small and Large Animals. US/USSR Scientific Exchange Program, Seattle, WA, June, 1979.
- Phillips, R. D. Biological Effects of 60-Hz Electric Fields on Rodents. DOE Electric Field Bioeffects Contractors' Review Meeting, Washington, D.C., November 14-15, 1979.
- Phillips, R. D. Health Aspects of Power Transmission. Symposium on Energy and Human Health: Human Costs of Electric Power Generation, University of Pittsburgh and Ohio River Basin Energy Study, Pittsburgh, PA, March 19-21, 1979.
- Phillips, R. D., J. H. Chandon, D. I. Hilton and R. L. Sheldon. Growth of Rats and Mice Exposed to 60-Hz Electric Fields. Bioelectromagnetics Symposium and International IEEE/APS Symposium and National Radio Science Meeting, Seattle, WA, June 18-22, 1979.
- Renne, R. A. Dermal Effects of SRC Materials. Seminar, Biology Department, Battelle, Pacific Northwest Laboratory, Richland, WA, April 24, 1979.
- Renne, R. A., J. E. Lund, K. E. McDonald and L. G. Smith. Morphologic Effects of Intratracheally Administered Oil Shale in Rats. Conference on Health Implications of New Energy Technologies, sponsored by The Society for Occupational and Environmental Health, Park City, UT, April 4, 1979.
- Robinson, A. V. and R. P. Schneider. Phagocytosis, Toxicity and Solubility of AmO_2 . 19th Annual Hanford Life Sciences Symposium, "Pulmonary Toxicology of Respirable Particles," Richland, WA, October 22-24, 1979.
- Sanders, C. L. Personal Abuse--Cigarette Smoking. Benton-Franklin Counties Juvenile Justice Center Safety Meeting, Kennewick, WA, October 15, 1979.
- Sanders, C. L. Pulmonary Carcinogenesis of Inhaled Transuranics. Seminar, Duke University, Durham, NC and University of Rochester, Rochester, NY, July, 1979.
- Sanders, C. L. Toxicological Aspects of Fossil and Nuclear Powered Plants. Radiological Sciences Seminar, Joint Center for Graduate Study, February 28, 1979.
- Sanders, C. L. Radiation Carcinogenesis. Nuclear, Chemical, and Mechanical Engineering Seminar, Joint Center for Graduate Study, March 7, 1979.
- Sanders, C. L. Radiation Biology. Health Physics Certification, Refresher Course, Joint Center for Graduate Study, May 9, 1979.
- Sanders, C. L. Animals in Biomedical Research. Presented to the students of Jefferson Elementary School, Richland, WA, February 13, 1979.
- Sanders, C. L. Basic Radiobiology. National Registry of Radiation Protection Technologists, Richland, WA, March 7, 1979.
- Sanders, C. L., A. W. Conklin and J. G. Hadley. Mixed Chelation Therapy for Removal of ^{109}Cd and ^{239}Pu . American Society for Pharmacology and Experimental Therapeutics, Portland, OR, August 19-23, 1979.

Sanders, C. L., J. A. Mahaffey and J. G. Hadley. Inhalation Toxicology of Transuranics in Rodents. IAEA International Symposium on Biological Implications of Radionuclides Released from Nuclear Industries, Vienna, Austria, March 26-30, 1979.

Sikov, M. R., D. D. Mahlum, G. M. Zwicker, D. B. Carr and J. O. Hess. Relationships Between Age at Exposure to ^{239}Pu and the Pathogenesis of Late Effects. 6th International Congress of Radiation Research, Tokyo, Japan, May 13-19, 1979.

Smith, V. H. Therapy for incorporated radionuclides: General problems, current states of solutions and need for a future. Workshop on "Measurements and Interpretation of Actinide Accumulation by Man." Snowbird, UT, October 15-17, 1979.

Smith, V. H. and R. A. Gelman. Relative Solubility of Simulated Biological Fluids of PuO_2 on Air Sampler Filters. 25th Annual Environmental and Analytical Chemistry and Bioassay Conference, Las Vegas, NV, Oct. 31 - Nov. 2, 1979.

Springer, D. L. Metabolism and Biological Effects of Hydrazine in Rats. Seminar, Biology Department, Pacific Northwest Laboratory, Richland, WA, February, 1979.

Sullivan, M. F. Actinide Absorption from the GI Tract. Workshop on "Measurement and Interpretation of Actinides in Man," Snowbird, UT, October 14-17, 1979.

Sullivan, M. F. The Influence of Oxidation State on Absorption of Plutonium from the Gastrointestinal Tract. Presented to the DOE and Rockwell Officials, Rocky Flats, CO, June; also presented to the Colorado Department of Health, Denver, CO, June, 1979.

Sullivan, M. F., T. R. Garland, D. A. Cataldo and R. G. Schreckhise. Absorption of Plant-Incorporated Nuclear Fuel Cycle Elements from the Gastrointestinal Tract. IAEA International Symposium on Biological Implications of Radionuclides Released from Nuclear Industries, Vienna, Austria, March 26-30, 1979.

Sullivan, M. F., L. S. Gorham and E. F. Blanton. Absorption of Plutonium by the Neonatal Animal after Inhalation. 19th Annual Hanford Life Sciences Symposium, "Pulmonary Toxicology of Respirable Particles," Pacific Northwest Laboratory, Richland, WA, October 22-24, 1979.

Sullivan, M. F. and V. H. Smith. Plutonium Removal by Continuous Chelate Administration. ASPET Meeting, Portland, OR, August 19-23, 1979.

Thompson, R. C. A Consideration of the Wider Impact of Present Trends in Radiation Protection Systems. IAEA Seminar on the Practical Implications of the ICRP Recommendations (1977) and the Revised IAEA Basic Standards for Radiation Protection, Vienna, Austria, March 5-9, 1979.

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Seattle, WA 98195

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Washington, DC 20545

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University of California
Los Alamos Scientific
Laboratory
P. O. Box 1663
Los Alamos, NM 87545

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Los Alamos Scientific
Laboratory
P.O. Box 1663
Los Alamos, NM 87545

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