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PNL-4600 PT1
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Pacific Northwest Laboratory Annual Report for 1982 to the DOE Office of Energy Research

Part 1 Biomedical Sciences February 1983

Prepared for the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
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Printed in the United States of America
Available from
National Technical Information Service
United States Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22151

NTIS Price Codes
Microfiche A01

Printed Copy

Pages	Price Codes
001-025	A02
026-050	A03
051-075	A04
076-100	A05
101-125	A06
126-150	A07
151-175	A08
176-200	A09
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H. Drucker and Staff Members
of Pacific Northwest Laboratory

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Pacific Northwest Laboratory
Richland, Washington 99352

PREFACE

This 1982 annual report from Pacific Northwest Laboratory (PNL) to the Department of Energy (DOE) describes research in environment, health, and safety conducted during fiscal year 1982. The report again consists of five parts, each in a separate volume.

The five parts of the report are oriented to particular segments of our program. Parts 1 to 4 report on research performed for the DOE Office of Health and Environmental Research in the Office of Energy Research. Part 5 reports progress on all research performed for the Office of the Assistant Secretary for Environmental Protection, Safety and Emergency Preparedness. Each part consists of project reports authored by scientists from several PNL research departments, reflecting the interdisciplinary nature of the research effort.

The parts of the 1982 Annual Report are:

Part 1: Biomedical Sciences	
Program Manager - H. Drucker	D. L. Felton, Editor
Part 2: Environmental Sciences	
Program Manager - B. E. Vaughan	B. E. Vaughan, Report Coordinator C. M. Novich, Editor
Part 3: Atmospheric Sciences	
Program Manager - C. E. Elderkin	N. S. Laulainen, Report Coordinator E. L. Owczarski, Editor
Part 4: Physical Sciences	
Program Manager - J. M. Nielsen	J. M. Nielsen, Report Coordinator J. E. Danko, E. M. Toomey, Editors
Part 5: Environmental and Occupational Protection, Assessment, and Engineering	
Program Managers - S. Marks W. A. Glass	W. J. Bair, Report Coordinator R. W. Baalman, Editor

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

Credit for this annual report goes to many scientists who performed the research and wrote the individual project reports, to the program managers who directed

the research and coordinated the technical progress reports, to the editors who edited the individual project reports and assembled the five parts, and to Ray Baalman editor in chief, who directed the total effort.

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

Previous reports in this series:

Annual Report for

1951	W-25021, HW-25709
1952	HW-27814, HW-28636
1953	HW-30437, HW-30464
1954	HW-30306, HW-33128, HW-35905, HW-35917
1955	HW-39558, HW-41315, HW-41500
1956	HW-47500
1957	HW-53500
1958	HW-59500
1959	HW-63824, HW-65500
1960	HW-69500, HW-70050
1961	HW-72500, HW-73337
1962	HW-76000, HW-77609
1963	HW-80500, HW-81746
1964	BNWL-122
1965	BNWL-280; BNWL-235, Vol. 1-4; BNWL-361
1966	BNWL-480, Vol. 1; BNWL-481, Vol. 2, Pt. 1-4
1967	BNWL-714, Vol. 1; BNWL-715, Vol. 2, Pt. 1-4
1968	BNWL-1050, Vol. 1, Pt. 1-2; BNWL-1051, Vol. 2, Pt. 1-3
1969	BNWL-1306, Vol. 1, Pt. 1-2; BNWL-1307, Vol. 2, Pt. 1-3
1970	BNWL-1550, Vol. 1, Pt. 1-2; BNWL-1551, Vol. 2, Pt. 1-2
1971	BNWL-1650, Vol. 1, Pt. 1-2; BNWL-1651, Vol. 2, Pt. 1-2
1972	BNWL-1750, Vol. 1, Pt. 1-2; BNWL-1751, Vol. 2, Pt. 1-2
1973	BNWL-1850, Pt. 1-4
1974	BNWL-1950, Pt. 1-4
1975	BNWL-2000, Pt. 1-4
1976	BNWL-2100, Pt. 1-5
1977	PNL-2500, Pt. 1-5
1978	PNL-2850, Pt. 1-5
1979	PNL-3300, Pt. 1-5
1980	PNL-3700, Pt. 1-5
1981	PNL-4100, Pt. 1-5

FOREWORD

This volume describes progress on biomedical and health effects research conducted at PNL in 1982. 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 activities for three major energy technologies: nuclear fuel cycle; fossil fuel cycle (oil, gas, and coal process technologies, mining, and utilization; synfuel development), 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 energy activities. The coal-based synfuel research project, for example, is 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 qualitative validity of applying results from animal experimentation to man is firmly based on empirical observations. However, as indicated in reports on animal lifespan 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 synfuels. 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 synfuels.

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 and Chemistry Department are the principal contributors to the volume. Requests for reprints from the list of publications for 1982 on pages 145 to 149 will be honored while supplies last.

CONTENTS

Coal

COAL

Studies in the area of coal energy technologies are relevant to potential occupational and environmental health concerns of coal combustion and coal synfuel processes.

In the area of coal synfuels 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.

• Mutagenicity of SRC Materials

Principal Investigators: R. A. Pelroy and M. E. Frazier

Other Investigators: P. A. Lacy, J. E. Samuel, and D. L. Stewart

Two carcinogenic chemical fractions from a high-boiling coal liquid were compared in microbial and mammalian in vitro bioassay systems for mutagenic activity. The coal liquid was the >850°F distillate cut from a wide-boiling-range solvent refined coal (SRC) liquid. For the microbial test, reverse mutation was measured in *Salmonella typhimurium* TA-98 (Ames test). For the mammalian test, forward mutation was the endpoint, using cultured Chinese hamster ovary (CHO) cells. *S. typhimurium* responded very strongly to primary aromatic amines (PAAs) in the nitrogen polycyclic aromatic compound (NPAC) chemical fraction and much less strongly to neutral polyaromatic hydrocarbons (PAHs). The CHO cells responded strongly to PAHs. Within NPAC, the CHO assay appeared to respond much more strongly to azaarene components than to carbazoles or PAAs. There appeared to be a quantitative correlation between mutagenic response in the CHO assay and initiation of tumorigenesis in mouse skin for both the crude SRC-II distillate cuts and their PAH fractions. The PAH fraction from the 850°F distillate was more active against *S. typhimurium* than other, less carcinogenic, PAH fractions from the SRC-II distillate cuts. Thus, Ames test mutagenicity of the PAH components of the SRC coal liquids was also ranked in the same order as the carcinogenicity of these chemical fractions in the initiation/promotion (I/P), mouse skin-painting assay.

The mutagenicity of two coal-derived liquids (the SRC-II 800 to 850°F and >850°F distillates) were compared in microbial and mammalian cellular (in vitro) bioassays. The microbial bioassay consisted of the standard Ames (or histidine reversion) system using Salmonella typhimurium TA-98. The mammalian in vitro system was the Chinese hamster ovary (CHO) forward mutation to thioguanine resistance at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus. Both distillates were highly carcinogenic to mouse skin in chronic and initiation/promotion (I/P) skin-painting assays. Previous work showed that neutral polyaromatic hydrocarbon (PAH) components determined most of their carcinogenic activity; however, the nitrogen polycyclic aromatic compound (NPAC) constituents were also important as carcinogens in these distillate cuts. The standard Ames test detects mutagens in the PAH and NPAC fractions of the two distillate cuts; however, NPACs are much more potent than PAHs as mutagens to S. typhimurium. Thus, the microbial genetic potency of these fractions is the reverse of their carcinogenic potency as determined by mouse-skin response.

One of our major program objectives has been to determine whether the mutagenic and carcinogenic potency of coal-derived fuels is correlated. In other words: Do large numbers of induced mutants in the in vitro tests imply high tumor incidence in mouse skin? This is obviously not the case for the response of S. typhimurium TA-98 to chemical fractions from coal liquids compared with tumorigenic response in the I/P skin-painting assays to the same fractions (Figure 1). Conversely, the level of induced mutation in CHO cells appears to be roughly correlated with skin tumorigenesis in the sense that chemical fractions that contain the most active mutagens also contain the most active skin carcinogens (Figure 1).

For comparisons of the specific mutagenic activities of PAH fractions from the various distillate cuts in the Ames and CHO assays, the picture changes (Figure 2). Although the CHO assay is more responsive than the Ames assay to PAH fractions (i.e., a higher mutation rate is correlated with strong tumorigenesis response), the relative increase in Ames specific mutagenic activity (small numbers) for PAH fractions from different distillate cuts was also nearly proportional (i.e., correlated) with tumorigenesis. Stated in another way: Given two PAH fractions, the relative increase in specific mutagenic activity in the Ames and CHO assays are both correlated with the initiation of mouse-skin carcinogenesis. However, the absolute mutagenic response per target cell per equivalent weight of PAH fraction (S. typhimurium versus CHO) was clearly much greater for the CHO than for the Ames test.

The magnitude of this differential sensitivity to PAH and NPAC fractions is shown more clearly by the data in Figure 3. In these experiments, we have extended the comparison of the mutagenicity of chemical fractions of the >850°F cut between the S. typhimurium TA-98 and CHO mammalian in

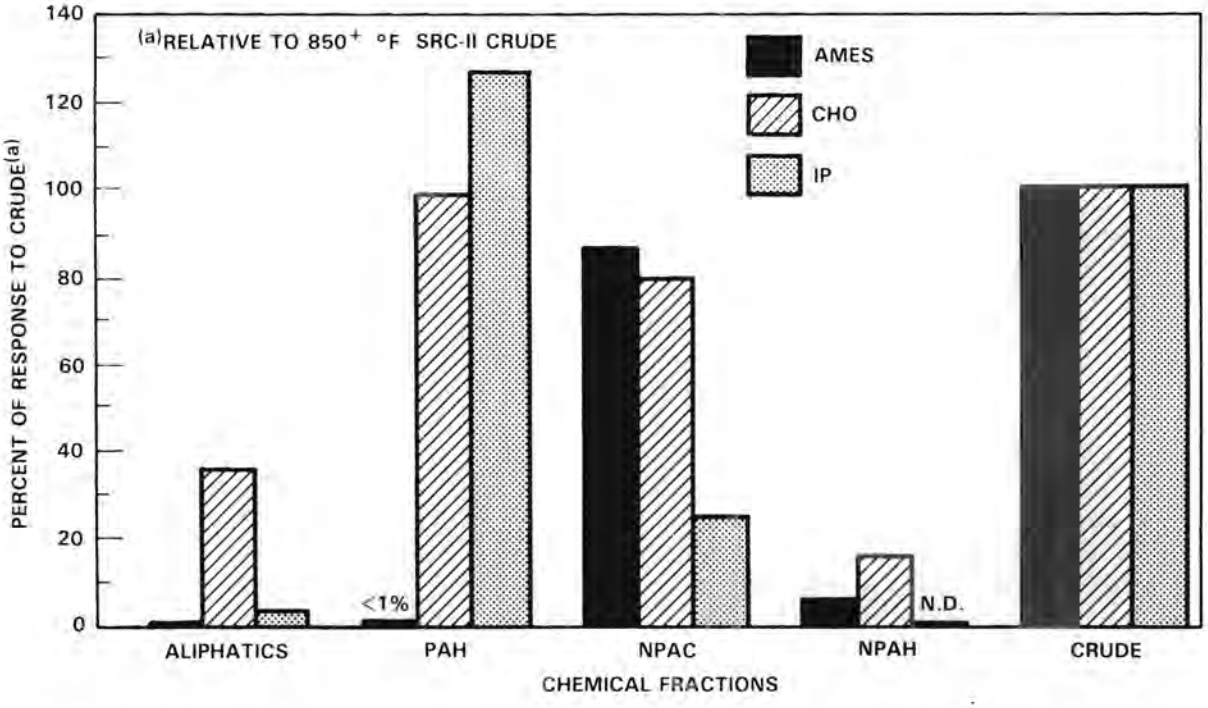

FIGURE 1. Relative Activity of Chemical Fractions From the SRC-II >850°F Distillate Cut in the Ames and CHO Mutation and Initiation-Promotion Carcinogenesis Assays.

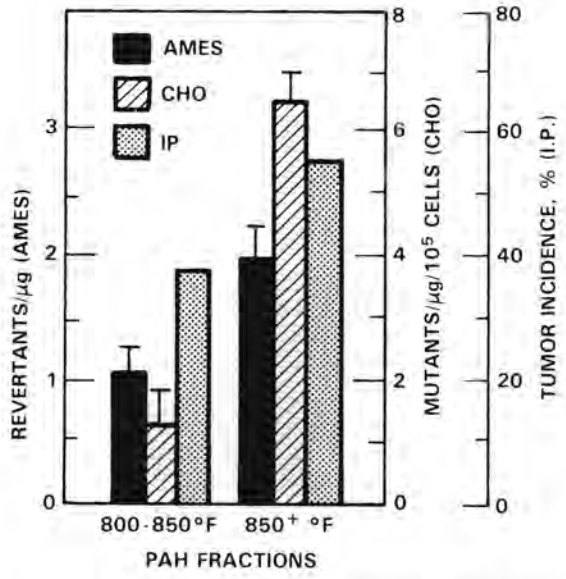

FIGURE 2. Comparison of Specific Mutagenic Activities of PAH Fractions in the Ames Assay With S. typhimurium TA-98 and CHO Assay With Tumor incidence in the Initiation-Promotion (I/P) Mouse-Skin Carcinogenesis Assay. Specific mutagenic activities were determined by linear regression analysis of dose-response data and represent the average slope values for 3 experiments with each material and each assay system. Tumor incidence was measured 15 days after the appearance of the first tumor. All responses were significant at $P<0.01$.

vitro test systems. In order to make these comparisons, the SRC-II >850°F distillate cut was fractionated by semipreparative, high-pressure liquid chromatography (HPLC), and the resulting fractions were analyzed in both the standard Ames and CHO in vitro assays. The HPLC procedure employed in this study separates the components in the distillate into chemical classes. The PAHs were contained in HPLC fractions (LC) 1 and 2; the NPACs were contained in LC 3 and 4 (Table 1). Specific mutagenic activity for the individual HPLC fractions and activities, weighted for composition, are shown in Figure 3. As can be seen, the activity of the PAH-rich LC 1 and 2 fractions in the Ames test was comparatively weak. LC fraction 4 was strongly mutagenic in the Ames test, accounting for more than 90% of the total response from the LC fractions. Most of the response in the CHO mutational assay was obtained for the PAH-enriched LC 1 and 2 and the azaarene-containing LC 3 (Figure 3). Little CHO response was observed for LC 4 (or for other LC fractions).

Since the response in the CHO assay was based on 10^6 target cells/assay and response in the Ames test on roughly 10^8 target cells/assay, the differential sensitivity (defined as the ratio of CHO to Ames response) for PAH fractions was on the order of 100 to 1000 times. In other words, for a given amount of complex PAH

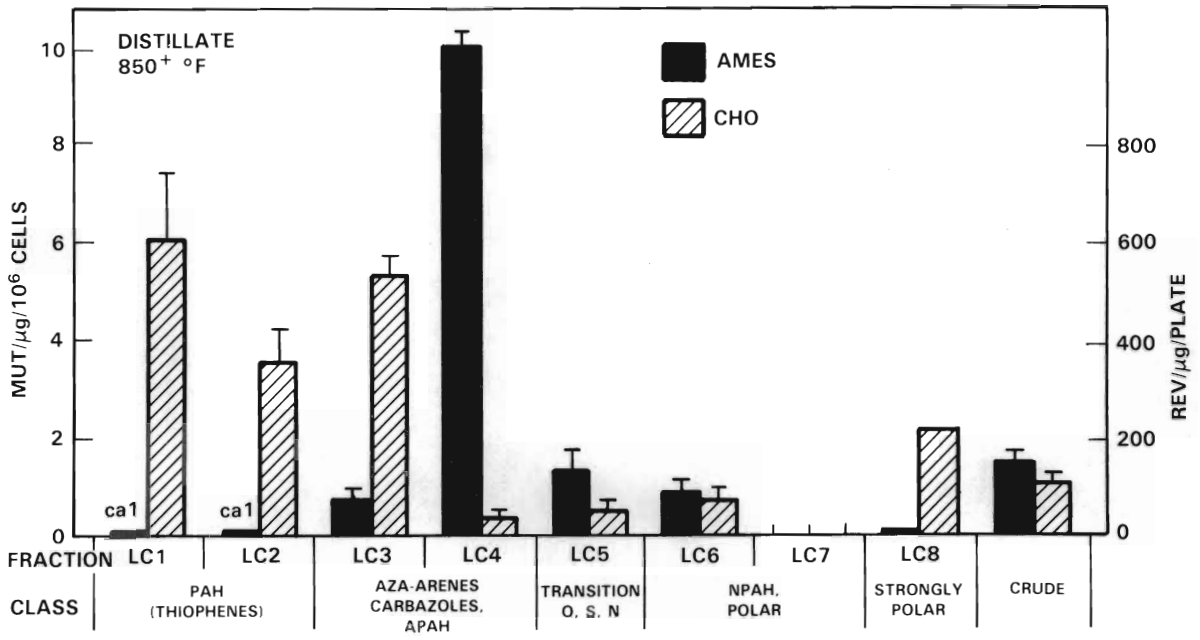

FIGURE 3. Comparison of Mutagenic Activity of Chemical Class Fractions From the SRC-II 850°F Distillate Cut in the Standard Ames Test With S. typhimurium TA-98 and CHO Cells.

fraction, the CHO assay appears to be 100 to 1000 times more sensitive to mutagenic insult. However, the relative increase in mutagenic specific activity for the PAH fractions from different distillate cuts was similar for both assays.

In summary, both the Ames and CHO in vitro assays detect PAH and NPAC mutagens in the 850°F SRC-II distillate cut; however, the Ames test is more sensitive to aminopolycyclic aromatic hydrocarbons within the NPAC fraction, than to PAH. The CHO assay appears to detect PAH and, possibly, azaarene constituents in the coal liquids with greater sensitivity than the Ames test. Since the PAHs and NPACs appear to account for most of the initiation of carcinogenesis in mouse skin, the ability to adequately screen for mutagens in these chemical fractions is important. The differential sensitivity of the Ames and CHO tests to PAH and NPAC mutagens makes them complementary for screening potentially carcinogenic compounds in the high-boiling coal liquids.

TABLE 1. Concentration in ppm of Selected Polycyclic Aromatic Hydrocarbons, Azaarenes, Aminopolycyclic Aromatic Hydrocarbons and Benzocarbazoles in LC 1 Through LC 4 of >850°F Cut.

Compound	LC 1	LC 2	LC 3	LC 4
Pyrene	385	253	ND[a]	ND
Benz(a)anthracene	308	120	ND	ND
Dimethylbenzanthracene (Isomers)	394	269	ND	ND
Benzo(a)pyrene	3037	3861	ND	ND
Benzo(g,h,i)perylene/Ananthrene	2929	10,688	ND	ND
Azachrysenes/Benzanthracene	ND	ND	1039	t[b]
Azabenzopyrenes/Azabenzofluoranthenes	ND	ND	4431	t
Azabenzo(g,h,i)perylenes/Azaananthrenes	ND	ND	2396	t
Aminopyrene/Fluoranthenes	ND	ND	t	25
Aminochrysene/Benzoanthracene	ND	ND	t	53
Aminobenzopyrenes/Perylenes	ND	ND	t	32
Benzocarbazoles	ND	ND	257	t

[a] ND = not detectable
[b] t = trace amounts; i.e., <1 ppm

• Solvent Refined Coal Biostudies

Principal Investigator: D. D. Mahlum

Other Investigators: M. E. Frazier, H. A. Ragan, and D. L. Springer

Technical Assistance: C. J. Gerdes, L. W. McGee, and J. E. Samuel

Coal liquids obtained from the SRC-I and -II processes were selectively distilled to provide cuts with 50°F boiling ranges. These cuts were studied in the Syrian hamster embryo (SHE) transformation, the Chinese hamster ovary (CHO) mutation, and the initiation/promotion skin carcinogenesis assays. The results from all assays showed that activity of the cuts increased as the boiling range increased. Further fractionation of some of the higher-boiling cuts by alumina or high-pressure liquid chromatography revealed that the highest activity was associated with the neutral polyaromatic hydrocarbon fraction, although the nitrogen-containing polycyclic aromatic fraction also had significant activity.

Rats and mice were exposed by inhalation to SRC-II heavy distillate (HD) aerosols (0.70, 0.14, and 0.03 mg/L) for 6 hr/day, 5 days/wk, for 13 wk. Weight gain of the rats was inhibited in the two highest doses. Liver weights were increased in all dose groups; thymus and ovary weights decreased in the mid and high dose groups. Erythrocyte, leukocyte, and lymphocyte values all decreased in a dose-dependent manner. Total serum protein decreased in the high-level rats; serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), cholesterol, and triglyceride levels increased in exposed rats.

MAMMALIAN CELL ASSAYS

As indicated in last year's Annual Report, we have tested a number of coal-derived liquids in mammalian-cell culture assays to assess the potential genotoxicity of these complex organic mixtures in mammalian systems. The results obtained are compared with those obtained in the Ames assay and those obtained in whole-animal studies. Two mammalian cell systems have been used: the Chinese hamster ovary (CHO) forward mutation and the Syrian hamster embryo (SHE) transformation assay. In the transformation assays, SHE cells were exposed to the test materials and monitored to determine whether they underwent morphologic and biological changes that resulted in nonrestrictive growth analagous to that of the cancer cell. Chemicals capable of transforming cells in this manner were considered to be candidate carcinogens. Many of the same materials were assayed in the CHO cell system, which measures the forward mutation of the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus.

Results from assays of distillation cuts prepared from an SRC-II broad-boiling-range liquid showed that the transformation activity of these cuts increased dramatically with increasing temperature range. We have added another set of materials to the protocol to confirm these findings. Table 1 shows that, for these materials, the frequency of transformation is again higher for materials of the highest-boiling ranges. Therefore, distillation cuts were subjected to further fractionation to determine whether the transforming activity could be segregated according to chemical classification. Samples of material boiling from 300-700°F and from a fraction boiling above 850°F were subjected to fractionation on alumina columns. This fractionation procedure yields four fractions: A-1, enriched in aliphatics and olefins; A-2, with neutral polycyclic aromatic hydrocarbons (PAHs); A-3, with nitrogen polycyclic aromatic compounds (NPAC); and A-4, in hydroxy-polyaromatic compounds (HPAC). In some cases, the third fraction was subjected to further fractionation on silicic acid, yielding three additional groups of materials. The first (S-1) and the third fractions (S-3) contain carbazoles and azaarenes, respectively; the second fraction, S-2, is greatly enriched in aromatic amines. These materials were assayed in the SHE cell transformation system. The results (Table 2) indicate that even for the 300-700° material, there is some degree of activity in both the neutral PAHs and the nitrogen-containing fraction. When the >850°F distillation cut is assayed, the activity is much higher than for the lower-boiling materials. Again, substantial activity is found in the neutral PAHs and the nitrogen-containing fractions. Subfractionation shows that S-2, containing the aromatic amines, is the most active of the nitrogen fractions (A-3).

The distillation cuts boiling from 800-850° or at >850°F were also subjected to fractionation, using high-pressure liquid chro-

TABLE 1. Influence of Boiling Point on the Transforming Activity of Solvent Refined Coal Liquids as Assayed in Syrian Hamster Embryo Cells.

Boiling Range, °F	Percent Transformation (at 10 μg/ml)
300-700	0.2
700-750	0.3
750-800	3.7
800-850	6.8
>850	8.9
BaP (Positive Control)	10.3

TABLE 2. Transforming Activity of Chemical Class Fractions Prepared From Selected Distillation Cuts of a SRC-II Liquid as Assayed in Syrian Hamster Embryo Cells.

Boiling Range, °F	Fraction	Chemical Class	Percent Transformation (at 10 μg/ml)
300-700°	--	--	0.4
	A1	Aliphatics and Olefins	0
	A2	Neutral Polyaromatic Hydrocarbons	0.6
	A3	Nitrogen-Polyaromatic Compounds	0.3
	A4	Hydroxy-Polyaromatic Compounds	0
850°	--		6.2
	A1	See Above	0.6
	A2		5.9
	A3		3.1
	A4		0.2
	S1	Carbazoles	0
	S2	Aromatic Amines	2.4
	S3	Aza-arenes	1.6

matography (HPLC). When fractions were analyzed in the SHE cell system, the neutral PAHs were, again, the most active from both boiling-point (bp) materials, with substantial activity in the fraction containing the primary aromatic amines. Thus, for two fractionation systems, the neutral PAHs appear to be the most active of the transforming principles in the distillation cuts studied.

Several of these materials were then analyzed, using the CHO cell system. Results (Table 3) showed that the distillation cut boiling above 850° was more active than that boiling from 800-850°F. Further examination of the results from the HPLC fractions prepared from the >850° cut demonstrated that the highest activity was found in fractions 1 and 2, which contain primarily neutral PAHs. Significant but lower activity was found in fractions 3 through 6. There was also measurable activity in a very polar fraction, which probably contained hydroxy PAHs.

TABLE 3. Mutagenic Activity of Selected Coal Liquid Distillates and Their High-Pressure Liquid Chromatographic Fractions in Chinese Hamster Ovary Cells.

Boiling Range, °F	HPLC Fraction	Chemical Class	Mutants/μg
800-850	--		0.45
>850	--		1.05
800-850	1	Neutral Polyaromatic Hydrocarbons (PAHs)	2.9
	2		3.8
	4	Polyaromatic Amines (PAAs)	0.9
>850	1	Neutral PAHs	5.9
	2		2.5
	3	Carbazoles	1.2
	4	PAAs	0.4
	5	O-, S- and N-Containing Heterocycles	0.5
	6		0.6
	7	Hydroxy PAHs	0
	8		~2

In summary, results obtained from both the SHE and CHO cell systems indicate that the higher-boiling coal liquids contain more active material than those with lower boiling points. Moreoever, the neutral PAHs appear to account for a substantial part of the activity found in these cuts; however, significant activity is also found in the nitrogen-containing fraction.

Initiation/Promotion (I/P) Studies. The I/P studies have continued in an effort to identify components of coal liquids responsible for carcinogenesis. We have also obtained data which can be compared to results from microbial and mammalian-cell mutagenesis and chronic skin-painting studies.

Materials tested for initiating activity were applied to the backs of shaved female CD-1 mice in a volume of 50 μl. Two weeks after initiation, the mice received twice-weekly applications of 5 μg of phorbol myristate acetate (PMA) in 50 μl of acetone. Negative controls were initiated with acetone; positive controls were initiated with benzo[a]pyrene (BaP) or dimethylbenzanthracene (DMBA).

The studies of 50°F bp cuts derived from a broad bp range (300 to >850°F) of SRC-II liquid were completed this year, substantiating the results reported last year. The 700-750° and 750-800° cuts had similar initiating activity. The 800-850° and the 300 to >850° liquids also had activities similar to one another, but the activities were significantly higher than those of the lower bp cuts.

An experiment similar to that described above was performed, in which 50°F bp cuts derived from the SRC-I process were tested for initiating activity. The results, shown in Figure 1, are similar to those obtained with the SRC-II materials. Although there is some activity in fractions boiling below 700°F, the activity increased substantially above 700° and continued to increase as the bp increased.

Since the highest-initiating activity was found in the highest-bp cuts, the 800-850° and >850°F cuts from SRC-II material and the 800 to >850°F cut from SRC-I process solvent were subjected to further fractionation on alumina. These fractions were applied to mouse skin in amounts equivalent to those found in the parent material, and the tumor response after PMA promotion was determined relative to that produced by the parent material. The results (Figure 2A-C) indicate that the neutral PAH fraction contains the highest activity in all three distillation cuts. Substantial activity was also found in the NPAC fraction, with only low amounts of activity in the other two fractions. Comparison of the three graphs also shows that the >850°F cut from SRC-II material has the highest activity of the distillation cuts tested. Since the neutral PAH fractions from all three distillation cuts contain the highest activity, they are being further fractionated by HPLC to determine if the initiating activity can be further segregated.

INHALATION STUDIES

Exposures. A 90-day inhalation exposure to SRC-II HD was conducted on groups of 32 Fischer rats and 15 CD-1 mice of each sex. Ten of these animals were randomly selected for bleeding and sacrifice after 30 days of exposure; the remaining animals were bled and necropsied after 90 days of exposure. These samples were evaluated hematologically and for changes in chemical parameters. Blood samples from mice were collected only after 90 days of exposure and were evaluated hematologically. Extensive necropsies (35 tissues) were performed on all sacrificed animals, and the weights of major tissues were determined.

For this study, the animals were exposed 6 hr/day, 5 days/wk, for 13 wk. The animals were continuously housed in four exposure chambers. Aerosol was produced by a Solosphere® nebulizer and the appropriate amount of aerosol was delivered to each

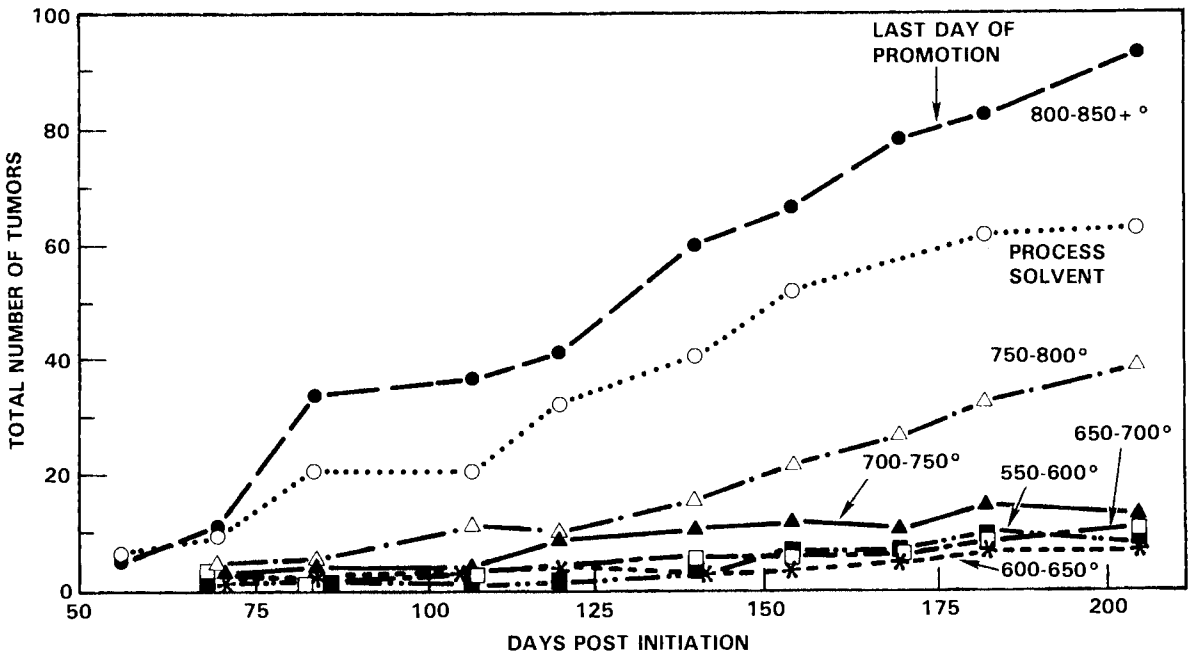

FIGURE 1. Initiating Activity of SRC-1 Distillation Cuts.

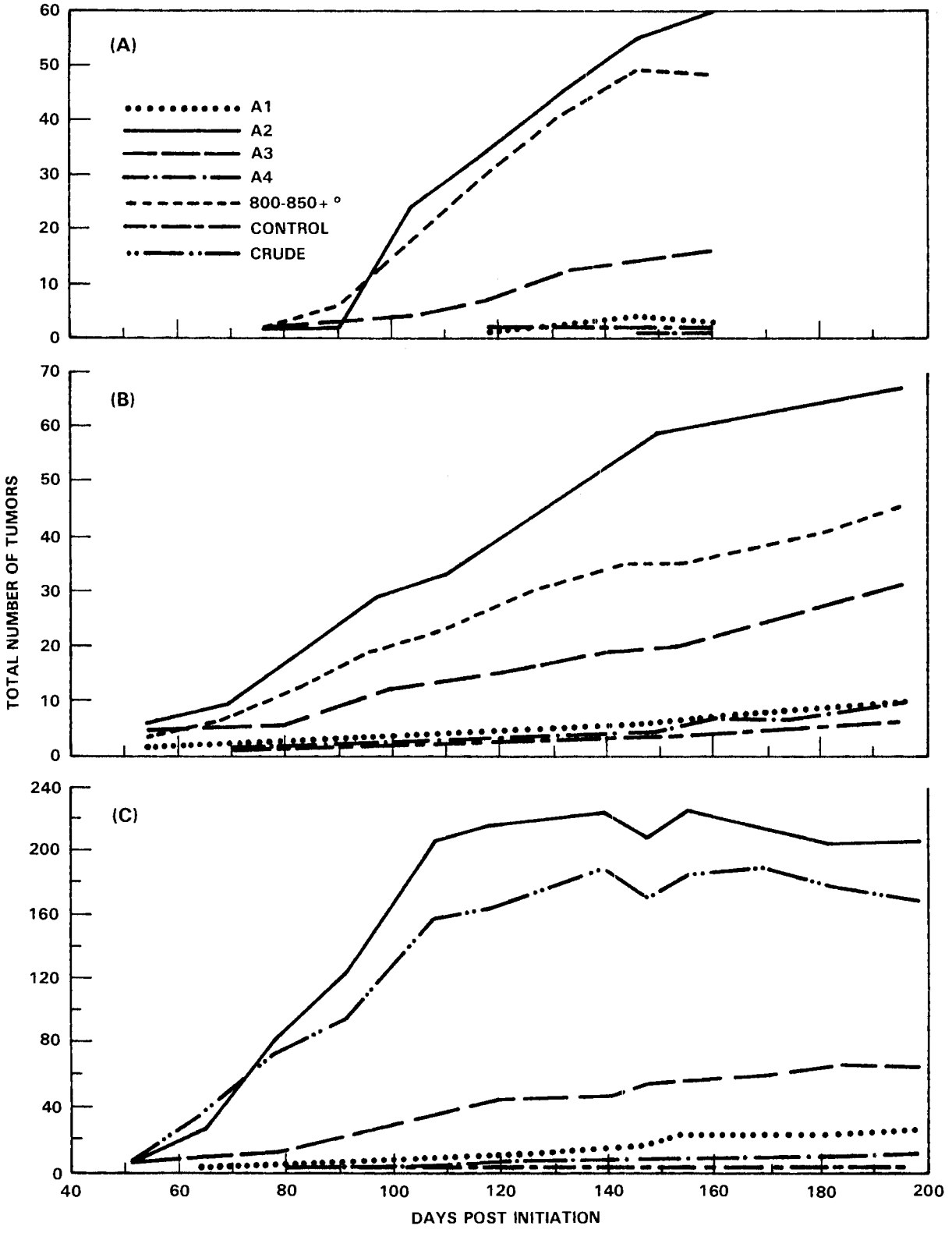

FIGURE 2. Initiating Activity of Fractions Prepared by Alumina Chromatography From: (A) SRC-1 800 to >850 °F Distillation Cut, (B) SRC-II 800 to 850 °F Distillation Cut, (C) SRC-II >850 °F Distillation Cut.

chamber by an orifice-controlled manifold. Mean aerosol concentrations during the 13-wk exposure were 0.70 ± 0.03, 0.14 ± 0.01, 0.03 ± 0.003, and 0.0 mg HD/L of air in the high-, middle-, and low-exposure and control groups, respectively. The mass medium aerodynamic diameter for the aerosol was approximately 1.7 μm, with a geometric standard deviation of 2; there was no significant difference in particle sizes among chambers.

Body and Tissue Weights. Body weights of male rats, measured during exposure, decreased in a dose-dependent fashion (Figure 3A). Male rats in the high-exposure group lost an average of 20 g each during the first week of exposure and thereafter failed to gain weight. They weighed 125 g less than controls at the end of exposure. Male rats in the mid-exposure group consistently weighed less than controls even though they gained weight during exposure. The average weight of animals in the low-exposure group was not significantly different from that of the control group. The pattern for body weight gain of female rats during the exposure period was similar to that for males, although differences between treated groups and controls were smaller due to the slower growth rate of the females (Figure 3B). There were no significant differences in body weight between the treatment and control groups for mice of either sex.

Mortality data for exposed rats indicated that HD was not particularly lethal at the doses employed. One female from the high-exposure group died during the first week of exposure, most probably from choking. All other deaths occurred during the last 3 wk of exposure, also apparently from choking, since food was present in the esophagus and back of the mouth. Although choking is not often reported in the literature, it has been observed in other inhalation studies in our laboratory. The condition of the animals in the high-exposure group, observed at the 90-day sacrifice, suggested that many animals were nearly ready to succumb due to treatment, and that they probably would not have survived many more weeks of exposure.

Evaluation of tissue data from rats exposed for 30 or 90 days indicated that some tissues were significantly affected by exposure (Table 4). Mean liver weights increased in a linear, dose-dependent manner for male rats exposed for 90 days; liver weights in all three treatment groups were significantly above those of the control group. Significant increases in liver

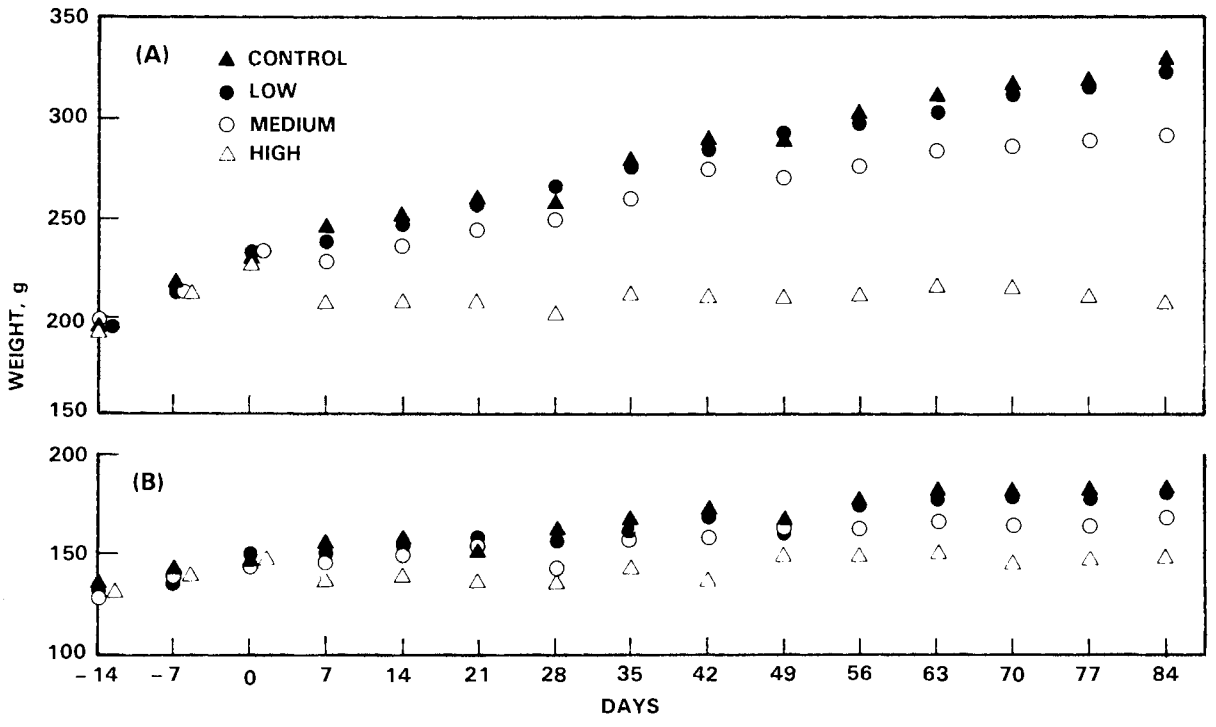

FIGURE 3. (A) Weight Changes in Male Rats Exposed to 0.7, 0.14 or 0.03 mg/L of SRC-II Heavy Distillate Aerosols, (B) Weight Changes in Female Rats Exposed to 0.7, 0.14 or 0.03 mg/L of SRC-II Heavy Distillate Aerosols.

weight for females were observed in the middle and high groups. These increases were also apparent on both an absolute and a relative (tissue weight as percent of body weight) basis. Similar increases were observed for both sexes of rats after 30 days of exposure and for both sexes of mice following 90 days of exposure.

Thymus weight for both male and female rats decreased after 30 and 90 days of exposure. This dose-related decrease was also apparent on both an absolute and relative weight basis. Similar reductions in the size of thymus were observed for mice after 90 days of exposure. Mean ovary weights, on absolute and relative bases, decreased relative to those of controls for rats and mice in the high exposure group. Statistical analysis of the data indicated that the effect was linearly related to dose.

Significant increases were observed for heart, spleen, brain, testes, and adrenals when calculated on relative weight bases; however, absolute weight indicated either no change in weight or a slight decrease for these tissues. Thus, the apparent increase in the size of these tissues on a relative basis appears attributable to decreases in body weight.

HEMATOLOGY

Mean erythrocyte values are shown in Figure 4 for male and female rats following 30 or 90 days of exposure to the three levels of SRC HD, and for the control group. There is a dose-dependent decrease in erythrocyte numbers for both the males and females at 30 and 90 days of exposure. Similar dose-related changes were also evident in other erythrocyte parameters, i.e., hemoglobin concentrations and volume of packed red blood cells.

The leukocyte concentrations (Figure 5) were sex-related. The values for female rats were generally lower than those for males. The females showed no effect of exposure to HD at 30 days of exposure; however, there was an apparent increase in leukocytes in high-exposure-group males after 30 days. This was due to one animal with a white cell count of 23 x 10^3/ml. When this rat is eliminated from the calculations, the group mean falls within the standard error of the other groups, i.e., there is no longer a statistically significant difference between this group and the others. After 90 days of exposure, there was a dose-dependent reduction in leukocytes in both sexes.

TABLE 4. Tissue Weight as Percent of Body Weight for Rats Sacrificied After 90 Days of Exposure to Heavy Distillate.

Tissue	Sex	Treatment Group			
		Control	Low	Mid	High
Heart	M	0.30 ± 0.004	0.31 ± 0.005	0.33[b] ± 0.07	0.47 ± 0.02[c]
	F	0.36 ± 0.007	0.35 ± 0.006	0.37 ± 0.01	0.44 ± 0.01
Liver	M	3.35 ± 0.05	3.73 ± 0.08	4.28 ± 0.10	6.42 ± 0.15
	F	3.54 ± 0.08	3.55 ± 0.08	4.18 ± 0.12	6.37 ± 0.11
Kidney	M	0.67 ± 0.009	0.69 ± 0.01	0.73 ± 0.01	1.05 ± 0.03
	F	0.74 ± 0.01	0.77 ± 0.02	0.81 ± 0.01	0.97 ± 0.02
Spleen	M	0.20 ± 0.002	0.21 ± 0.004	0.21 ± 0.003	0.24 ± 0.005
	F	0.25 ± 0.006	0.25 ± 0.007	0.27 ± 0.007	0.28 ± 0.006
Thymus	M	0.089 ± 0.005	0.082 ± 0.006	0.065 ± 0.003	0.039 ± 0.004
	F	0.13 ± 0.008	0.17 ± 0.005	0.09 ± 0.005	0.05 ± 0.006
Brain	M	0.59 ± 0.006	0.60 ± 0.01	0.64 ± 0.01	0.91 ± 0.02
	F	0.99 ± 0.019	1.00 ± 0.009	1.08 ± 0.02	1.15 ± 0.03
Gonads	M	0.93 ± 0.010	0.95 ± 0.01	1.00 ± 0.01	1.24 ± 0.04
	F	0.038 ± 0.002	0.036 ± 0.001	0.037 ± 0.001	0.026 ± 0.002
Adrenals	M	0.016 ± 0.0008	0.017 ± 0.001	0.018 ± 0.0007	0.028 ± 0.002
	F	0.033 ± 0.001	0.034 ± 0.001	0.041 ± 0.002	0.038 ± 0.001

(a) $\bar{x}$ ± SEM
(b) Underlined values are significantly different from the control group using Dunnetts range test ($P<0.05$)
(c) Significant trend with dose ($P<0.05$)

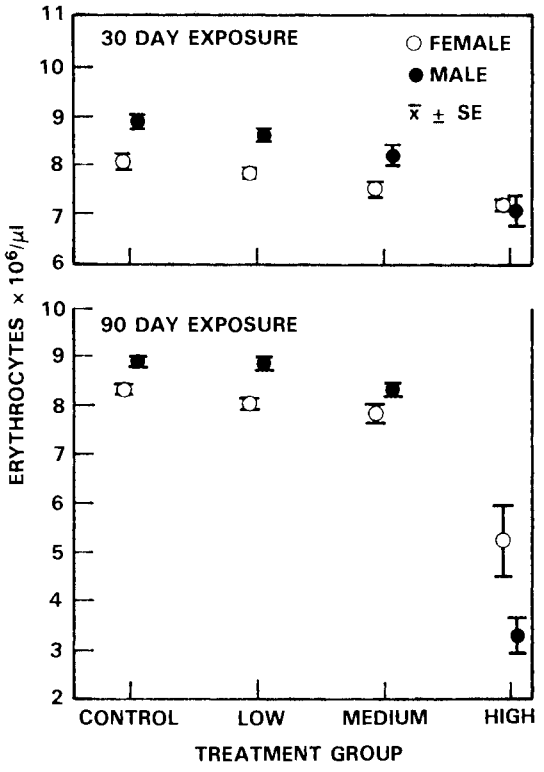

FIGURE 4. Effect of Inhalation Exposure to SRC-II Heavy Distillate on Rat Erythrocyte Levels.

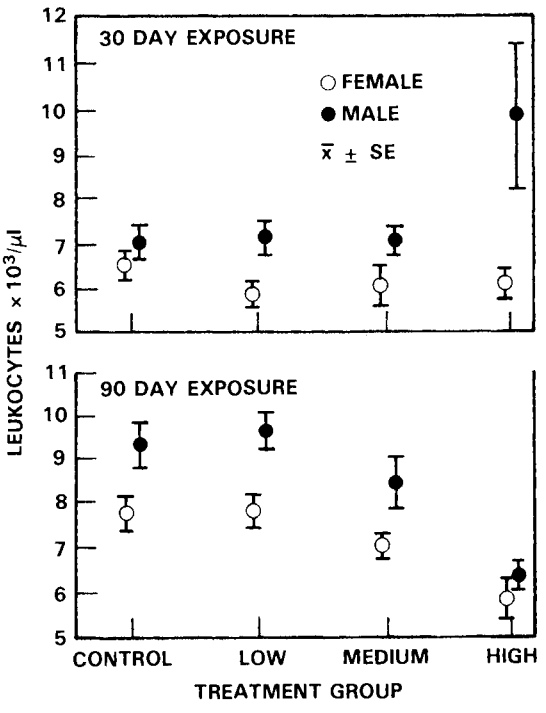

FIGURE 5. Effect of Inhalation Exposure to SRC-II Heavy Distillate on Rat Leukocyte Levels.

Mean lymphocyte values did not change significantly in any of the exposure groups after 30 days, although there was a tendency for a reduction in the females exposed to the high dose (Figure 6). After 90 days of exposure, however, there was a marked depression ($P < 0.05$) in lymphocyte values for both sexes in the high-level exposure groups. The reduction in lymphocyte numbers could result from reduced lymphopoiesis but is more likely the result of increased levels of adrenal corticosteroids, resulting from chronic stress. This speculation is substantiated, to an extent, since there is also a significant reduction in eosinophils, another cell type very susceptible to corticosteroids.

Hematology data for mice were obtained only after 90 days of exposure. The sex-related difference in erythrocyte concentrations observed in the rats was also found in the mice, i.e., female mice of all groups had significantly higher values than male mice. A treatment-related reduction of erythrocytes ($P < 0.05$) was observed only in the high-level exposure group of both sexes. Also in contrast to the findings in rats, no significant differences or treatment-related trends were seen in total leukocyte, neutrophil, or lymphocytes as compared to control rats or among the SRC exposure groups.

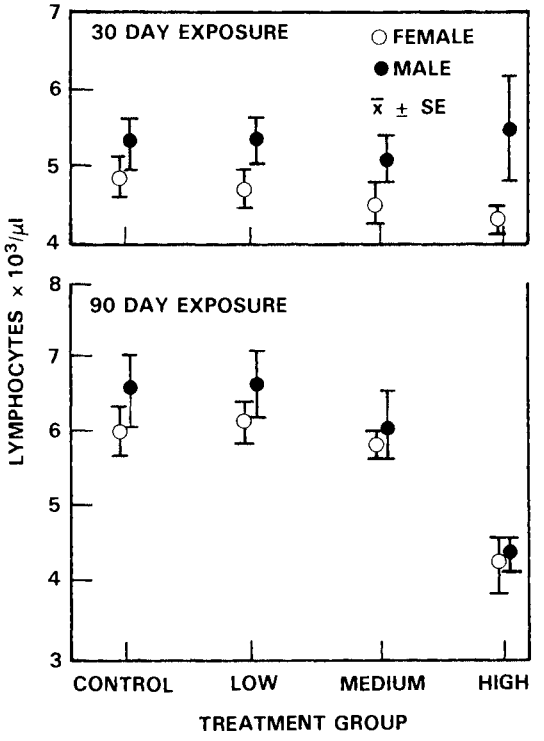

FIGURE 6. Effect of Inhalation Exposure to SRC-II Heavy Distillate on Rat Lymphocyte Levels.

Clinical Chemistry. The following tests were performed after the rats were exposed for 30 and 90 days: protein, albumin, globulin, cholesterol, triglycerides, BUN, glucose, SGPT, lactic dehydrogenase, and total bilirubin. After 30 days of exposure, only cholesterol values seemed to be affected by the SRC exposure, i.e., mean values were significantly higher ($P < 0.05$) in the high-level group than in the controls, or medium- and low-exposure groups.

After 90 days of exposure, total protein concentrations were significantly lower ($P < 0.05$) in both sexes of the high-level group when compared to the control, low- and medium-exposure groups. The rats exposed to high levels of SRC also had significantly lower ($P < 0.05$) albumin and globulin ratios, which were due primarily to reduced albumin concentrations. The latter may be the result of reduced hepatic synthesis (males of the high-exposure group had significantly increased SGPT values) and/or a result of increased urinary clearance, since BUN concentrations were significantly elevated ($P < 0.05$) in the same group of males.

There was a sex-related, statistically significant difference in cholesterol levels: values were higher in female rats of the control, low and medium groups. There was a treatment-related increase in cholesterol in the high-level males that made this group significantly higher ($P < 0.05$) than the other three groups. For females, only the mid-exposure level had cholesterol values significantly higher than controls.

A significant ($P < 0.05$) sex-related increase in triglyceride levels was seen in male rats. In addition, the high-exposure group of both sexes had significantly higher ($P < 0.05$) triglyceride values than their respective controls.

• Health Effects of Synthetic Fuels

Principal Investigator: R. A. Renne

Other Investigators: L. G. Smith and H. D. Tolley

Technical Assistance: S. M. Baze, J. C. Chapman, V. L. Dedmond, S. R. Eldridge, J. S. Hammack, and B. B. Paas

The purpose of this project is to study the potential human health hazards associated with synthetic fossil-fuel technologies. Studies in progress are investigating the carcinogenic potential of cutaneous exposure to various chemically derived fractions or boiling-point distillates of materials and products from the solvent-refined coal technology.

Epidermal carcinogenesis studies are in progress on chemically derived fractions and boiling-point distillates from the solvent-refined coal (SRC) technology. Exposure is complete, and histopathologic examination is in progress of tissues from animals exposed to the chemically derived fraction of SRC-II heavy distillate. Skin-tumor incidence, based on gross observations of these animals, was reported in the 1981 Annual Report.

Studies are in progress on recombined polyaromatic hydrocarbons (PAH) and basic fractions of SRC-II heavy distillate. Results to date (Table 1) do not indicate synergistic activity in the carcinogenic response to these two fractions; skin-tumor latency is similar to that observed in response to the PAH fraction alone (Annual Report, 1981).

Studies are also in progress on samples of nitrosated basic tar fractions of SRC-II heavy distillate to determine if its carcinogenicity is reduced by destruction of primary aromatic amines. This study was prompted by the observation that the mutagenicity of aromatic amine-containing fractions of SRC materials was radically decreased by destroying aromatic amines with nitrosation (Pelroy, PNL Annual Report, 1980). Results to date (Table 1) indicate that nitrosation does not decrease the carcinogenicity of basic tar. This suggests that primary aromatic amines are not the determinant class of carcinogens in this sample.

Epidermal carcinogenesis studies are also in progress on boiling-point distillates of whole-boiling-range material from an SRC-II development unit. Figures 1 and 2 present data on the boiling-point ranges of the samples being assayed, the doses of each material being applied three times weekly, and tumor incidence/latency data as of 9/30/82. These results indicate that the carcinogenic potency of the various boiling-point distillates of this material varies directly with the boiling point.

TABLE 1. Incidence of Grossly Observed Skin Tumors in Mice After Exposure to Recombined or Nitrosated Chemical Fractions of SRC-II Heavy Distillate.

Material	Dose, mg/50 μl		Days on Study	Mice at Risk[a]	No. of Mice with Tumors	Days to First Tumor	Days to Median Tumor	Days to Final Tumor
SRC-II HD: PNA + Basic Fraction	L	0.10	351	47	0	--	--	--
	M	0.50	351	50	5	260	--	--
	H	2.50	351	49	49	143	253	325
Basic Tar + DMSO	H	4.00	267	50	50	78	176	225
Nitrosated Basic Tar + DMSO	H	4.00	267	50	50	94	157	218

[a]Excludes mice that died without developing a tumor at the application site

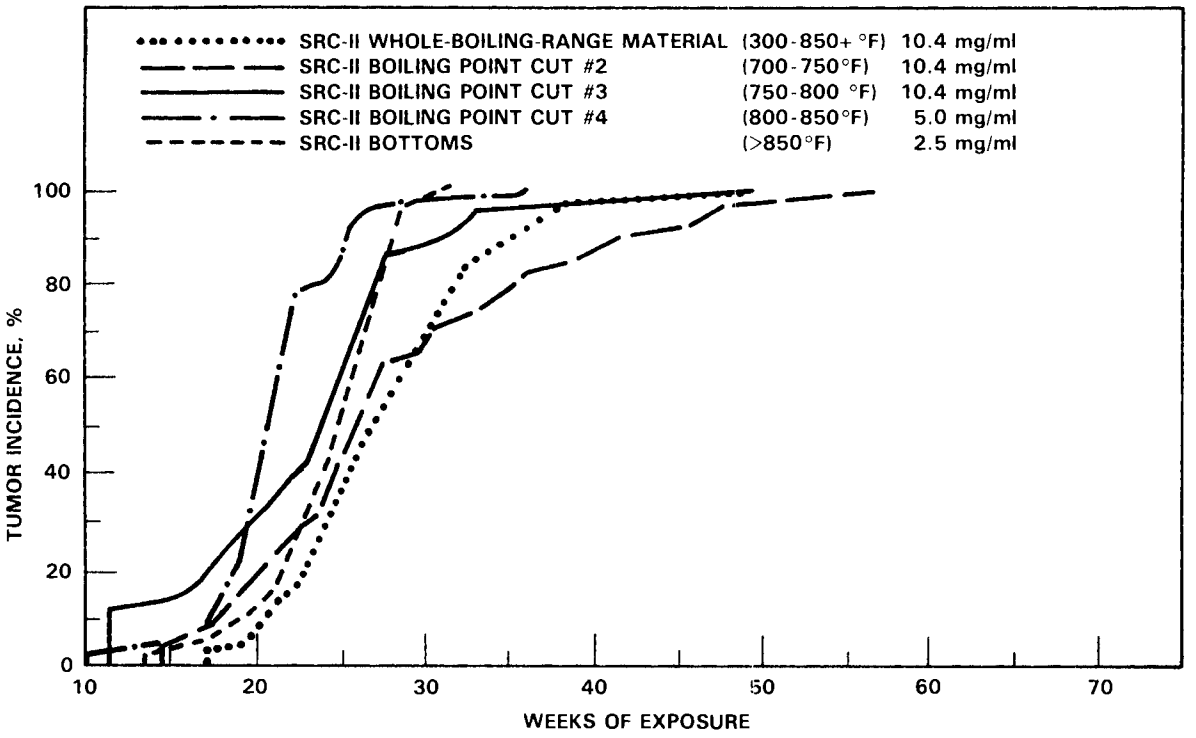

FIGURE 1. Incidence of Grossly Observed Skin Tumors in Mice After Exposure to Whole Material or Boiling-Point Distillates From an SRC-II Development Unit: High Dose Levels.

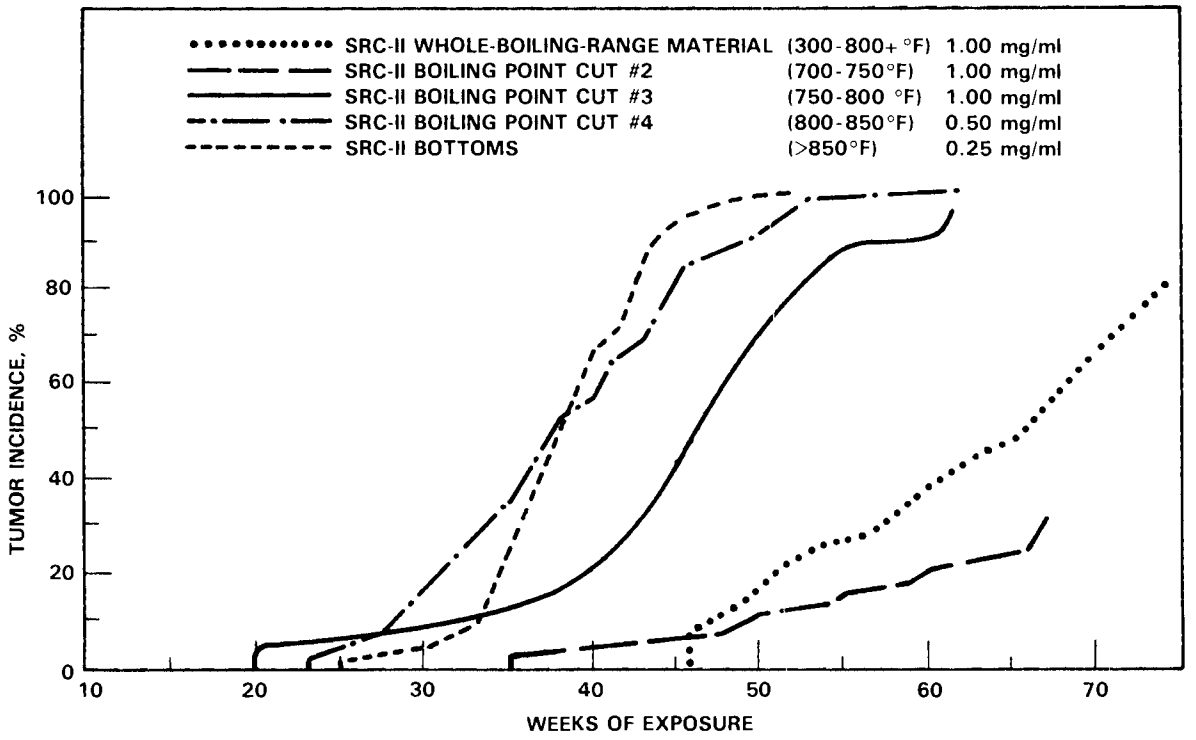

FIGURE 2. Incidence of Grossly Observed Skin Tumors in Mice After Exposure to Whole Material or Boiling-Point Distillates From an SRC-II Development Unit: Middle Dose Levels.

• Perinatal Effects of SRC

Principal Investigator: D. L. Springer

Other Investigators: R. L. Buschbom, D. D. Mahlum, and J. E. Morris

Technical Assistance: J. A. Brower, C. J. Gerdes, T. A. Graham, P. S. Lytz, L. W. McGee, and L. F. Montgomery

Studies were conducted to determine whether exposure in utero to high-boiling coal liquids initiated animals toward tumor development and whether the immune system, in rats, was adversely affected by prenatal exposure to these materials. Heavy distillate (HD) is an active skin carcinogen and contains a high percentage (40%) of polyaromatic hydrocarbons (PAH). Since in utero exposure to PAHs such as dimethylbenzanthracene (DMBA) results in initiation of skin tumor development in the offspring, we conducted studies to determine whether HD also acts as a cross-placental carcinogen. Pregnant mice were gavaged with HD; after delivery, offspring were treated either with dermal applications of 12-0-tetradecanoylphorbol-13-acetate (TPA) or intraperitoneal injections of butylated hydroxyltoluene (BHT), both promoters. Applications of these promoters to mice exposed in utero failed to result in significant increases in skin tumors or lung adenomas.

To evaluate the effects of the coal liquids on the immune system, 2-day-old rats were gavaged with HD, and the responses of peripheral blood, spleen and thymus cells to mitogens were determined. Statistical analysis of these data failed to demonstrate significant changes in the responsiveness of cellular preparations to these mitogens.

Exposure of pregnant rats or mice to heavy distillate (HD), the high-boiling coal liquid (550-850°F) from the SRC-II process, results in cleft palate, small lungs, and diaphragmatic hernia in the offspring (Andrew and Mahlum, Annual Report, 1980); those most severely affected usually die. In order to determine some of the potential consequences of prenatal exposure to HD, we conducted studies to determine whether HD acts as a cross-placental carcinogen and whether the response of the immune system in animals exposed in utero is altered.

Skin-Tumor Experiments

Pregnant CD-1 mice were treated by gavage with 0.5 g/kg of body weight/day of HD in milk on days 14-18 of gestation. After the dams delivered, each litter was randomly assigned to either the 12-0-tetradecanoylphorbol-13-acetate (TPA) or the butylated hydroxytoluene (BHT) experiment. Pups from 4 to 5 litters were assigned to each experimental group to give a total of approximately 40 pups/group. When pups reached 4 wk of age they were treated with the potent skin tumor promoter, TPA. Five micrograms of TPA were applied twice weekly to the shaved dorsal surface, and the animals were observed over a period of 6 mo for the appearance of papillomas.

At 2 days of age, the average number of live pups per litter was 10.2 and 7.6 for the control and treated groups, respectively, indicating that in utero exposure to HD resulted in a mortality rate of 25%. Furthermore, 2-day-old pups from HD-treated dams weighed 25% less than those from the control groups.

After 6 mo of promotion, papillomas were observed on three animals that received in utero exposure to HD; one papilloma developed on a control animal that received only TPA (Table 1). Papillomas were not observed on animals exposed to HD that did not receive the promoter. It therefore appears that in utero exposure to HD is not very effective as an initiator of skin tumor development.

TABLE 1. Skin Tumor Development in Mice Exposed In Utero to Heavy Distillate and Promoted With TPA.[a]

Initiator	Promoter	No. Mice	No. Mice w/Tumors	Average No. Tumors/ Tumor-Bearing Mouse
Control	Control	34	0	--
--	TPA	40	1	1
HD[b]	--	34	0	--
HD	TPA	40	3	1

[a] 12-0-Tetradecanoylphorbol-13-Acetate
[b] Heavy distillate

Lung-Tumor Experiments

For the BHT experiment, dams were exposed in a manner identical to those in the first experiment. Offspring were given weekly intraperitoneal (IP) injections of BHT in corn oil (300 mg/kg) to promote lung tumor development. This treatment began when pups were 4 wk of age and continued for 18 wk. One week after the last BHT treatment, pups were killed, and the number of adenomas located on the surface of the lung was determined. A positive control group of adult animals was given a single IP injection of urethane, followed by weekly injections of BHT. Urethane was selected because it is known to initiate the development of lung adenomas. The HD-treated dams were also treated with BHT as an additional positive control group, starting at 4 wk after delivery.

The average number of live pups per litter at 2 days of age was 18% less for animals in the treated group when compared to the controls. In addition, body weights for 2-day-old animals in the HD-treated group were less than those of controls.

Examination of animals from the positive control group indicated that 57% (8 of 14) of the urethane-initiated mice developed adenomas, averaging six adenomas per tumor-bearing mouse (Table 2). Administration of BHT alone failed to result in the appearance of tumors. These data indicate that urethane is an initiator of lung adenomas, and that BHT promotes adenoma development.

Adult animals treated by gavage with HD failed to develop lung adenomas. Two animals that received in utero exposure to HD followed by BHT promotion had one adenoma each at sacrifice; this tumor incidence was not statistically significant. These data suggest that in utero exposure to HD is not very effective as an initiator of either skin or lung tumor development for mice. It should be noted that this lack of initiating activity occurred at HD doses that resulted in reduced body weight of the animals at birth and caused substantial mortality (20%) of the neonates.

TABLE 2. Lung Tumor Development for Mice Exposed by Gavage or In Utero to Heavy Distillate and Promoted With BHT.[a]

Initiator	Promoter	No. Mice	No. Mice w/Tumors	Average No. Tumors/ Tumor-Bearing Mouse
Urethane[b]	BHT	14	8	6
--	BHT	36	0	--
HD[c]	--	8	0	--
HD[d]	BHT	6	0	--
HD	--	40	0	--
HD	BHT	35	2	1

[a] Butylated hydroxytoluene
[b] Adult animals were given a single ip injection of urethane (1 mg/g body weight) to serve as a positive control group.
[c] Heavy distillate
[d] Adult animals exposed by gavage to 0.5 g/kg/day of HD.

Immunological Studies

The effects of in utero exposure to high-boiling coal liquids on the immune system were reported last year. Those studies have been extended to include treatment of neonates and observation for growth, survival, and response of the immune system to mitogens. In addition, another group of animals were given Aroclor-1254; this material was chosen as a positive control since it is known to cause changes in the response of the immune system. Lymphocyte function was measured by mitogen-induced lymphocyte activation assays (in vitro correlate of cell-mediated immunity), using cells from peripheral blood, spleen, and thymus. Mitogens used in the study were concanavalin-A (Con-A) and phytohemagglutinin (PHA), both specific for T-cell populations; and pokeweed mitogen (PWM), which stimulates B-cells in addition to a small fraction of the T-cell population. Cell activation was measured by assessing the incorporation of ^{125}I-iododeoxyuridine (^{125}I-UdR) into newly synthesized DNA during the final 20 hr of a 3-day culture period. The distribution of T-lymphocytes in spleen-cell preparations was determined by measuring the uptake of ^{3}H-uridine.

Immunological measurements were conducted on 42-day-old male rats that had been gavaged with HD at 2 days of age at doses of 0.25, 0.50, 0.65, or 1.0 g/kg, or with Aroclor at doses of 0.5 or 1.0 g/kg. Rat pups were randomly assigned so as to include one pup from each litter in each treatment group. They were given a single gavaged dose of HD in approximately 0.1 ml of milk at 2 days of age, and were observed and weighed at 7, 14, 28, and 42 days of age. At sacrifice, body weight and the weights of spleen, lung, thymus, brain, kidney, and liver were determined.

Body weights of male rats treated with HD (Table 3) decreased in a dose-dependent manner. Statistically significant differences were observed between controls and groups treated at doses of 0.5 g/kg and greater through 14 days of age, and at sac-

TABLE 3. Body Weight and Survival of Male Rats Through 42 Days of Age After Exposure to Heavy Distillate or Aroclor at 2 Days of Age.

		Heavy Distillate						Aroclor	
Dose, g/kg Body Weight:	0	0.25	0.50	0.65	0.85	1.0	1.25	0.5	1.0
No. Animals Dosed:	10	12	12	4	5	15	9	3	6
No. Days After Dosing	Body Weight, kg								
2	8.3 ± 0.2	8.0 ± 0.1	8.2 ± 0.2	8.1 ± 0.3	7.9 ± 0.2	8.2 ± 0.1	8.3 ± 0.2	8.2 ± 0.3	8.1 ± 0.3
7	18.5 ± 0.3	16.3 ± 0.4	15.0 ± 0.5[b]	13.4 ± 0.5[b]	10.6	10.4 ± 0.8[b]	9.5	18.2 ± 0.4	16.1 ± 0.5[b]
14	37.2 ± 0.8	34.0 ± 0.6	32.5 ± 0.7[b]	31.2 ± 0.7[b]	28.6	26.4 ± 1.9[b]	23.5	36.1 ± 0.9	32.3 ± 1.0[b]
28	99.2 ± 2.6	91.1 ± 1.9	89.6 ± 2.1	87.6 ± 2.5	84.1	73.0 ± 7.2	62.7	92.2 ± 6.2	83.0 ± 3.7[b]
42	208 ± 4.4	193 ± 3.6	185 ± 2.8[b]	197 ± 9.2	189	166 ± 13.2	162	205 ± 3.8	183 ± 5.9
Percent Surviving	100	100	100	100	20	20	10	100	100

[a] x ± SEM; statistically analyzed using a two-tailed *t*-test
[b] $P \leq 0.05$

rifice. Similar effects were observed for female rats exposed to HD and for males exposed to 1.0 g/kg Aroclor. All animals survived doses of 0.65 g/kg and below. At doses of 0.85 g/kg and greater, the survival rate was 20%, indicating that the LD_{50} for the 2-day-old rat is between these doses. Mahlum (Annual Report, 1978) reported an adult LD_{50} of 3.2 g/kg for HD; our data indicate that the neonatal LD_{50} is about one-fourth that for the adult. Analysis of tissue weight data as percent of body weight did not indicate any significant changes in tissue weights attributable to the treatment.

Immunological data were analyzed using a two-tailed t-test, with independent comparisons of each treatment group to the control group. Using this procedure, significant decreases in the responses of thymus cells to PHA and PWM were observed in rats treated with 1.0 g/kg HD (Figure 1). The response of these cells to Con-A was not significantly different from that of the control group. The mitogen responses of peripheral blood lymphocytes to Con-A and PWM for animals in the 0.65-g/kg group were significantly increased relative to the control group (Figure 2). The response of these cells to PHA also increased when compared to that of controls, although this increase was not statistically significant. There were no significant changes in the response of spleen cells to any of the mitogens as a result of exposure to HD (Figure 1). In Aroclor-treated rats (1.0 g/kg), there was a significantly decreased response for thymus cells to Con-A, PHA, and PWM; at 0.5 g/kg there were no significant changes in the response to the same mitogens. Peripheral-blood lymphocyte responses to Con-A in rats treated with 0.5 g/kg Aroclor were significantly increased when compared to control values. The response to PHA for this group also increased but not significantly.

Even though the altered mitogen responses were observed at certain concentrations of HD and Aroclor, these responses did not appear to follow a dose-response relationship. Statistical analysis of these data for trends with dose confirmed that the effects were not dose-related. Since the observed effects were not dose-related, the data were further analyzed using a one-way analysis of variance. Analysis of the data in this manner indicated that there were no significant differences between the responses of HD and control animals to any of the mitogens for the three tissues studied. Similar calculations for Aroclor-treated animals indicated that the only significant difference was a decreased response for thymus cells to PHA; this effect was apparent for animals given 1.0 g Aroclor/kg body weight. It therefore appears that exposure of 2-day-old animals by gavage to HD failed to identify any dose-related alterations in the responsiveness of T- and B-cells in selected tissues.

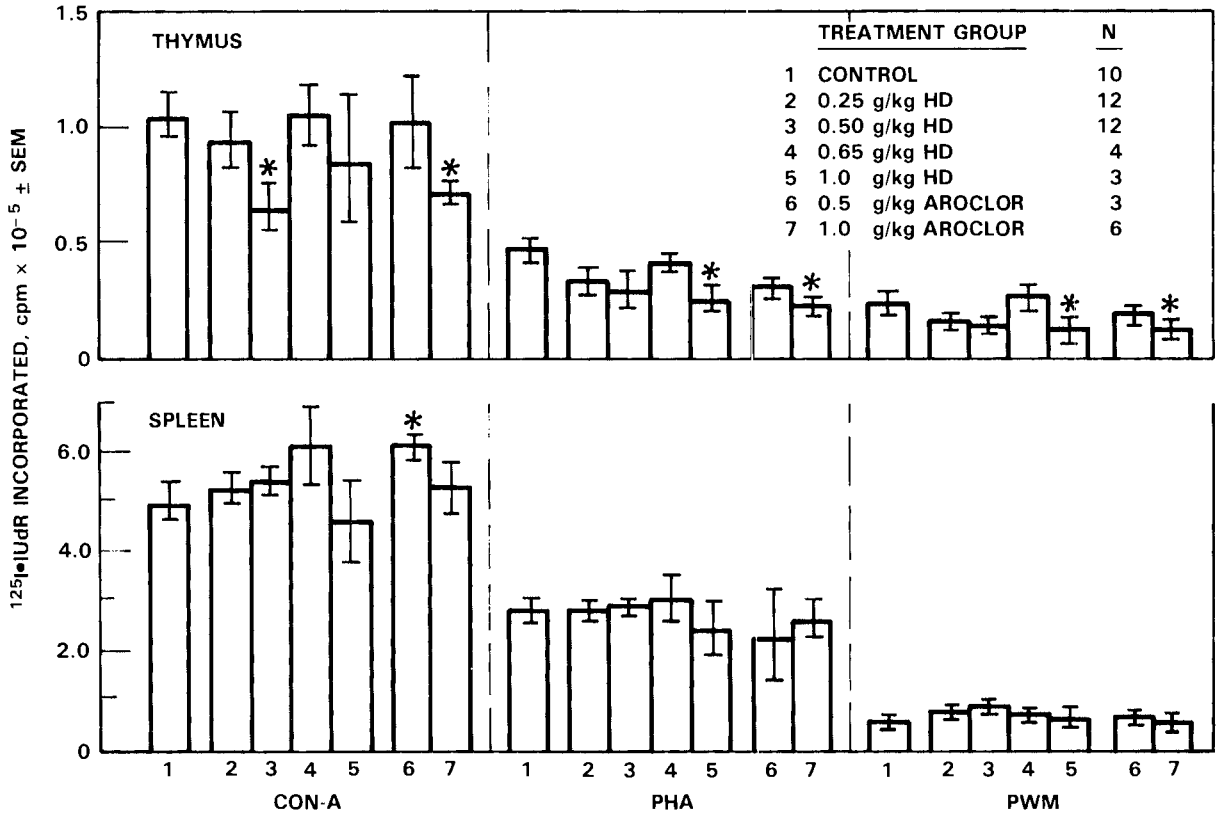

FIGURE 1. Response of Mitogen-Activated Cells From Lymphoid Tissues of 42-Day-Old Rats Gavaged at 2 Days of Age With Heavy Distillate or Aroclor-1254. Data statistically analyzed using two-tailed *t*-test and one-way analysis of variance. The *t*-test analyses calculated as independent comparisons for each treatment group against the appropriate control group (* indicates $P < 0.05$).

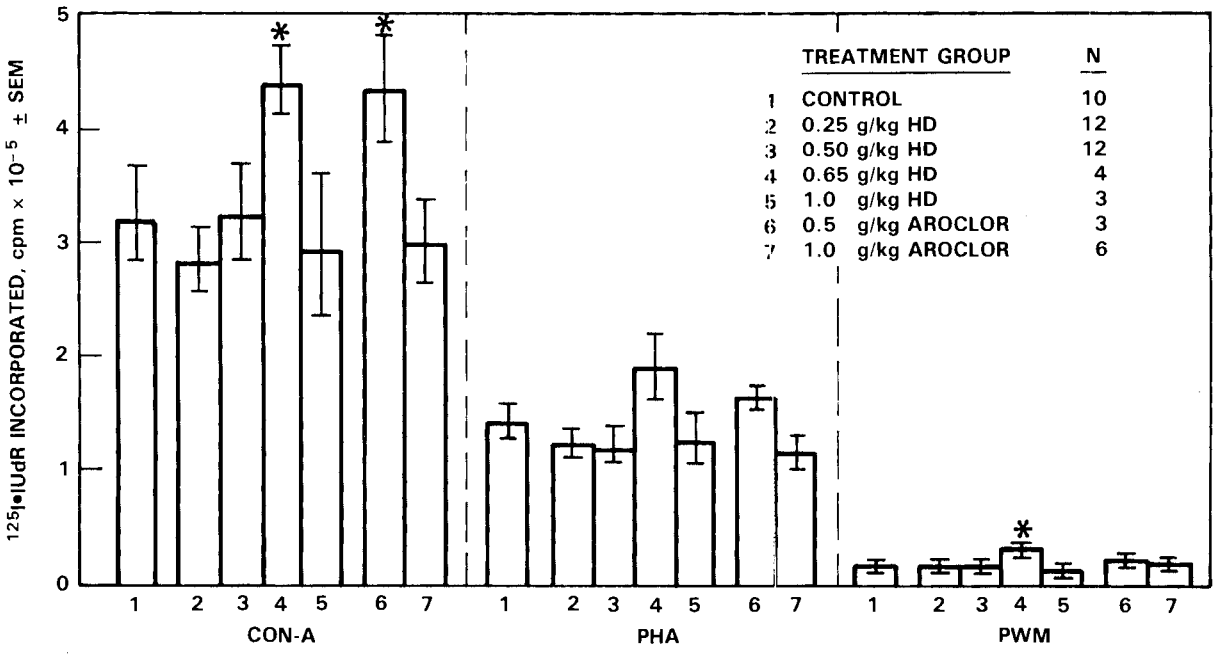

FIGURE 2. Response of Mitogen-Activated, Peripheral-Blood Lymphocytes From 42-Day-Old Rats Gavaged With Heavy Distillate or Aroclor-1254. Data statistically analyzed using two-tailed *t*-test and one-way analysis of variance. The *t*-test analyses calculated as independent comparisons for each treatment group against the appropriate control group (* indicates $P < 0.05$).

• Teratology of SRC-II Materials

Principal Investigator: P. L. Hackett

Other Investigators: M. R. Sikov and D. D. Mahlum

Technical Assistance: R. L. Music, S. A. Watson, J. A. Hastings, L. A. Shippert, and L. I. Benton

Concern over the availability of adequate sources of petroleum has led to a search for alternatives, including development of coal liquefaction methods such as those used in the solvent-refined coal (SRC) process. Data from survey experiments indicated that some SRC-II materials of pilot-plant origin were embryocidal and teratogenic when administered to pregnant rats at doses approximating the maternally toxic level. This project was initiated to quantify the developmental toxicity of these materials. The approach is to develop dose-response relationships, in rodents, to determine if levels of materials that are subthreshold for maternal toxicity can affect intrauterine growth and survival or alter morphogenesis so as to result in malformations or other morphologic changes. These experiments may provide indications of the minimal toxic levels of SRC materials, since the embryo has been found to be a sensitive indicator of the toxicity of many substances.

Initial developmental toxicology survey studies performed with SRC-II coal-liquid samples demonstrated that a high-boiling-range material, designated as heavy distillate (HD), possessed embryotoxic and teratogenic properties when administered to rodents. These biological responses were observed following exposure at midgestation (12 to 16 days) rather than during early organogenesis (9 to 11 days). Subsequent studies employing midgestational exposures of rats to HD by inhalation or by intragastric (IG) intubation provided more definitive information concerning the minimal doses required to produce developmental and maternal toxicity. The studies reported here, utilizing the IG route of administration, were initiated to define the period of embryonic development most sensitive to the exposure of these materials. A separate study that seeks to relate embryotoxic and teratogenic activities of SRC-II materials with their physicochemical properties is also in progress. This study will utilize five limited-boiling-range fractions of an SRC-II material designated as Harmarville process solvent (HPS).

In general, protocols for both studies are similar. The SRC-II materials, their boiling-point ranges, and the administered dose levels are shown in Table 1. Female rats (Sprague-Dawley CD, Charles River) of known gestational age were assigned to treatment groups by formal randomization based on body weight. On each dosing day, suspensions of the HD, HPS, or HPS fractions in milk were prepared immediately prior to IG intubation of a constant-volume dose. All animals were weighed at intervals; at sacrifice, the gravid uterus, with products of conception, was also weighed. This value was subtracted from the body weight to obtain the "extragestational body weight." The excised uterus was opened and the contents examined for number and location of resorptions and live and dead fetuses. Mortality in utero was estimated to have occurred during early, mid-, or late gestation. Live fetuses were weighed and examined for gross defects, visceral malformation, and altered morphologic development of the skeleton. Fetal lungs were examined in situ and were then removed and weighed.

TABLE 1. Boiling-Point Ranges and Dose Levels of SRC-II Materials Administered in Developmental Toxicology Studies.

SRC-II Material	Boiling-Point Range, °F	Fraction of HPS, %	Administered Dose, g/kg/day
Heavy Distillate	550-850	--	0.37
Harmarville Process Solvent (HPS)	300->850	100	0.74
HPS Fraction I	300-700	78.9	0.58
II	700-750	5.5	0.041
III	750-800	7.4	0.055
IV	800-850	3.5	0.026
V	>850	4.7	0.035

HPS Dosing During Midgestation

Since previous developmental toxicology studies with SRC-II materials have been performed with HD, a direct comparison of the effects of HPS with those of HD was necessary. Examination of the boiling ranges of the two materials (Table 1) suggested that comparable responses for HPS and HD could be obtained if HPS doses were at least twice those of HD. At the same

time, studies were initiated to determine the effect of altering the period during which the materials were administered. For these studies, the daily dose level of HPS was held constant, and the animals were dosed either on 12 through 14 days of gestation (dg) or on 15 to 16 dg, so that they were receiving 60% or 40%, respectively, of the total amount of HPS delivered from 12 to 16 dg, the usual dosing interval. Results from these studies are summarized in Tables 2 and 3.

Neither extragestational nor discrete body weight gains were altered by HD or HPS for any dosing regimen (Table 2). The percentage of resorptions, especially during midgestation, appeared to be higher than control values for animals dosed with HD or HPS between 12 and 16 dg or with HPS between 12 and 14 dg; however, differences were not significant (Table 2). Body and lung weights for the 20-dg fetuses tended to be depressed in HD-dosed rats and in rats dosed with HPS from 12 to 14 dg. Those weights were significantly lower in HPS animals dosed from 12 to 16 dg (Table 3). Fetal lung-to-body-weight ratios were significantly lower than control values in all experimental groups except that in which rats were dosed with HPS on 15 to 16 dg.

The incidence of fetal malformations in HPS-treated animals exposed from 15 to 16 dg was similar to that of control rats, but malformations were commonly observed following all other dosing regimens (Table 3). The incidence of "small lungs" (less than 2% of the fetal body weight) in HD-exposed rats was equal to that of rats exposed to HPS from 12 to 16 dg (Table 3). Treatment with either compound affected an average of 70% of the fetuses in all litters. Dosing with HPS from 12 to 14 dg produced a lower incidence of small lungs (40% of the fetuses/litter in 75% of the litters) than did dosing between 12 and 16 dg. This effect is suggestive of a dose response. Preliminary evaluations of fetal small-lung incidence in males and females indicate a possible, but not a statistically significant, sex difference in response to HD or HPS exposure. Following HD exposure, 65% of both sexes had small lungs; however, HPS exposure produced a 70% incidence of small lungs in females but only a 50% incidence in males. Cleft palates occurred more frequently in HPS-dosed than in HD-dosed rats on similar regimens. The incidence of this anomaly in rats exposed to HPS for 3 days (12 to 14 dg) was similar to that of the animals receiving HD for 5 days (75 and 71% of the litters, respectively).

These data indicate that exposure to HD or HPS from 12 to 16 dg produces qualitatively, but not quantitatively, similar results. These variations in response may be due to differences in the concentration of biologically active material in the two SRC-II mixtures. Exposure to HPS during the gestational interval of 12 to 14 days produced a high incidence of fetal anomalies; exposure during 15 to 16 dg caused no apparent fetal response. Further studies are in progress to determine whether an acute teratogenic dose of HPS can be delivered without producing excessive maternal mortality, or whether teratogenicity depends upon a gradual accumulation of metabolically activated SRC-II materials in the fetus.

Dosing with Boiling-Range Fractions of HPS

For studies to determine the toxicity and teratogenicity of five boiling-range fractions of HPS, pregnant rats were dosed, between 12 and 14 dg, with 0.74 g/kg body

TABLE 2. Weight Gains and Intrauterine Mortality Following Administration of Heavy Distillate (HD) or Harmarville Process jSolvent (HPS) to Pregnant Rats.

Material	Dose, g/kg/day	dg Dosed	Number of Rats	Weight Gain, 0-20 dg[a]		Resorptions/Implants, %[a]			
				Body Weight, g	EG Body Weight, g[b]	Early	Mid	Late	Total
Vehicle	--	12-16	6	101 ± 11	34 ± 8	4.8 ± 3.1	0	0	4.8 ± 3.1
HD	0.37	12-16	7	94 ± 7	45 ± 4	7.3 ± 2.5	4.7 ± 3.2	2.6 ± 1.7	14.5 ± 4.3
HPS	0.74	12-16	12	84 ± 6	37 ± 5	6.9 ± 3.5	10.9 ± 2.7	2.3 ± 1.3	20.1 ± 5.3
HPS	0.74	12-14	6	84 ± 4	27 ± 3	4.5 ± 2.0	12.9 ± 5.8	0	17.4 ± 5.7
HPS	0.74	15-16	6	84 ± 5	24 ± 4	4.1 ± 2.8	1.1 ± 1.1	2.7 ± 1.7	7.9 ± 4.2

[a] Mean ± SE

[b] Extragestational (EG) body weight = body weight − weight of pregnant uterus.

TABLE 3. Fetal Body and Lung Weights, and Incidence of Commonly Observed Malformations, Following Exposure to Heavy Distillate (HD) or Harmarville Process Solvent (HPS).

Material	Dose, g/kg/day	dg Dosed	Number of Fetuses/ Litters	Body Weight, g[a]	Lung Weight, mg[a]	Lung/Body Weight, × 100[a]	Incidence of Malformations[b]		
							Small Lung	Cleft Palate	Syn/ Ectrodactyly
Vehicle	--	12-16	83/7	3.3 ± 0.2[c]	110 ± 6[c]	3.4 ± 0.1[c]	1/1	0	0
HD	0.37	12-16	73/7	3.1 ± 0.1[c,d]	54 ± 5[d]	1.7 ± 0.1[d]	54/7	10/5	0
HPS	0.74	12-16	127/12	2.9 ± 0.1[d]	50 ± 3[d]	1.8 ± 0.2[d]	92/12	49/12	10/4
HPS	0.74	12-14	74/6	3.0 ± 0.1[c,d]	76 ± 5[d]	2.3 ± 0.2[d]	23/5	21/4	0
HPS	0.74	15-16	74/6	3.3 ± 0.1[c]	91 ± 2[c]	2.9 ± 0.1[c]	1/1	0	0

[a] Mean ± SE
[b] Number of affected fetuses/number of affected litters
[c,d] Values that do not share a common superscript letter are significantly different ($P < 0.05$) from one another.

weight/day of HPS or with a dose of each boiling-point cut equivalent to its percentage concentration in HPS (Table 1). The animals were sacrificed on 21 dg and evaluated for toxicity and teratogenicity.

Preliminary results from this study are summarized in Table 4. Increased embryolethality and decreased fetal lung weights were obtained following HPS exposure, but no evidence for developmental toxicity or teratogenicity was observed following dosing with any one of the five boiling-range fractions. Since suspensions of the two higher-boiling fractions appeared to coalesce rapidly or, in the case of Fraction V, to be particulate in nature, work is in progress to improve the solubility (and thus, perhaps, the biological availability) of the dosing mixtures. Once this is accomplished, we plan to obtain dose-response relationships and to determine if the biological effects are due to solubility interactions of the individual fractions.

TABLE 4. Intrauterine Mortality and Fetal Measures (Mean ± SE) Following Exposure to Five Boiling-Range Fractions of Harmarville Process Solvent (HPS).

SRC-II Material	Dose, g/kg/day	Number of Fetuses/ Litters	Resorptions/Implants, %				Body Weight, g	Lung Weight, mg	Lung/Body Weight Ratio ×100
			Early	Mid	Late	Total			
Vehicle	--	58/5	4.4 ± 1.8	0	0	4.4 ± 1.8	4.8 ± 0.3	133 ± 5	2.8 ± 0.1
HPS	0.74	15/2	3.6 ± 2.9	37 ± 11.9	0	40.6 ± 4.0	3.5 ± 0.5	67 ± 16	1.8 ± 0.2
HPS-I	0.58	31/3	12.8 ± 6.8	2.6 ± 2.6	0	15.4 ± 8.9	4.5 ± 0.3	125 ± 3	2.8 ± 0.1
HPS-II	0.041	63/5	6.2 ± 2.9	0	0	6.2 ± 2.9	4.5 ± 0.1	126 ± 4	2.8 ± 0.1
HPS-III	0.055	57/5	7.9 ± 3.8	5.0 ± 5.0	0	12.9 ± 7.2	4.4 ± 0.3	124 ± 16	2.8 ± 0.1
HPS-IV	0.026	60/5	9.2 ± 1.4	0	0	9.2 ± 1.4	4.5 ± 0.2	122 ± 5	2.8 ± 0.1
HPS-V	0.035	13/3	7.4 ± 7.4	0	0	7.4 ± 7.4	4.5 ± 0.3	138 ± 5	3.1 ± 0.1

• Effects of Pollutant Metals

Principal Investigator: H. A. Ragan

Other Investigators: G. A. Apley and B. A. Denovan

Technical Assistance: K. H. Debban, M. C. Perkins, and J. K. Sweeney

The in vitro cloning efficiency of granulocytic stem cells from mouse bone marrow was completely inhibited or markedly reduced by lead and by materials from shale oil and coal liquefaction processes.

The emphasis on this project has been changed from absorption of pollutant metals by intact animals to a study of the effects of metals and fossil-fuel materials on hematopoietic stem cells.

Information regarding the hematologic effects of fossil-fuel materials is scarce. However, it is known that the general lipid solubility of hydrocarbon mixtures allows them to be absorbed through respiratory epithelium, mucous membranes, gastrointestinal epithelium and epidermis. Following these various routes of exposure, petroleum products have been found in most tissues of rodents, primates and man. Thus, it is reasonable to assume that hematopoietic tissue is at risk from exposure to fossil-fuel products.

We have performed pilot studies investigating the potential myelotoxic effects of synthetic fuels and metals on the hematopoietic stem cells in culture systems. These pilot studies were concerned with the growth and differentiation of granulocytic progenitor cells from mouse bone marrow, using modifications of a soft-agar culture technique. The colony stimulating factor (CSF) used in these assays was serum from mice injected with Salmonella endotoxin, i.e., post-endotoxin serum (PES). Each lot of PES was assayed and added to culture plates so that 50-75 x 10^3 nucleated marrow cells would result in 25-50 colonies in 1.0-ml cultures of marrow from control mice. For these studies, marrow was flushed from the femurs of several mice, using tissue culture media devoid of magnesium and calcium, to help prevent clotting. Cells were pooled and counted using a model ZH Coulter Counter. Eight cultures per material to be tested were incubated for 7 days at 37°C in 5% CO_2 and 100% humidity. The numbers of colonies (>50 cells) and clusters (<50 cells) were then determined.

A known myelotoxic pollutant (lead) was incorporated into the culture media to evaluate the effect on mouse marrow cells in vitro. It has been shown that children intoxicated by lead have marrow concentrations of lead ranging from 4-35 mg/100 g of marrow. After determining that young adult mice had a femoral marrow weight of about 2.1 ± 0.4 mg, lead (as the acetate) was added to mouse marrow cultures at concentrations equivalent to those reported in children, i.e., 12.5, 25.0, and 50.0 mg of lead/100 g of marrow. These concentrations reduced granulocytic colony formation by 40, 54, and 60%, respectively, compared to control cultures without lead.

As a continuation of these pilot studies, the effects of various fractions and materials from shale oil and coal liquefaction processes were determined in the marrow culture system. Complete inhibition of granulocytic colony formation occurred with the following shale oil fractions: basic fraction at 620 mg/100 g of marrow, polynuclear aromatic hydrocarbons at 1240 mg/100 g of marrow, and the neutral fraction at 4960 mg/100 g of marrow. Crude shale oil at 4960 mg/100 g of marrow reduced the number of granulocytic colonies by 60% compared to control cultures of the same bone marrow suspension. Next, fractions from the SRC-I process were evaluated in the culture system. The following process materials completely inhibited colony growth: wash solvent at 4960 mg/100 g of marrow, process solvent at 12,400 mg/100 g of marrow, and light oil at 12,400 mg/100 g of marrow. The concentrations of shale oil and SRC fractions selected were those reported by investigators at this laboratory to reduce growth 50% in VERO monkey kidney cells in tissue culture (Frazier et al., Annual Report, 1979).

It should be noted that the synfuel materials used in this study are of pilot-plant origin and may not be representative of materials that would be produced in commercial facilities.

Because the fossil-fuel stock-solution fractions used for these studies were in a dimethylsulfoxide (DMSO) solution, it was important to determine if DMSO might be inhibitory to granulocytic colony formation

in vitro. The final concentration of DMSO in the culture system, when included with the fossil-fuel fractions, was 0.12%. To test the effects of DMSO, mouse bone marrow was cultured with 0.12% DMSO, along with control cultures not containing DMSO. There was a significant reduction in numbers of colonies formed in those culture dishes containing DMSO:

Control (n = 20 cultures)	DMSO (n = 16 cultures)	P
No. Colonies: 25.2 ± 7.5	No. Colonies: 14.9 ± 3.1	<0.01
No. Clusters: 47.3 ± 10.3	No. Clusters: 36.1 ± 8.9	<0.001

In addition to the culture of granulocyte-macrophage precursors, techniques for cloning erythrocyte and megakaryocyte progenitor cells have now been established in our laboratory. This will permit a comparison of in vitro effects on hematopoietic stem cells with those observed in intact animals.

Recently, mice were administered 0.8 mg/kg of heavy distillate from SRC-II process in DMSO by intraperitoneal injection. Control mice received DMSO only. Portions of each group were killed 4, 10 and 19 days after injection to examine the effects on blood, bone marrow, and the cloning efficiency of granulocyte, erythrocyte and megakaryocyte precursors. In addition, syngeneic mice were given 900 R total body irradiation, then injected with bone marrow from the SRC- or DMSO-injected mice to examine effects on the ability of the pluripotential hematopoietic stem cell to repopulate the spleen (colony-forming-unit-spleen assay of Till and McCulloch). These data are presently being evaluated.

Conservation

CONSERVATION

This section reports on progress made on projects concerned with potential health effects of exposure to static and 60-Hz electric fields from high-voltage transmission systems. They seek to determine whether such fields can produce effects in biological systems and develop a dosimetric basis for extrapolating effects from laboratory studies on animals and cellular systems to humans.

These projects are part of the Pacific Northwest Laboratory bioelectromagnetics program and are complemented by ongoing 60-Hz electric fields in laboratory rodents, sponsored by the DOE Office of Electric Energy Systems, and in miniature swine, sponsored by the Electric Power Research Institute.

• Teratology of Guinea Pigs Exposed to Electric Fields

Principal Investigator: L. B. Sasser

Technical Assistance: J. A. Cushing and T. A. Pierce

The objective of this research is to determine whether chronic exposure of two successive generations of guinea pigs to 60-Hz electric fields will produce birth defects and/or fetal anomalies among their offspring. Experiments will investigate development of a guinea pig model to evaluate factors involved in producing teratogenic effects observed in swine exposed to electric fields. Currently, 144 female guinea pigs are being exposed or sham-exposed to electric fields; the exposure will continue for 9 mo, through two pregnancies. The female offspring of the first pregnancy will be reared in the field and mated at 5 mo of age. Mating behavior, reproductive performance, and teratology of offspring will be evaluated. If the model proves successful, results will be helpful in understanding the effects of electric fields on reproduction and development.

In a previous study of swine exposed to 60-Hz electric fields, the main effects were an apparently increased incidence of teratism in fetuses of the second litter of F_0 sows (conceived after 1.5 years of exposure), a mating deficit in females of the F_1 generation, and an increased incidence of birth defects among the subsequent offspring of females of the F_1 generation.

It would be advantageous, from the standpoint of cost and space requirements, to study these phenomena in a smaller species. The guinea pig was preferred to the rat because it has a longer gestation period (68 versus 20 days), is more mature at birth, and has a longer period of tissue differentiation during development. Also, in the guinea pig, malformations are induced by a variety of teratogens.

This study is designed to mimic conditions in the previous swine study using an appropriate factor to scale physiological events in swine to the guinea pig. Female guinea pigs (72 exposed and 72 sham-exposed) are currently maintained in a 60-Hz electric field at 100 kV/m (unperturbed) for 19 hr per day. After 40 days of exposure, females (F_0) are mated with males that have not been previously exposed to the field. Female offspring from this mating (F_1) will be reared in the electric field and mated with unexposed males at 5 mo of age. The dams (F_0) will be bred for a second time 168 days after the first breeding. Mating behavior, reproductive performance, and teratology of near-term fetuses will be evaluated.

The 40-day premating exposure period is now complete, and mating is in progress. Each female is examined daily for evidence of vaginal membrane opening, an event associated with estrus that lasts for 3 to 4 days of the 16-day estrus cycle. These females are caged with males during a 5-hr, field-off period each day. The restricted cohabitation period (due to exposure requirements) results in reduced breeding efficiency, therefore mating will continue through at least five estrus cycles (80 days) to maximize the fraction of the population that mate successfully. Pregnant guinea pigs will litter early in FY 1983; F_0 females will be mated for a second time in mid-FY 1983. The F_1 females will be mated during the second half of FY 1983 at 5 mo of age. The experiment will probably be completed in FY 1983.

The results of this study should provide adequate data to decide whether the guinea pig is a good system in which to perform in-depth studies. If the guinea pig model is validated, it will possible to perform a variety of experiments to evalute the factors contributing to effects and to establish a dose-response curve (i.e., relationship of exposure intensity and duration to the effect). If this species does not show effects similar to those seen in swine, the data may provide suggestive evidence that the effects observed are unique to swine and cannot be extrapolated to other species, including man.

• Genetic Effects of Electric Fields

Principal Investigator: G. L. Williams

Technical assistance: L. K. Fritz

Sixty-hertz electric fields at strengths up to 100 V/m have been shown to produce no detectable mutagenic effects as measured by the classical Ames *Salmonella* test. Previously demonstrated effects of electric field exposure on the spontaneous induction of lysogenic viruses are shown to result from irreversible changes confined to the first few minutes of exposure following induction of the virus genome. The phenomenon is due to increased production of virus particles by cells already induced rather than to induction of additional cells. We propose an increase in the efficiency of virus DNA replication to account for our observations.

Experimentation on other molecular processes involving genetic material suggests that there is no generalized effect from the 60-Hz electric field on recombination, replication and repair of DNA. The virus-related effects appear to be highly specific and may be related to the specific membrane linkage of virus DNA during its replication.

Results from some experiments at this laboratory indicate that electric fields may act synergistically with mutagenic chemicals as a result of an effect on specific cellular DNA repair systems (recombination repair). More data are required, however, to support this speculation.

Single-burst experiments were conducted to determine the number of viable virus particles produced by induction of integrated lambda virus in exposed and sham-exposed Escherichia coli cells. The following results were obtained:

- Exposed: 289 ± 17 viruses produced by single induced cell
- Sham-exposed: 124 ± 21 viruses produced by single induced cell.

This difference accounts fully for the observed increase in production of viable virus particles which we reported last year. No evidence for induction of additional cells above the spontaneous level was observed for electric-field-exposed cells. The effect is due entirely to increased productivity of cells already induced. The previously observed substrain specificity of the effect (Figure 1) and the absolute upper limit (Figure 2) of a threefold increase in virus yield are consistent with such a process.

Large numbers of cells are synchronously induced by ultraviolet (UV) exposure. By exposing UV-induced cells (at various times and durations prior to, during and after

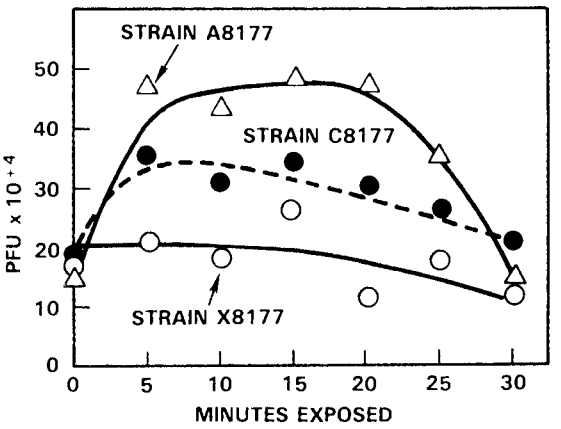

FIGURE 1. Output of Progeny Phage Measured in Plaque-Forming Units (PFU), From Three Substrains of 8177 Cells Exposed to 60-Hz AC Electric Field, 120 V/m. Cells were exposed between time 0 and 27 min.

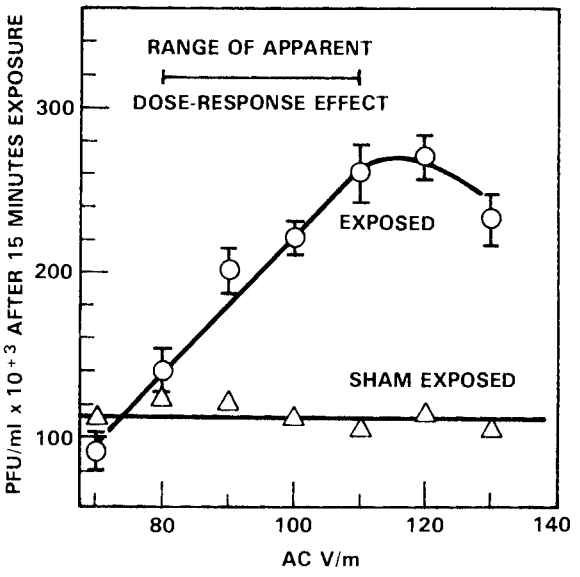

FIGURE 2. Output of Progeny Phage From Strain 8177 Cells Exposed to Various Voltages of AC Current.

the UV exposure) to electric fields and comparing the increase in virus yield, we determined that only electric field exposure during the first 2 min following induction produced the characteristic observable effect (Table 1).

At a molecular level, the only events occurring in this time interval are: 1) the induction event itself, and 2) the onset of replication of virus DNA. The latter requires a specific, stable association of viral DNA with the cell membrane. Progeny of field-exposed bursts are identical to progeny of sham-exposed bursts (Table 2).

TABLE 1. Induced PFU/ml Following 20-sec UV Irradiation. Samples were exposed to inducing effects of short-wave UV for 20 sec at t = 0 min, and exposed to electric field (60 Hz, 120 V/m). See test for details.

Sample	Duration of Electric Field Exposure,[a] min	PFU/ml (Phage produced)
Control	None	$436 \pm 25 \times 10^6$
Pre-exposed	−5 to 0	$479 \pm 61 \times 10^6$
Induction-Exposed	0 to + 2	$975 \pm 118 \times 10^6$
Postexposed	+2 to +10	$487 \pm 35 \times 10^6$
Continuous	−5 to +10	$1122 \pm 87 \times 10^6$

[a]Induction by UV light occurred in all cases at t = 0 min

TABLE 2. Infectivity of Phage Progeny From Electric-Field-Exposed Cells. Phage derived from exposed or sham-exposed 8177 cells were tested for lytic infectivity in 6340 cells and for lysogenic infectivity in K12 cells.

	Test Strain	Burst Size
Exposed Parental Host	6340	162 ± 27
	K12	96 ± 18
Sham-Exposed Parental Host	6340	154 ± 35
	K12	110 ± 21

Lysogenic viruses in Bacillus subtilis, which replicate DNA independently of the cell membrane, were not noticeably affected by electric field exposure. Phage phi 105, and SP01 were tested in appropriate host strains.

Other DNA-related molecular events that showed no detectable field-associated effects were:

- UVR excision repair (error-free) in E. coli
- SOS DNA repair (error-prone) in E. coli
- Uptake and expression of homologous transforming DNA (in B. subtilis).

Experiments were inconclusive in the following areas:

- Postreplication (recombination) repair in E. coli
- Integration of transforming DNA (genetic recombination) in B. subtilis
- Synergistic effects in E. coli (simultaneous exposure to chemical mutagens and the electric field).

In this respect, it is interesting that recombination repair, but not excision repair or SOS repair, involve obligatory association with the cell membrane, as does the integration of transforming DNA. These associations are structurally analogous to the membrane association of replicating lambda virus. Synergistic effects on mutagenicity, if they exist, might be explained mechanistically by effects on postreplication or by some other DNA repair system.

• Electric Field Dosimetry: Characteristics of the Fields Induced in a Human Phantom Exposed to 60-Hz Electric Fields

Principal Investigator: W. T. Kaune

Technical Assistance: W. C. Forsythe

Data on electric fields and current densities inside the bodies of animals and humans are needed to relate electric-field exposures in laboratory experimental animals to those experienced by humans in the vicinity of electric-power generation, transmission, and distribution systems. We report here extensive data on current densities induced in models of humans exposed to 60-Hz electric fields.

A number of projects are underway to investigate whether electric fields can produce biological effects in laboratory animals. However, before data from these projects can be used for the assessment of possible risks to humans exposed to power-frequency electric fields, dosimetric characterization for humans and animals is needed. The purpose of this project is to determine the electric fields and current densities induced inside the bodies of humans and animals exposed to such electric fields.

Preliminary measurements in human, pig, and rat phantoms were given in the 1981 Annual Report. In this report we give a summary of our experimental technique and present the results of a detailed characterization of the induced fields in human phantoms.

Conducting Models

Conducting models were made by filling hollow, styrofoam molds (which do not significantly perturb an applied electric field) with saline solution. The styrofoam molds are prepared in halves, corresponding to the two halves of a human body divided along the frontal plane, or to the two halves of a rat or pig, divided along a sagittal plane. A field probe was positioned inside the mold, and the two halves were reassembled, using paraffin as a glue/sealant. Stainless steel pads, attached to the bottom of each foot, were instrumented so that the current passing through each foot could be measured and controlled.

Electric-Field Measurement System

Electric-field measurements in grounded saline models were made using probes that sensed, by conductive coupling, the voltages at three points forming an equilateral right triangle (Figure 1). Wires from the probe were routed vertically down through the feet of the model and to a remote, lock-in amplifier, which was used to measure the magnitude and phase of the differential voltages between the probe tips. With this system we have measured induced vertical and horizontal electric fields in hemispherical, hemispheroidal, and cylindrical saline models; for these models, measured electric fields agree with theoretical calculations to within 5%, proving, experimentally, the accuracy of the measurement technique.

Induced-Current Measurements

Our data are presented in terms of induced current density, rather than induced electric field strength, because current density is independent of the particular conductivity of the saline-filled model (typically, 0.01 S/m for our model). Current density can, therefore, be more easily extrapolated to other models or to live animals. (Current density is the current crossing a unit area, oriented perpendicularly to the direction of current flow.) Electric field strength can be obtained by dividing the current density by the conductivity of the body.

Vertical and horizontal current densities measured in the neck and upper torso of the human body exposed to a 10-kV/m electric field are given in Figures 2-4. Data were taken with the body grounded equally through both feet and also grounded through only one foot. Induced current densities for these two grounding configurations were almost equal; only the data with both feet grounded are given in the figures. Very substantial enhancements in the horizontal current density were observed in the axillae.

Figures 5-8 give vertical and horizontal current densities induced in the lower torsos of the human body exposed to a 10-kV/m electric field. Since that region of the body is closer to the legs, whether the body is grounded through one or two feet is significant. This is most clearly illustrated in Figures 7 and 8. With both feet grounded (Figure 7), the horizontal induced current density in the lower pelvic area is almost zero, as would be expected on the basis of symmetry considerations. However,

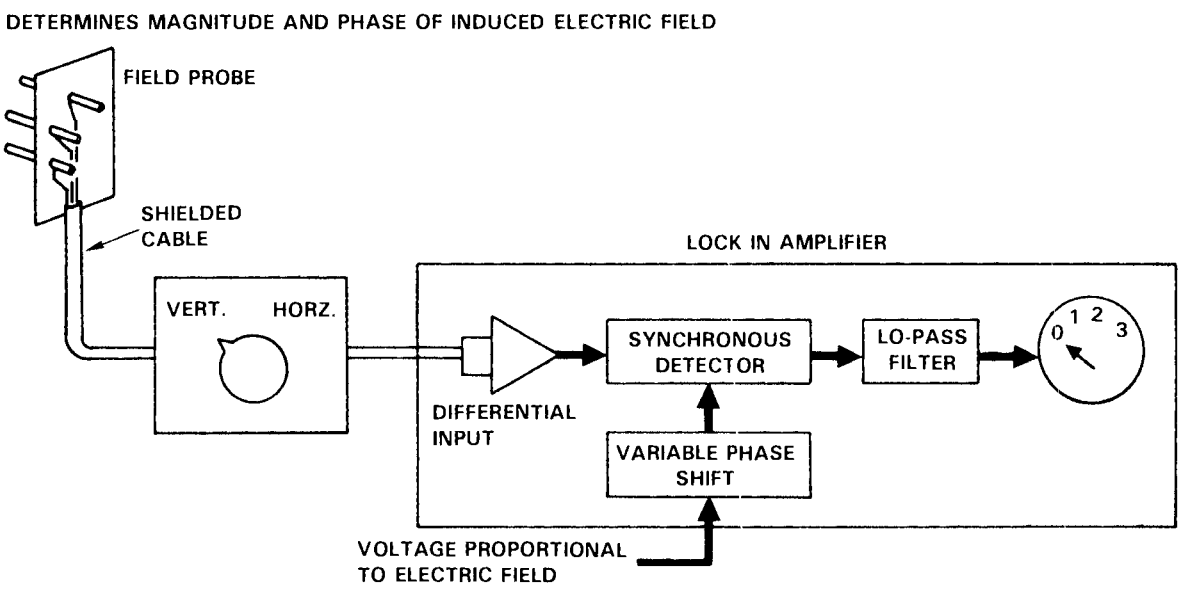

FIGURE 1. System for the Measurement of Electric Fields Induced in Saline Models Exposed to External 60-Hz Electric Fields. A lock-in amplifier was recently added to the system to enable us to measure the phase of the induced electric field, as well as its magnitude, and to give better rejection of noise signals.

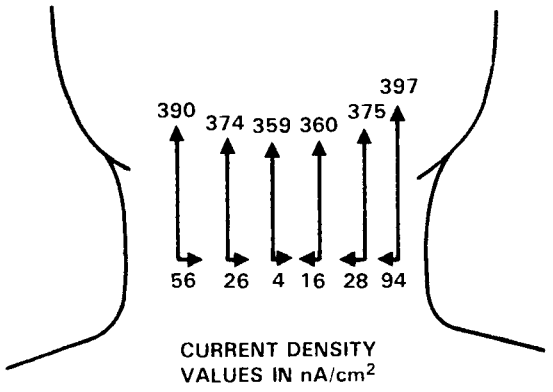

FIGURE 2. Measured Current Densities (nA/cm^2)in the Midline Frontal Plane of a Conducting Model of a Human Neck Exposed to a 10-kV/m, 60-Hz Electric Field. Both feet of the model were grounded so that equal currents passed through them.

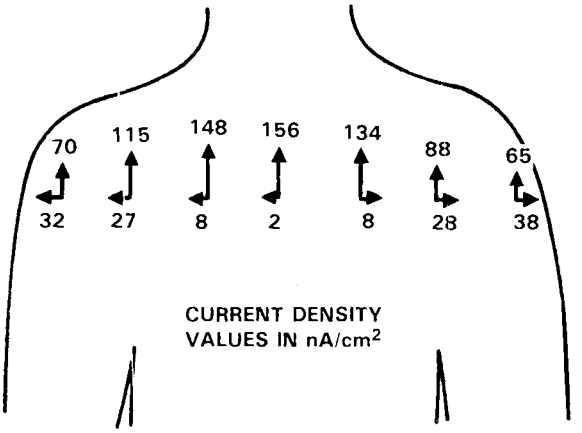

FIGURE 3. Measured Current Densities (nA/cm^2) in the Midline Frontal Plane of a Conducting Model of a Human Chest Exposed to a 10-kV/m, 60-Hz Electric Field. Both feet of the model were grounded so that equal currents passed through them.

with only one foot grounded, the value rises to 770 nA/cm^2. It is noteworthy that this value is the largest value observed in the torso!

Discussion and Future Plans

Typical tissue conductivities in the human body are about 0.1 S/m. Thus, current densities observed in the torso of the human model (100-800 nA/cm^2) exposed to a 10-kV/m electric field correspond to induced electric fields in the approximate range of 0.01 to 0.08 nA/cm^2.

Initial measurements in rat and pig phantoms were presented in the 1981 Annual Report. Comparison of these data to the hu-

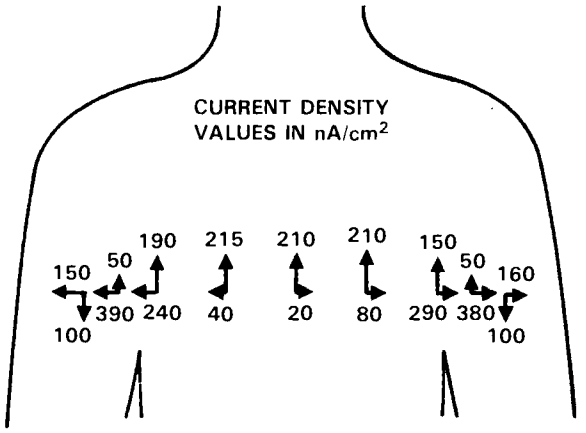

FIGURE 4. Measured Current Densities (nA/cm^2) in the Midline Frontal Plane of a Conducting Model of a Human Torso Exposed to a 10-kV/m, 60-Hz Electric Field. Both feet of the model were grounded so that equal currents passed through them.

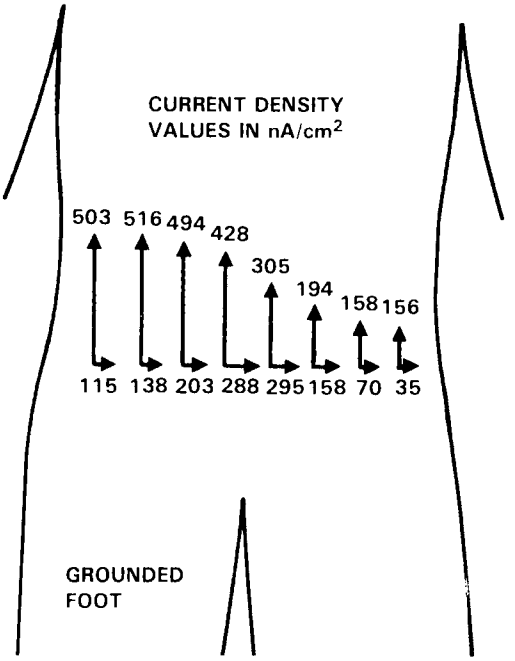

FIGURE 6. Measured Current Densities (nA/cm^2) in the Midline Frontal Plane of a Conducting Model of a Superior Human Pelvis Exposed to a 10-kV/m, 60-Hz Electric Field. Only one foot was grounded.

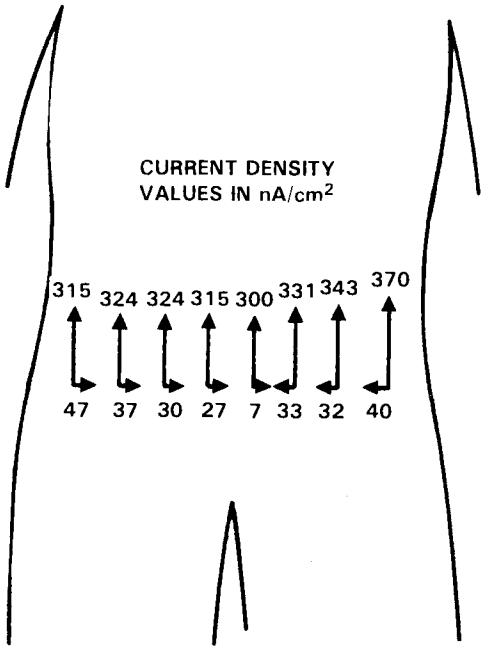

FIGURE 5. Measured Current Densities (nA/cm^2) in the Midline Frontal Plane of a Conducting Model of a Superior Human Pelvis Exposed to a 10-kV/m, 60-Hz Electric Field. Both feet of the model were grounded so that equal currents passed through them.

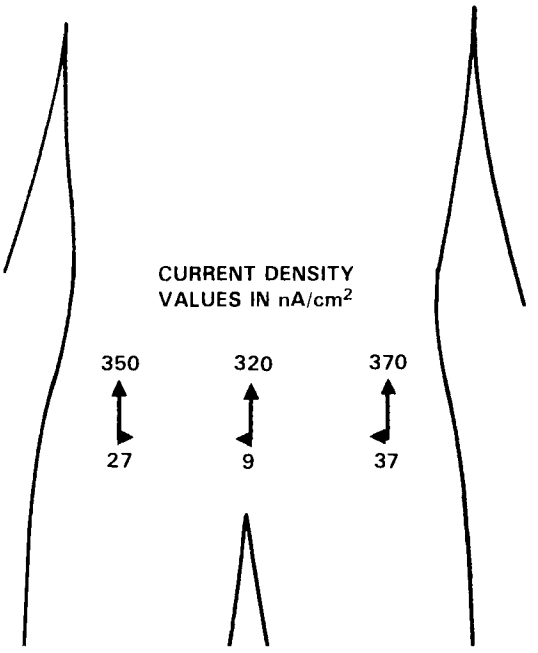

FIGURE 7. Measured Current Densities (nA/cm^2) in the Midline Frontal Plane of a Conducting Model of an Inferior Human Pelvis Exposed to a 10-kV/m, 60-Hz Electric Field. Both feet of the model were grounded so that equal currents passed through them.

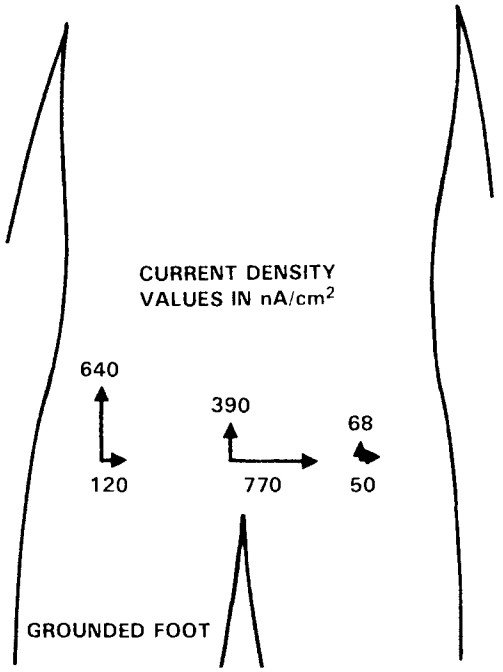

FIGURE 8. Measured Current Densities (nA/cm^2) in the Midline Frontal Plane of a Conducting Model of an Inferior Human Pelvis Exposed to a 10-kV/m, 60-Hz Electric Field. Only one foot was grounded.

man data given here demonstrate that induced currents in the human are much larger, by about a factor of 10, than induced currents in rats and pigs exposed to the same external field.

In the next year, we plan to characterize the fields in rats and pigs to a level of detail comparable to that given here for the human body. With these data, quantitative dosimetric comparisons between animals and humans can be made. In addition, we plan to begin measurements in live rats to determine the effect of varying tissue impedances on the distribution of induced currents.

• In Vitro Effects of Electric Fields

Principal Investigator: M. E. Frazier

Other Investigators: W. T. Kaune and J. E. Samuel

This project seeks to measure cytotoxicity, DNA damage and/or specific gene mutation resulting from exposure of mammalian cells (Chinese hamster ovary) in suspension to 60-Hz electric fields. Using an exposure system without electrodes, which induces an electric field in the cell culture medium via a magnetic field, we have been able to expose cells to electric field strengths (60 Hz) from 0.15 to 10.5 V/m for 48 hr. The results of these studies indicate that electric fields equal to or greater than 0.7 V/m significantly decreased cell viability as measured by comparing the cloning efficiencies of exposed cells to those of control cells. Conversely, electric field exposures up to 10.5 V/m did not increase the mutation frequency of these cells as measured at the hypoxanthine guanine phosphoribosyl transfer (HGPRT) level. These data indicate that the observed effect on cloning efficiency is not due to a direct interaction between the electric field and cellular DNA.

Considerable concern exists about the possible health hazards of electromagnetic fields associated with high-voltage transmission lines. While a number of literature reports indicate effects in whole animals, the results are often controversial and conflicting. The one indisputable fact is that documenting repeatable effects of electric fields on biological systems has been difficult. Our approach has been to use a relatively simple system (individual cells in culture) to systematically examine the effects of an electric field on these cells. Initially, parameters of interest were effects on cell viability. In addition, we wanted to determine if a 60-Hz electric field could alter the inheritable information (i.e., cause mutation) within cells. Chinese hamster ovary (CHO-K1) cells were the system of choice.

Cell viability was determined using CHO cells in a clonal growth assay that involved the seeding of a known number of monodispersed cells (at low cell density) and counting the number of colonies (clones) present after 7 days. In the mutation assay, the remaining exposed CHO cells were then scored for mutations at a specific (hypoxanthine guanine phosphoribosyl transferase; HGPRT) locus, and the frequency of these mutational events was compared to those from sham-exposed and unexposed cultures.

In last year's report we described the system for exposing cells to electric fields without using any kind of electrode. In that system, the cells are exposed in a toroidal chamber which encircles the ferromagnetic core. The exposure electric field is inductively generated by a 60-Hz magnetic field contained within the core. The exposure chamber is divided into two sections: the exposure chamber (Figure 1), which actually contains the cells, and the

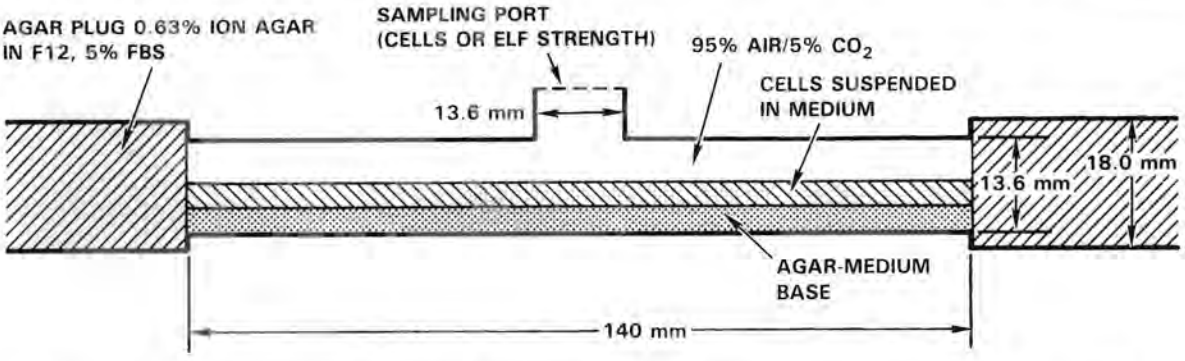

FIGURE 1. Cell Exposure Chamber. The actual cell exposure chamber consists of a glass section (140 mm long x 13 mm diameter) that contains 7.5 ml of an agar (0.63%) medium (F12 FBS) mixture, 7.5-ml medium (F12 FBS) with cells and a 7.5-ml air space that contains a 95% air/5% CO_2 mixture. The sampling port is closed with a rubber stopper that can be used to remove cells or to insert a probe for measuring electric field strength. Attached to the ends of the cell exposure chamber is the return leg (not shown), which consists of a piece of Tygon tubing filled with conducting medium. This return leg loops around the ferromagnetic core, thus completing the electric circuit.

return leg, which is filled with conducting medium and completes the electrical circuit around the core. The exposure section (Figure 1) has an agar bed under a layer of culture medium; the remaining volume contains 95% air and 5% CO_2. The electrical conductivities of the agar bed and culture medium are adjusted so that they are equal. Cells are seeded onto the agar surface and are surrounded on all sides by an electrically homogenous material. This greatly simplifies dosimetric calculations.

The exposure electric field is determined by the following parameters: 1) the electromotive force induced in the exposure toroid by the alternating magnetic field, 2) the circumference of the exposure toroid, and 3) the relative conductivities and cross-sectional areas of the exposure section and return leg. Because all of these parameters are somewhat variable across a series of exposures, it is important to determine the actual electric field through direct measurement. Unfortunately, it is not practical to make such measurements during actual exposure because of the risk of biological contamination from the field probe. Therefore, we characterized the electric field in 10 exposure setups prepared in exactly the same way as the actual exposures.

The field probe used for making measurements in the conducting medium consisted of two parallel, stainless-steel needles spaced 3.1 mm apart and supported by an insulated glass handle. Two wires within a shielded cable were routed from the probe tips through the handle to a remote differential amplifier. The electric field was determined by inserting the probe in the culture medium (with the tips perpendicular to the electric field direction) and dividing the measured differential voltage by the separation between the probe tips. The electromotive force induced in the exposure toroid was determined by measuring the voltage generated in a wire loop that surrounded the transformer core. This voltage is designated V_{sec}.

Averages and standard deviations of the field measurements made in the 10 exposure chambers are given in Figure 2. These data show the expected linear relationship between the electric field and V_{sec}. A straight-line fit to the data, also shown in Figure 2, was used to determine exposure fields for all our experiments with cell cultures. (V_{sec} was always monitored.)

In initial cell exposures, CHO-K1 cells were exposed to 3.5 V/m for 24 or 48 hr. Cloning efficiency of exposed cells was significantly reduced. Four out of five experiments were significant at the 0.001 level; the remaining experiment was significant at the 0.05 level. After a reproducible effect was demonstrated, we attempted to determine the minimum length of exposure required to produce effects. At least 1 hr (probably more) was necessary to consistently produce the observed cytotoxic effect (Table 1). Following 8 hr of exposure (to 3.5 V/m), a highly reproducible effect was observed; the effect could not be enhanced by increasing the duration of exposure beyond 24 hr.

A second series of experiments were conducted to see if varying the electric field strength (from 0.15 to 10.5 V/m) would alter the cell's response. Using a 24-hr exposure, the effect was clearly reproducible at 1.4 V/m and above but was not detectable

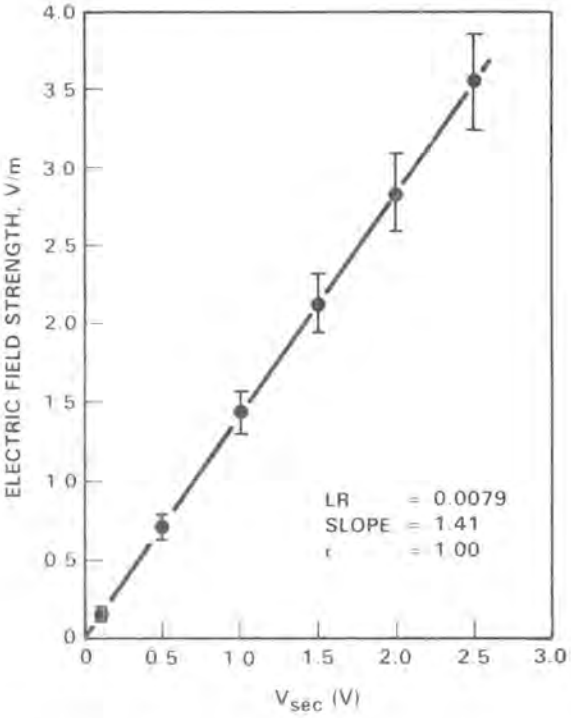

FIGURE 2. Cell Culture Dosimetry as Determined by ELF Probe Measurements. The electric field strength for each experiment was determined by measuring the parallel current (V_{sec}) produced in a wire below the cell exposure chamber. This indirect measurement was necessary in order to avoid contamination of the cultured cells. In order to validate our dosimetry measurements, a series of experiments were conducted in which the currents produced in cell exposure chambers were measured directly in V/m using a probe inserted in the cell culture chamber. This electric field strength was designated V_{probe}. The probe measurement (exposure electric field in V/m) and parallel-wire (V_{sec}) electric field measurements had a linear relationship and direct correlation ($r = 1.0$).

TABLE 1. Effect of 60-Hz ac Electric Field on the Absolute Cloning Efficiency of Chinese Hamster Ovary (CHO-K1) Cells. Electric field fixed (3.5 V/m) and exposure time variable.

Exposure Duration, hr	Absolute Plating Efficiency[a] (± S.E.) Electric Field Exposed	Sham-Exposed	P[b]
0 (48)[c]	50.0 ± 1.7	48.0 ± 1.4	
1	66.4 ± 1.3	69.2 ± 1.6	N.S.[d]
1	59.4 ± 1.9	63.7 ± 1.4	0.05
8	75.3 ± 1.6	81.3 ± 1.4	0.001
8	59.9 ± 1.4	75.8 ± 1.5	0.001
16	53.4 ± 1.2	58.3 ± 1.1	0.001
24	43.1 ± 1.2	52.2 ± 1.4	0.001
24	61.7 ± 1.1	73.4 ± 1.3	0.001
24	73.9 ± 1.5	84.1 ± 1.3	0.001
24	74.2 ± 2.5	98.0 ± 2.0	0.001
48	58.4 ± 2.1	72.0 ± 1.8	0.001
48	69.2 ± 2.1	73.8 ± 2.3	0.05

[a] Number of clones formed/100 cells seeded
[b] Significant difference between exposed and sham-exposed, using a two-tailed, unpaired *t*-test. Values obtained were from published tables of the distribution of *t*.
[c] Cells in exposure chambers for 48 hr, but magnet was not turned on and there was no measurable electric current in either the "electric-field-exposed" or the "sham-exposed" culture chambers.
[d] Not significant at 0.05 level

TABLE 2. Effect of 60-Hz ac Electric Field on the Absolute Cloning Efficiency of Chinese Hamster Ovary (CHO-K1) Cells. Exposure Duration Fixed (24 hr) and Electric Field Strength Variable.

Exposure Level, V/m	Absolute Plating Efficiency[a] (± S.E.) Electric Field Exposed	Sham-Exposed	P[b]
0.15	80.4 ± 1.4	78.8 ± 1.5	N.S.[c]
0.15	66.7 ± 1.3	65.1 ± 1.4	N.S.
0.7	68.4 ± 1.4	71.0 ± 1.7	N.S.
0.7	51.8 ± 1.2	54.9 ± 1.2	0.05
0.7	65.6 ± 1.5	79.2 ± 1.5	0.001
0.7	62.5 ± 2.6	77.2 ± 1.4	0.001
1.4	60.7 ± 1.2	65.4 ± 2.2	0.001
1.4	56.8 ± 2.2	68.7 ± 1.5	0.001
3.5	43.1 ± 1.2	52.2 ± 1.4	0.001
3.5	61.7 ± 1.1	73.4 ± 1.3	0.001
3.5	74.2 ± 2.5	98.0 ± 2.0	0.001
3.5	73.9 ± 1.5	84.1 ± 1.3	0.001
10.5	87.7 ± 1.1	101.7 ± 1.3	0.001
10.5	69.5 ± 1.3	76.9 ± 1.1	0.001
10.5	64.6 ± 2.1	76 ± 1.9	0.001
10.5	85.8 ± 1.7	97.6 ± 1.4	0.001
10.5	69.5 ± 2.2	94.2 ± 2.1	0.001
10.5	63.2 ± 1.7	98 ± 1.9	0.001

[a] Number of clones formed/100 cells seeded.
[b] Significant difference between exposed and sham-exposed, using a two-tailed, unpaired *t*-test. Values obtained were from published tables of the distribution of *t*.
[c] Not significant at 0.05 level

at 0.15 V/m (Table 2). In three of four experiments at 0.7 V/m, significant differences in cell cloning efficiency were apparent. It is possible that ~0.7 V/m is near the minimum field strength necessary to decrease cloning efficiency.

Finally, mutation frequencies of CHO cells exposed to 60-Hz electric fields were measured, using the HGPRT locus. Exposed cells did not exhibit increased mutation frequencies relative to control or sham-exposed cells (Table 3). In fact, part of the data indicate that exposure may reduce the spontaneous mutation frequency.

Research is in progress to explain the mechanism by which 60-Hz electric fields reduce cell survival (as measured by cloning efficiency) and to determine whether a dose-response relationship can be established.

TABLE 3. Effect of 60-Hz ac Electric Fields on Mutation Frequency of Chinese Hamster Ovary (CHO-K1) Cells.

	Exposure Duration, hr	Mutation Frequency/10^6 Viable Cells: Electric Field Exposed	Control	P
3.5 V/m	24	7.8	6.2	N.S.[a]
	24	2.1	2.3	N.S.
	24	3.8	3.2	N.S.
	24	0.87	0.93	N.S.
	48	10.3	11.3	N.S.
10.5 V/m	24	0	11.2	0.001 (?)
	24	1.0	15.5	0.001 (?)
	24	17.8	23.6	0.05 (?)
	24	2.5	2.3	N.S.
	24	2.8	3.0	N.S.
	24	3.5	3.1	N.S.

[a] Not significant at 0.05 level

Fission

FISSION

Biomedical studies in the fission energy technology area are, for the most part, directed toward evaluation of long-term effects from internal exposure to radionuclides. 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, including an attempt to evaluate the carcinogenicity of inhaled plutonium at life-span radiation doses to the lung as low as 2 to 10 rad. Both dogs and rodents are employed in continuing studies of the effects of chronic exposure to simulated uranium mine atmospheres.

Studies in rats and dogs have shown that cigarette smoke enhances retention of inhaled plutonium in the lung, and are being extended to investigate the mechanism of these effects. The toxicity of sodium or lithium that might be accidently released, from a liquid metal fast breeder reactor or from a fusion reactor, and the toxicity of chronically inhaled krypton-85 which may be released in small amounts from nuclear reactors are being studied, as are the biological behavior and carcinogenicity of high-specific-activity uranium and protactinium isotopes that might be released from reactors employing a thorium fuel cycle.

Studies related to the exposure of the general public include those 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. In the medical area, a device developed to irradiate the blood, which may be a useful treatment for leukemia or for the prevention of transplant rejection, will soon be ready for clinical trials.

In all of these areas, the development of new experimental techniques plays an important role. This has included major efforts in aerosol generation and exposure technology.

• Aerosol Technology Development

Principal Investigator: W. C. Cannon

Other Investigators: E. F. Blanton, J. K. Briant, J. R. Decker, E. G. Kuffel, K. E. McDonald, A. L. Moen, O. R. Moss, E. J. Rossignol, and D. L. Stevens

Technical Assistance: B. W. Killand

A variety of tasks are included in this project, all designed to improve the capabilities of this laboratory to conduct animal inhalation exposures to airborne pollutants and to characterize the aerosols used. These tasks include development of an improved, automated, nose-only, aerosol exposure system for rodents, testing and evaluation of an improved respirable-aerosol sampler, and further improvements in surface-area measurements and particle-size analysis methods.

Nose-Only Exposure System Development

Because of our past success in achieving uniform aerosol exposure in a new chamber (Annual Report, 1981), this year we converted a standard chamber to a flow-past configuration by adding a branched inlet and modifying the exposure ports to include an exhaust return tube (Figure 1). Prior to this conversion we experimented with a scale model to establish its feasibility. The model experiments and aerosol tests with the converted chamber indicate that converted chambers will perform as well as flow-past chambers with respect to exposure port isolation and rapid build-up and clearance of aerosol.

Converting existing standard chambers will be significantly less expensive than building new flow-past chambers. The necessary parts will be made in house, and they will be assembled and installed inside the exposure glove boxes. For completely new exposure systems, however, we will build new flow-past chambers, since they are more economical to build than the standard type.

We have also improved the accuracy of delivering a prescribed aerosol dose in nose-only exposures, using a particulate air monitor (PAM) that measures radioactive concentrations in real time. We have exposed several groups of 35 rats each to $^{239}PuO_2$ tagged with ^{169}Yb. A target value for the 14-day body burden was selected for each group. Actual 14-day burdens were measured by in vivo counting the ^{169}Yb tag.

Before developing the above method, we performed a pre-exposure test by generating the aerosol into an empty chamber and making the same aerosol concentration measurements as during an exposure. Assuming that aerosol concentrations would be the same during exposure, we timed the exposure accordingly (test aerosol method). In this method, errors resulted from differences between the test concentrations and actual exposure concentrations. In the newly devised "TWAC method," we measure aerosol concentration with the PAM several times during each exposure. We then calculate the time-weighted-average concentration (TWAC) and adjust exposure times accordingly. The TWAC method also corrects for concentration changes that may occur during exposure.

We have found that the coefficient of variation of body burdens in rats exposed in our flow-past chamber is approximately 25%. In an "ideal" exposure the mean lung burden would exactly equal the target value. We have calculated (Table 1) the number of rats with 14-day burdens within 10, 20, 30, 40 and 50% of the target value in 10 "ideal" exposures of 35 rats each. Comparison of the actual number obtained in 10 TWAC-method exposures with the numbers predicted in the test aerosol method have demonstrated the accuracy of the TWAC method.

In the TWAC method described above we previously used an HP-85 microcomputer to time the exposure and calculate the TWAC from PAM measurements entered on the keyboard. We have now designed an interface system to allow automatic control of nose-only rat exposures. The system (Figure 2) employs two Hewlett-Packard general-purpose, input-output (GPI/O) units to interface the PAM equipment and control valves to the HP-85. The HP-85 turns on the generator air, operates the PAM to measure aerosol concentration either on a prescribed schedule or at the operator's command, calculates the TWAC, and turns off the generator air when the product of TWAC and time reaches a predetermined value. It also provides the operator with printed data and a CRT display to monitor exposure progess.

Conducting Cyclone Sampler Evaluation

The 10-mm nylon cyclone is an instrument which is widely used in industry to measure

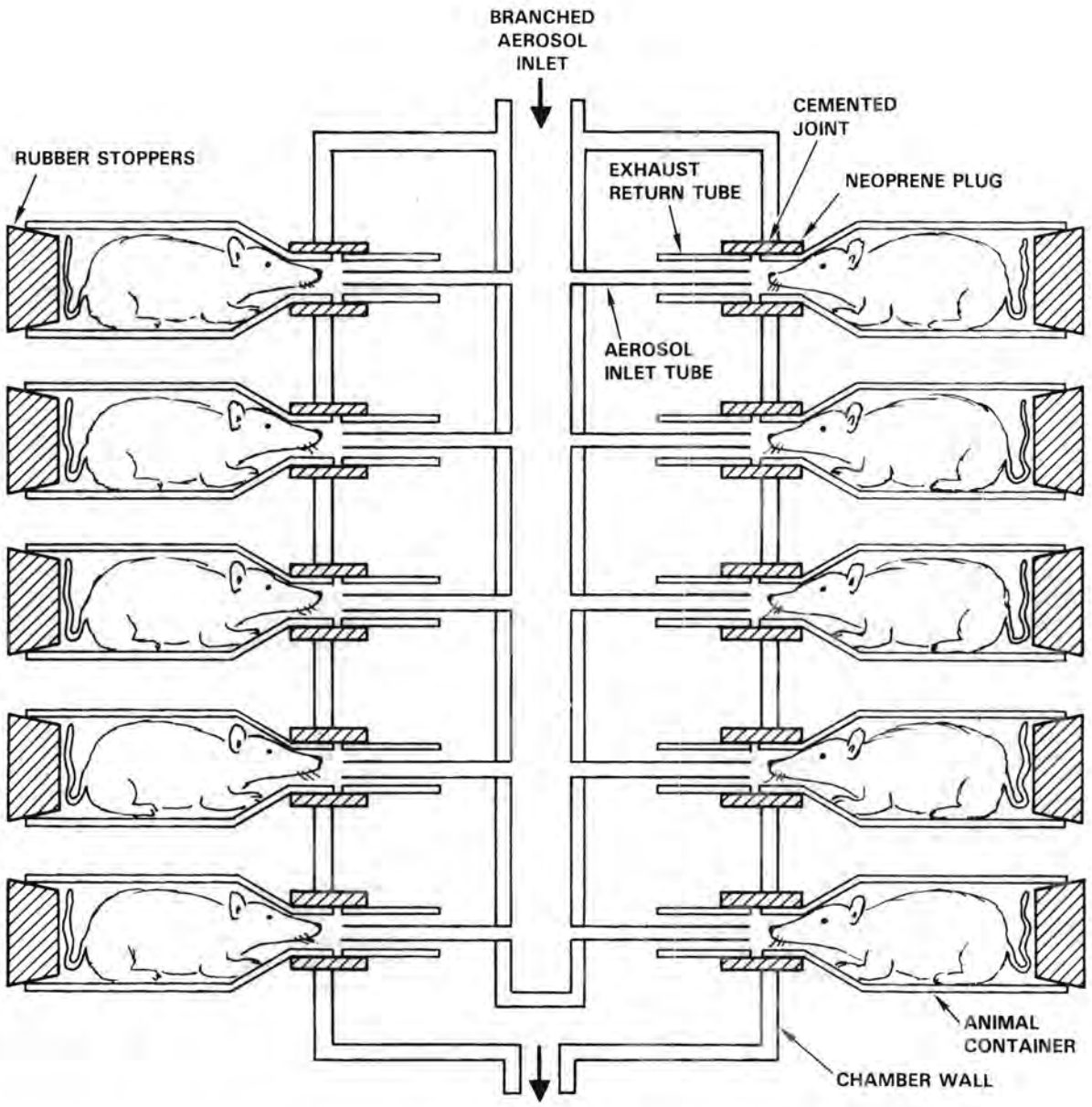

FIGURE 1. Diagram of a Standard, Nose-Only, Rat Exposure Chamber Converted to a Flow-Past Design.

the respirable fraction of airborne particles in the workplace. The standard instrument is made of nylon, which is subject to severe electrostatic charging. Electrostatic charge on either the cyclone or the particles influences collection efficiency and tends to reduce the measured respirable aerosol concentration. (The influence of electric charge in the human respiratory tract is minimal compared to its influence in the nylon cyclone.)

Knowing that graphite-filled conducting nylon could be used in manufacturing this instrument, we had two such instruments made to compare with the standard nylon cyclone to see if the conducting material would solve the electrostatic charge problem. We have found that, where no electrostatic charge is involved, the nonconducting nylon cyclone and the graphite-filled conducting nylon cyclone perform identically. Performance of the conducting cy-

clone is unaffected by inducing an electrostatic charge on an aerosol, but performance of the nylon cyclone is affected.

TABLE 1. Comparison of Body Burdens After Nose-Only Exposure by Three Methods (N = 350).

Percent of Target Values:	Number of Rats Having Burdens Within Stated Range of Target Value				
	10	20	30	40	50
Method					
Ideal	109	202	269	312	334
TWAC[a]	92	166	232	289	324
Test Aerosol	49	109	162	215	299

[a] Time-weighted average concentration

To measure cyclone charging, the cyclone was moved directly to a Faraday-cage charge-measurement apparatus without touching anything that could influence its charge. The aerosol was sampled with a nylon cyclone that was either initially charged or uncharged. The conducting cyclone was grounded and remained uncharged throughout the test. In the tests, initial charges on the nylon cyclones were on the order of 10^{-8} coulomb.

Either charged or uncharged aerosols of 2-μm polystyrene latex (PSL) and volcanic ash, were used in the experiment. Penetrations of the cyclones by the aerosols are listed in Table 2. The extreme case of a charged nylon cyclone sampling charged aerosol shows the most dramatic effect of electrostatic charge; however, the graphite-filled nylon cyclone is essentially unaffected by electrostatic charge conditions. Future tests will further characterize the performance of the conducting cyclone.

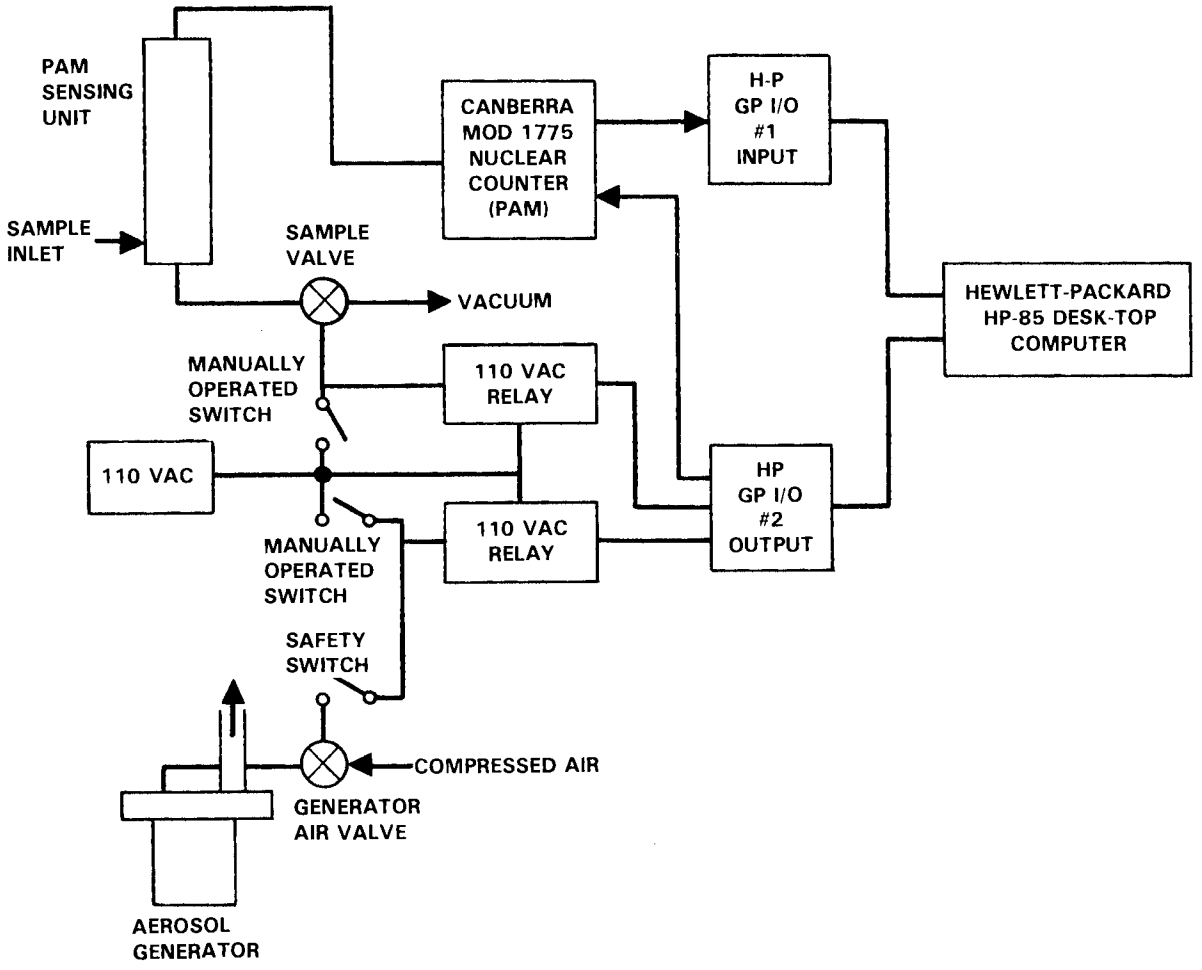

FIGURE 2. Control System for Nose-Only, Rat Exposures.

TABLE 2. Measurements of Penetration of Cyclones by Charged and Uncharged Aerosols.

Cyclone	Aerosol (PSL[b], 2 μm) Charged	Aerosol (PSL[b], 2 μm) Uncharged	Aerosol (Volcanic Ash, D ≐ 2 μm, GSD ≐ 2) Charged	Aerosol (Volcanic Ash, D ≐ 2 μm, GSD ≐ 2) Uncharged
Nylon Charged[a]	0.72	0.81	0.34	0.67
	0.55		0.25	0.61
Nylon Uncharged[a]	0.99	0.93	0.59	0.78
	0.95		0.57	0.66
	0.96			
Graphite Uncharged[a]	0.95	0.96	0.69	0.70
			0.74	0.71

[a] At start
[b] Polystyrene latex

Particle Size Analysis Development

We have continued our study of estimating particle-size-distribution parameters from cascade impactor data. A commonly used method minimizes the sum of squared differences between observed and expected mass fractions on cascade impactor stages. This method is sometimes improved by weighting the least squares, either with the variance in mass fraction estimated or by introducing total mass as an additional parameter in the sum of squares. However, solution of the weighted, least-squares problem is complex. We have demonstrated that application of the maximum likelihood method can transform the weighted, least-squares problem into the simpler, unweighted, linear least-squares problem, allowing use of widely available computer software and computers of smaller memory capabilities to obtain the same results.

We have incorporated the technology into a computer program which assumes infinitely sharp collection efficiency curves for impactor stages (the effective cut-off diameter method) and are presently applying it to a program which takes into account the actual collection efficency curves of the impactor.

Surface-Area Measurement Development

We have refined our ability to measure the specific surface area and density of powdered materials. The basic technique involves gas adsorption according to Brunauer, Emmet and Teller (commonly called the BET method). A microbalance measures the mass of nitrogen adsorbed onto the surface of a powder sample maintained at various nitrogen pressures. The change in mass of adsorbed nitrogen for each pressure determines the sample surface area. Measuring sample weight reductions due to buoyancy from argon gas determines density.

This year, the reproducibility of the density experiments was significantly increased by measuring the buoyancy due to argon at pressures below half an atmosphere. The reproducibility of the surface area measurements was increased by outgassing the volcanic ash sample at 300°C or higher temperatures. Outgassing times, determined by the stability of the sample mass, ranged from 1 to 64 hr. Samples of Mount St. Helens volcanic ash were measured following these procedural changes (Table 3). Density measurements have standard deviations within 5%; specific surface measurements have standard deviations less than 3%.

TABLE 3. Revised Specific Surface and Area Density of Mount St. Helens Ash.

Ash Collection Site	Size Fraction, μm	Specific Surface Area Measurements: SSA,[b] m^2/g	Specific Surface Area Measurements: Sample Size, mg	Density Measurements: ρ, g/cm^3	Density Measurements: Sample Size, mg
Composite[a]	< 3.5	9.8 ± 0.1	48.1	2.81 ± 0.09	47.6
Missoula, MT	< 3.5	8.9 ± 0.2	23.5	2.78 ± 0.06	22.6
Missoula, MT	>20	1.52 ± 0.01	187.7	2.56 ± 0.01	187.7
Spokane, WA	< 3.5	9.5 ± 0.3	21.8	2.79 ± 0.07	21.5
Yakima, WA	< 3.5	13.4 ± 0.2	15.0	3.02 ± 0.18	14.2
Yakima, WA	<20	9.8 ± 0.1	30.1	--	--
Yakima, WA	>20	1.43 ± 0.02	310.1	2.75 ± 0.01	310.1

[a] Mixed samples from Yakima, Ritzville and Spokane, WA
[b] Specific surface area

• Inhaled Plutonium Oxide in Dogs

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This project is concerned with long-term experiments to determine the lifespan dose-effect relationships of inhaled $^{239}PuO_2$ and $^{238}PuO_2$ in beagles. The data will be used to estimate the health effects of inhaled transuranics.

Beagle dogs given a single exposure to $^{239}PuO_2$ or $^{238}PuO_2$ aerosols to obtain graded levels of initial lung burdens are being observed for lifespan dose-effect relationships. Increased mortality due to radiation pnuemonitis and lung tumor was observed in the three highest dose-level groups exposed to $^{239}PuO_2$ during the 11-yr postexposure period. During the 8½ yr after exposure to $^{238}PuO_2$, increased mortality due to lung and/or bone tumors was observed in the two highest dose-level groups. Chronic lymphopenia was the earliest observed effect after inhalation of $^{239}PuO_2$ or $^{238}PuO_2$, occurring 0.5 to 2 yr after exposure, in the four highest dose-level groups with an initial lung burden ≧80 nCi.

To determine the lifespan dose-effect relationships of inhaled plutonium, 18-mo-old beagle dogs were exposed to aerosols of $^{239}PuO_2$ (mean AMAD, 2.3 μm; mean GSD, 1.9), prepared by calcining the oxalate at 750°C for 2 hr; or to $^{238}PuO_2$ (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 $^{239}PuO_2$ 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 lifespan dose-effect studies (Table 1). One hundred thirteen dogs exposed to $^{238}PuO_2$ in 1973 and 1974 were selected for lifespan dose-effect studies (Table 2). 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 3 summarizes, by dose-level group, the mortality and lesions associated with deaths through 11 yr after exposure to $^{239}PuO_2$. During the first 11 yr following exposure, all of the dogs in the highest-level dose group and in Dose Level Group 5, fifteen in Group 4, five in Group 3, three in Group 2, six in Dose Level Group 1 and four in the control group were euthanized when death was imminent. Fourteen dogs were sacrificed for comparison of plutonium tissue distribution. Table 4 and Figure 1 show the primary causes of death and the distribution of ^{239}Pu in the tissues of these animals.

As survival time increased, the fraction of plutonium in the lung decreased to ~10% of the final body burden by 10 to 11 yr after exposure. During the first postexposure year, plutonium was translocated primarily to the thoracic lymph nodes, with little plutonium translocated to other tissues.

TABLE 1. Lifespan Dose-Effect Studies with Inhaled $^{239}PuO_2$ in Beagles.[a]

Dose Level Group	Number of Dogs		Initial Alveolar Deposition[b]	
	Male	Female	nCi[c]	nCi/g Lung[c]
Control	10	10	0	0
1	10	10	3.5 ± 1.3	0.029 ± 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 postexposure and estimated lung weights (0.011 x body weight)

[c] Mean ± 95% confidence intervals around the means

TABLE 2. Lifespan Dose-Effect Studies with Inhaled $^{238}PuO_2$ in Beagles.[a]

Dose Level Group	Number of Dogs		Initial Alveolar Deposition[b]	
	Male	Female	nCi[c]	nCi/g Lung[c]
Control	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 postexposure and estimated lung weights (0.011 x body weight)

[c] Mean ± 95% confidence intervals around the means

Plutonium content of the thoracic lymph nodes was ~50% of the final body burden at 10-11 yr after exposure; the abdominal lymph nodes, principally the hepatic nodes, contained ~15%. The fraction of plutonium in liver increased, accounting for ~20% of the final body burden at 10 to 11 yr after exposure in the higher (0.1- to 0.4-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 11th 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 2% of the final body burden was in the skeleton at 10 to 11 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 804M also had a pulmonary tumor, classified as a bronchiolar-alveolar carcinoma.

Thirty-three of the 50 exposed dogs euthanized 3 to 11 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 exposed dogs with lung tumors were in Dose Level Groups 3, 4, 5, and 6. One control dog was euthanized due to a lung tumor. Dogs 794M, 803M, 809F, 824F, and 835F (Dose Level 4), 697M, 778M,

TABLE 3. Summary of Lesions in Dogs Euthanized During the 11-yr Period After Inhalation of $^{239}PuO_2$.

Dose Group	No. Dogs/ Group	No. Dead Dogs/ Group	Number of Dogs/Lesion Associated with Death																	
			Radiation Pneumonitis	Lung Tumor	Malignant Lymphoma	Hemangiosarcoma	Reticulum Cell Sarcoma	Pituitary Tumor, Cushing's	Ovarian Tumor	Oral Tumor	Round Cell Sarcoma	Pneumonia	Liver Cirrhosis	Thromboembolism	Septicemia	Epilepsy	Pyometra	Cardiac Insufficiency	Unknown
6	8	8	7	1																
5	21	21	1	20																
4	22	15		10			1	1				2	1							
3	20	5		2										1			1	1		
2	21	3							1							1				1
1	24	6			1	1		1			1				1					1
Control	20	4		1		1				1				1						

TABLE 4. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{239}PuO_2$.

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	0.18	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	Ovarium 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	0.613	14	27	6.9	41	8.1	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
834F	97	0.025	30	46	17	3.5	0.91	Pyometra
752M	98	0.055	24	62	1.2	7.7	0.98	Lung Tumor
864F	100	0.616	18	22	2.9	50	2.9	Lung Tumor
908F	101	0.0073	14	72	0.049	0.56	0.93	Unknown
778M	102	0.065	11	85	1.3	1.0	0.52	Pulmonary Thromboembolism
812M	103	0.288	15	36	29	16	2.2	Lung Tumor
814F	104	0.054	49	33	4.1	10	1.6	Lung Tumor
840F	107	0.389	17	35	5.8	37	2.0	Lung Tumor
777M	109	0.392	11	52	7.8	24	1.7	Lung Tumor
857M	109	0.333	20	39	9.4	27	2.4	Lung Tumor
898F	111	0.333	10	34	28	21	3.4	Lung Tumor
899F	113	0.0066	7.5	87	0.14	0.27	1.6	Hemangiosarcoma, Heart
697M	114	0.141	15	64	8.1	9.9	1.4	Cardiac Insufficiency
909M	115	0.444	16	46	11	25	1.2	Lung Tumor
824F	116	0.178	21	75	0.50	2.3	0.70	Pneumonia
891M	116	0.0023	11	84	0.064	0.48	1.5	Septicemia
836M	117	0.333	12	63	15	7.4	0.97	Lung Tumor
892M	120	0.348	10	47	18	20	3.7	Lung Tumor
794M	120	0.397	13	33	14	31	3.5	Pituitary Tumor, Cushing's
781F	122	0.034	37	59	0.25	1.1	0.72	Lung Tumor, Kidney Tumor
809F	123	0.120	12	36	18	28	3.3	Liver Cirrhosis, Thyroid Tumor
854M	124	0.435	12	66	15	3.8	1.3	Lung Tumor
807F	125	0.0021	10	71	0.55	1.2	1.3	Pituitary Tumor, Cushing's
810F	126	0.219	5.9	43	20	22	1.8	Lung Tumor
900M	126	0.0016	13	60	2.3	9.0	2.9	Round Cell Sarcoma
748F	127	0.0015	10	50	0.87	0.33	1.2	Unknown
860M	133	0.335	8.2	68	8.0	11	2.5	Lung Tumor

[a] Includes tracheobronchial, mediastinal and sternal lymph nodes
[b] Includes hepatic, splenic and mesenteric lymph nodes

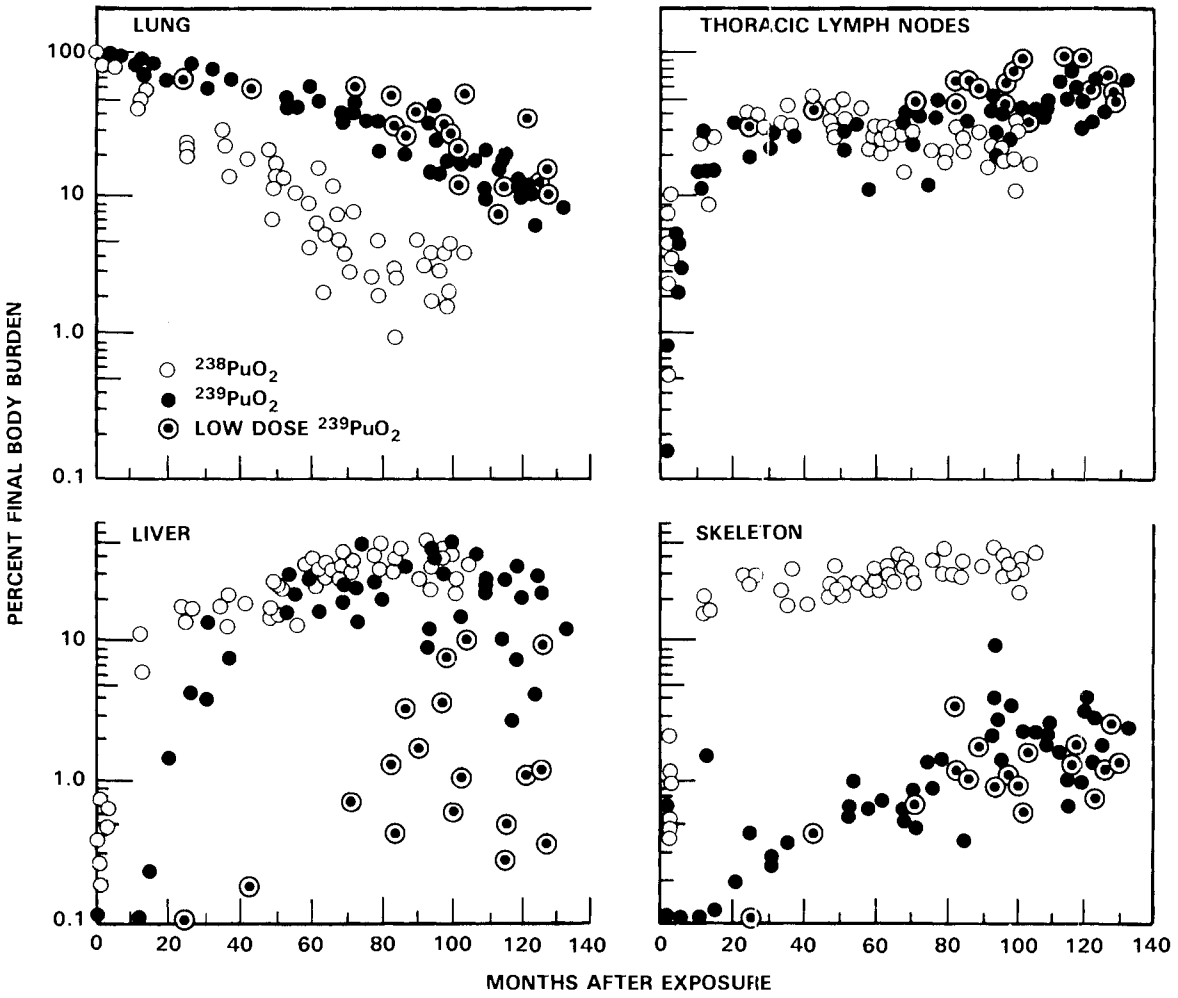

FIGURE 1. Plutonium in Tissues of Dogs After Inhalation of PuO_2.

and 834F (Dose Level 3), 748F, 754M and 769F (Dose Level 2), and 807F, 875M, 891M, 899F, 900M and 908M (Dose Level 1) died during the 7- to 11-yr postexposure period, of causes presently thought to be unrelated to plutonium exposure.

In 17 of the dogs, the lung tumors were classified as bronchiolar-alveolar carcinoma; in six dogs as adenosquamous carcinoma; in seven dogs, adenocarcinoma; in one dog, epidermoid carcinoma; in one dog, epidermoid and bronchiolar-alveolar carcinoma; and in another dog, adenocarcinoma, adenosquamous carcinoma and bronchiolar-alveolar adenocarcinoma. The epidermoid carcinoma metastasized to the skeleton; the bronchiolar-alveolar carcinomas metastasized only to the thoracic lymph nodes in eight dogs, and to several organs (including thoracic lymph nodes, mediastinum, kidney, thyroid, skeleton, heart, adrenal gland, aorta, and axillary, prescapular, cervical, splenic and hepatic lymph nodes) in four other dogs. Three of the adenosquamous carcinomas metastasized to thoracic lymph nodes, mediastinum and thoracic pleura, and one to the hepatic and tracheobronchial lymph nodes. The adenocarcinomas metastasized to the lungs, tracheobronchial lymph nodes, hepatic lymph nodes, heart and esophagus in one dog. The lung tumor in the control dog was classified as a bronchiolar-alveolar adenocarcinoma with metastases to thoracic and abdominal lymph nodes, trachea, esophagus and mediastinum. Three of the dogs had lesions of secondary hypertrophic osteoarthropathy. Sclerosing lymphadenopathy was associated with the high concentration of plutonium in the thoracic and hepatic lymph nodes of dogs in Dose Level Groups 3, 4, 5 and 6. 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 in Dose Level Groups 4 and 5, which were euthanized during the 4- to 11-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 $^{239}PuO_2$ in dose level groups with mean initial alveolar depositions of 79 nCi or more (Figure 2). Through 123 mo after exposure, mean lymphocyte values were significantly lower ($P < 0.05$) for Dose Level Groups 3 and 4 than for the control group. At 127 mo after exposure, mean lymphocyte values for Dose Level Groups 3 and 4 were not significantly different than for the control groups. The reduction in lymphocytes was dose-related, both in time of appearance and magnitude. Over the course of this study, there has been a slight age-related decrease in mean lymphocyte values of control dogs. In addition, mean lymphocyte concentrations in Groups 3 and 4 have tended to increase, making the differences between control dogs and these groups less significant than previously. 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 groups, which were also lymphopenic. No effects have been observed on red-cell parameters following $^{239}PuO_2$ inhalation.

Serum chemistry assays have been performed to detect organ-specific damage from plutonium that translocated from lung to extrapulmonary sites. No consistent, dose-related alterations have occurred in serum constituents (glutamic pyruvic transaminase [GPT], glutamic oxaloacetic transaminase, alkaline phosphatase [ALP], urea nitrogen, and serum protein fractions) of dogs exposed to $^{239}PuO_2$.

Table 5 summarizes, by dose-level group, mortality and lesions associated with death through 8½ yr after exposure to $^{238}PuO_2$. During the first 8½ yr following exposure, all of the dogs in the highest-level dose group, fifteen dogs in Dose Level Group 5, one dog in Group 4, three dogs in Group 3, one dog in Group 2, and two dogs in Dose Level Group 1 were euthanized when death was imminent. Two control dogs were euthanized during the 8½-yr postexposure period. Twenty-one dogs were sacrificed for comparison of plutonium tissue distribution. Table 6 and Figure 1 show the causes of death and the distribution of ^{238}Pu in the tissues of these animals.

Of the 35 exposed dogs euthanized, 21 were killed due to bone tumors (osteosarcoma), 3 due to lung tumors, and 3 due to Addison's disease. Ten of the dogs euthanized due to osteosarcoma also had lung tumors; two also had Addison's disease. All of the exposed dogs with osteosarcomas, lung tumors and Addison's disease were in Dose Level Groups 5 and 6. One Dose Level Group 1 dog (989F) had a fibrosarcoma in the ilium. Ten of the 21 osteosarcomas were in vertebrae; 2 in femora, 3 in ribs, 2 in the scapulae, 2 in the pelvis, 1 in the tibia and 1 in the humerus. Dog 1191F (Dose Level 5) 1081M (Dose Level 4), 960M, 1040M and 1043F (Dose Level 3) 1082M (Dose Level 2) and 1063M (Dose Level 1) died during the 3- to 8½-yr postexposure period, of causes presently thought to be unrelated to plutonium exposure.

The lung tumors were classified as bronchiolar-alveolar carcinomas in nine dogs, bronchiolar-alveolar adenoma in one dog, and adenosquamous carcinoma in two dogs. In one dog, three lung tumor types were observed: bronchiolar-alveolar, adenocarcinoma and fibrosarcoma. Lung tumor metastases were not observed. Bone tumor metastases were found in the lungs of six dogs; and in three dogs, the bone tumor metastasized to lungs, thoracic lymph nodes, liver, spleen and heart. The five 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 Dose Level Groups 5 and 6 dogs included focal alveolar histocytosis, 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 histocytosis 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 con-

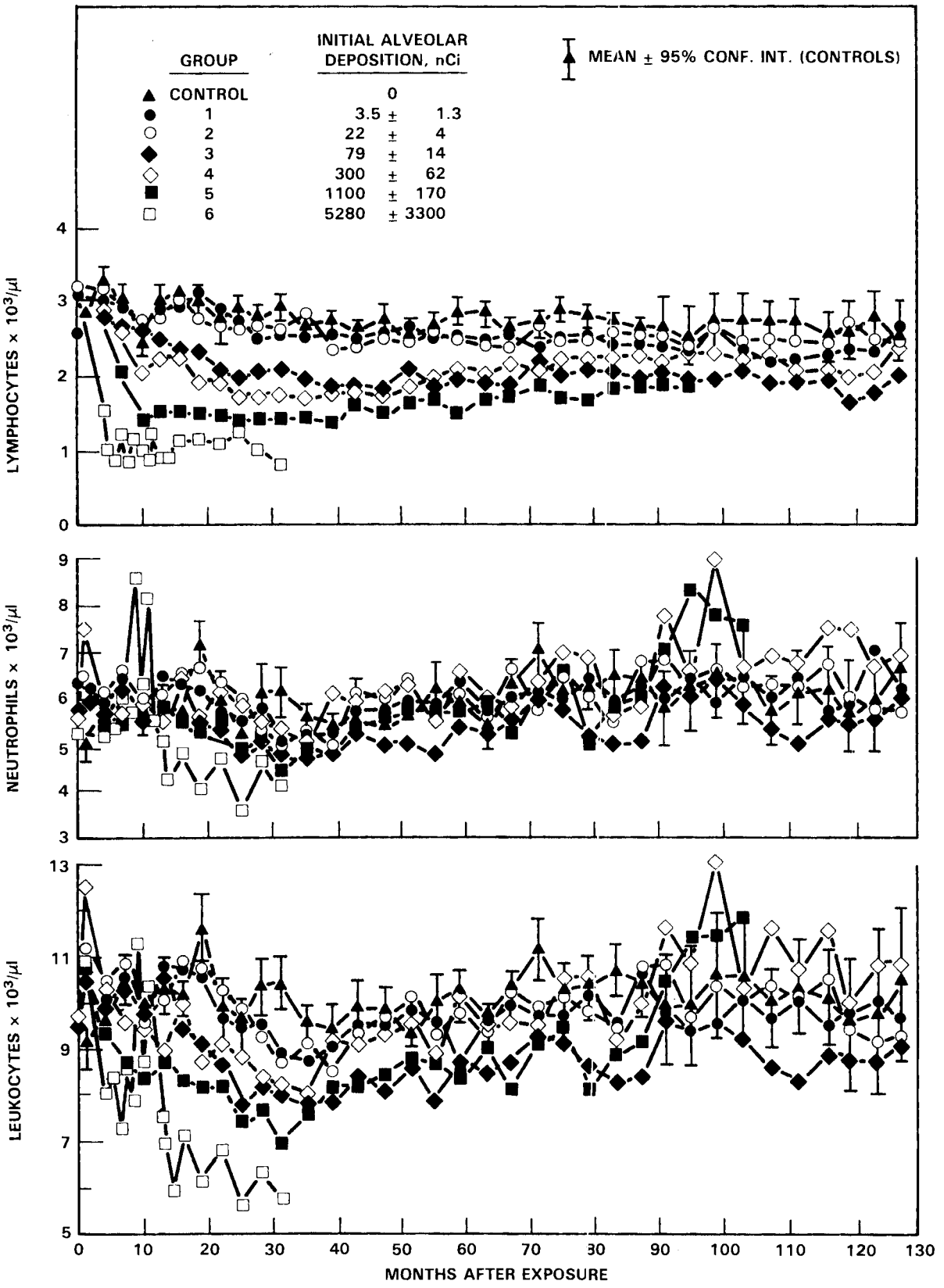

FIGURE 2. Mean Leukocyte, Neutrophil and Lymphocyte Values in Dogs After Inhalation of $^{239}PuO_2$.

TABLE 5. Summary of Lesions in Dogs Euthanized During the 8.5-yr Period After Inhalation of $^{238}PuO_2$.

			Number of Dogs/Lesion Associated with Death													
Dose Group	No. Dogs/ Group	No. Dead Dogs/ Group	Lung Tumor	Bone Tumor	Bone Tumor & Lung Tumor	Bone Tumor & Addison's Disease	Bone Tumor, Addison's Disease & Lung Tumor	Addison's Disease	Pituitary Tumor, Cushing's	Hemangiosarcoma, Heart	Malignant Lymphoma	Parathyroid Adenoma	Brain Tumor & Heart Tumor	Spinal Cord Degeneration	Pyometra	Pneumonia
6	13	13	3	2	6	1		1								
5	20	15		8	3		1	2								1
4	20	1								1						
3	22	3							1		1	1				
2	21	1												1		
1	20	2		1									1			
Control	20	2									1				1	

tained foci of hepatocellular fatty change, where small clusters of single tracks were seen. There was also mild, focal, nodular hyperplasia of hepatocytes. Elevated serum GPT levels, suggestive of liver damage, were observed in the Dose Level Groups 5 and 6 dogs.

Dose-related lymphopenia was observed in groups with mean alveolar $^{238}PuO_2$ deposition of 77 nCi or more (Figure 3). The lymphocyte depression was more pronounced, both in magnitude and earlier appearance, than in dogs exposed to similar doses of $^{239}PuO_2$. Through 98 mo after exposure, mean lymphocyte values were significantly lower ($P < 0.05$) for Dose Level Groups 3, 4 and 5 than for the control group. However, lymphocyte values in the $^{238}PuO_2$-exposed dogs tended to increase sooner after reaching a minimum than in $^{239}PuO_2$-exposed dogs and mean lymphocyte concentrations in Group 3 dogs were not significantly different from values of control dogs 86 to 94 mo following exposure. As with ^{239}Pu, lymphocyte values in the two lowest exposure groups (2.3 and 18 nCi) were not different from control values. A dose-related reduction in total leukocytes was evident, primarily due to lymphopenia, except in Groups 5 and 6, in which neutropenia was also observed. Through 98 mo after exposure, mean leukocyte and neutrophil values were significantly lower ($P < 0.05$) for Dose Level Group 5 than for the control group. 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 $^{238}PuO_2$ inhalation. No chronic effects have been observed in red-cell parameters.

Lymphopenia was the earliest observed effect after inhalation of either $^{239}PuO_2$ or $^{238}PuO_2$, 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.

In serum chemistry assays of $^{238}PuO_2$ dogs, ALP and GPT values were higher than those of the control group only in Dose Level Groups 4 and 5 dogs more than 90 mo following exposure. Elevations in GPT are consistent with liver histopathologic findings and radiochemical analyses indicating ^{238}Pu translocation to the liver. Alkaline phosphatase was elevated in some of the dogs with primary bone tumors. Several dogs have also had elevated ALP attributable (by heat inactivation of ALP) to the liver as the source of the largest portion of the ALP.

At 7½ to 8½ yr after exposure, the fraction of the final body burden in the lungs of the ^{238}Pu-exposed dogs was about 3%, compared to 22% in the ^{239}Pu-exposed dogs (Figure 1). At that time, ~21% of the ^{238}Pu was in the thoracic lymph nodes, compared to ~46% of the ^{239}Pu. Livers of the ^{238}Pu-exposed dogs contained ~32% of the plutonium burden, compared to 28% in the livers of the ^{239}Pu-exposed dogs. About

TABLE 6. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{238}PuO_2$.

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	1.515	2.1	26	9.1	29	30	Bone Tumor
975F	66	3.749	11	30	2.1	28	25	Bone Tumor, Lung Tumor
1042F	69	1.494	4.7	25	2.9	32	33	Bone Tumor, Lung Tumor
1037M	69	2.417	7.1	27	7.8	28	27	Bone Tumor
1027M	70	2.546	3.8	15	7.0	40	31	Bone Tumor, Lung Tumor
1006F	72	2.826	7.5	30	3.4	29	26	Bone Tumor, Lung Tumor
1057M	72	1.748	3.0	35	2.2	33	24	Bone Tumor
1082M	78	0.0083	2.4	20	0.31	40	34	Paralysis
1081M	80	0.361	4.6	15	0.48	47	29	Hemangiosarcoma, Heart
1058F	80	1.000	2.0	18	4.4	31	41	Bone Tumor, Adrenal Tumor
1002M	84	1.786	2.9	31	2.0	31	28	Bone Tumor, Lung Tumor
1109F	86	0.885	0.93	23	4.0	34	35	Bone Tumor, Addison's Disease, Lung Tumor
1218F	86	0.678	2.7	23	4.1	42	25	Bone Tumor
1071M	91	1.088	5.4	28	3.4	27	33	Bone Tumor, Lung Tumor
1063M	94	0.00060	3.4	15	1.3	22	43	Bone Tumor, Heart Tumor
1160F	95	0.956	1.6	21	0.91	43	30	Bone Tumor, Lung Tumor
960M	95	0.036	4.0	21	0.49	33	39	Malignant Lymphoma
1040M	96	0.059	3.0	17	0.96	40	35	Parathyroid Adenoma
1140M	97	0.504	3.8	18	7.7	37	30	Bone Tumor
989F	99	0.0017	5.1	11	1.2	22	29	Bone Tumor (Fibrosarcoma)
1211M	99	0.895	1.3	29	4.7	39	23	Bone Tumor
1173M	99	0.462	2.0	33	7.5	21	33	Bone Tumor
1043F	103	0.037	3.5	16	0.57	33	42	Pituitary Tumor, Cushing's

[a] Includes tracheobronchial, mediastinal and sternal lymph nodes
[b] Includes hepatic, splenic and mesenteric lymph nodes

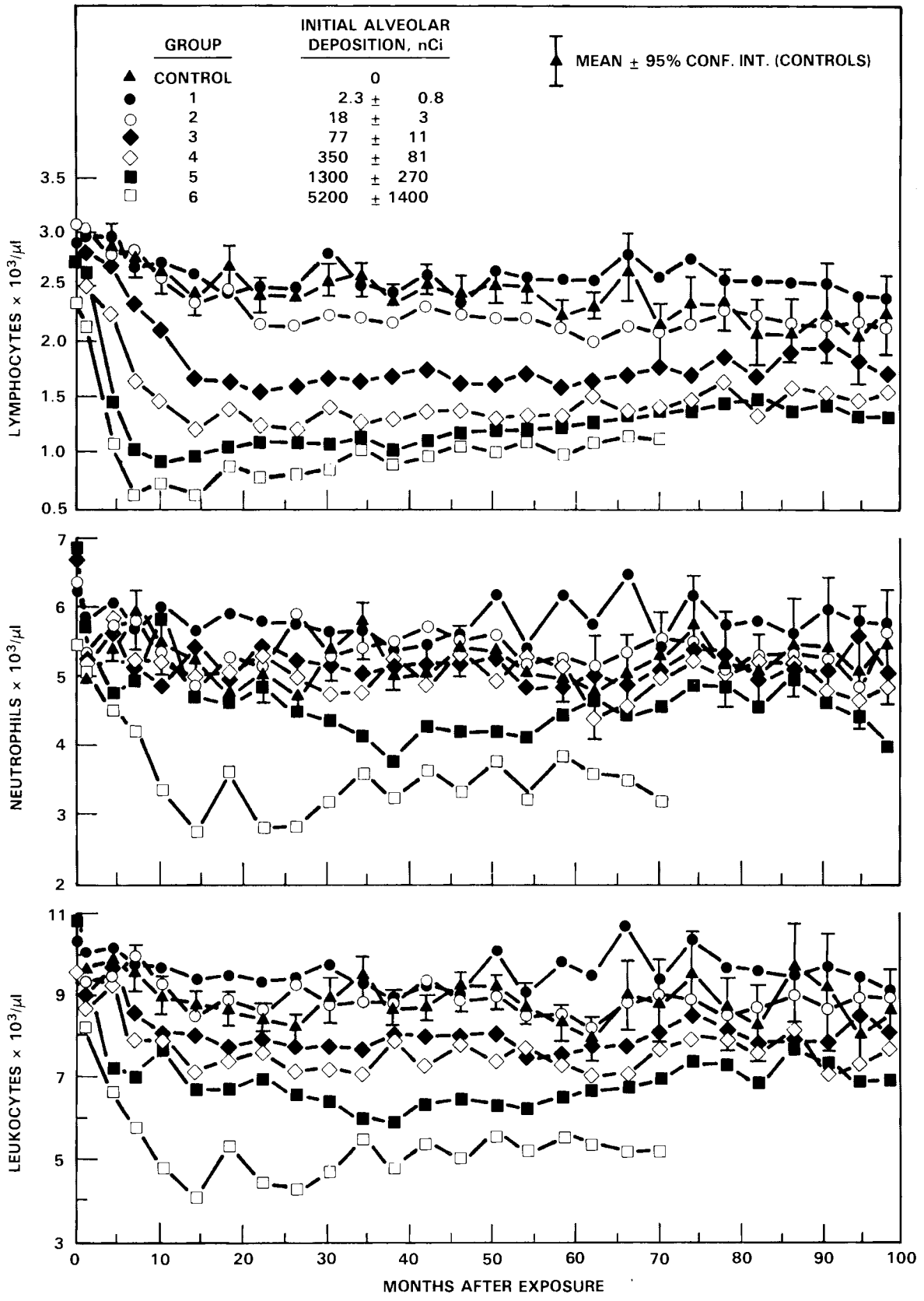

FIGURE 3. Mean Leukocyte, Neutrophil and Lymphocyte Values in Dogs After Inhalation of $^{238}PuO_2$.

33% of the final body burden was in the skeletons of the ^{238}Pu-exposed dogs, at that time, compared to less than 3% in the ^{239}Pu-exposed dogs. Tissue distribution of ^{238}Pu in low-dose-level dogs did not differ from that in high-dose-level dogs.

• Inhaled Plutonium Nitrate in Dogs

Principal Investigator: G. E. Dagle

Other Investigators: R. R. Adee, G. A. Apley, A. C. Case, J. F. McShane, G. J. Powers, H. A. Ragan, S. E. Rowe, R. E. Schirmer, D. L. Stevens, R. E. Weller, and E. L. Wierman

Technical Assistance: W. J. Chandon, J. C. Chapman, K. H. Debban, R. F. Flores, A. J. Kopriva, K. M. McCarty, B. G. Moore, C. L. Park, M. C. Perkins, and L. R. Peters

The major objective of this project is to determine dose-effect relationships of inhaled plutonium nitrate in dogs to aid in predicting health effects of accidental exposure in man. For lifespan dose-effect studies, beagle dogs were given a single inhalation exposure to $^{239}Pu(NO_3)_4$, in 1976 and 1977. The earliest biological effect was on the hematopoietic system; as described in previous Annual Reports, lymphopenia and neutropenia occurred at the two highest dose levels. We have also observed radiation pneumonitis, lung cancer, and bone cancer at the highest dose levels.

The skeleton and liver are generally considered the critical tissues after inhalation of "soluble" plutonium (e.g., plutonium nitrate), on the assumption that the 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. Lifespan studies are necessary to evaluate the complex interactions between tissues and organ systems directly or indirectly impaired by lower levels of exposure. Beagle dogs were chosen to correlate relative risks, determined in other studies, with different forms and routes of exposure to plutonium.

Six dose groups (105 dogs) were exposed, in 1976 and 1977, to aerosols of $^{239}Pu(NO_3)_4$ for lifespan observations (Table 1). In addition, 20 dogs were exposed to nitric acid aerosols as vehicle controls, 25 dogs were exposed to aerosols of $^{239}Pu(NO_3)_4$ for periodic sacrifice to study plutonium metabolism and the pathogenesis of developing lesions; seven dogs were selected as controls for periodic sacrifice; and 20 dogs were selected as untreated controls for lifespan observations. The dogs were exposed in aerosol chambers, using techniques described in previous reports. The Appendix (following the entire Annual Report) shows the current status of each dog on these experiments.

The initial deposition and early clearance of inhaled $^{239}Pu(NO_3)_4$ aerosols were discussed in previous Annual Reports. The fraction of plutonium in the lung decreased to less than 5% of the final body burden in dogs surviving 4 yr or more (Table 2). There was early translocation to the liver and skeleton, with an average of 35% and 52%, respectively, of final body burden present in these tissues in dogs surviving 4 yr or more. Only minimal amounts were translocated to thoracic or abdominal lymph nodes. This was in contrast to dogs that inhaled $^{239}PuO_2$, in which a considerable amount translocated to the thoracic lymph nodes, but only minimal amounts translocated to liver or skeleton at these time periods.

TABLE 1. Lifespan Dose-Effect Studies With Inhaled $^{239}Pu(NO_3)_4$ in Beagles.[a]

Dose Level Group	Number of Dogs		Initial Alveolar Deposition[c]	
	Male	Female	nCi[c]	nCi/g Lung[c]
Control	10	10	0	0
Vehicle	10	10	0	0
1	10	10	2 ± 2	0.02 ± 0.02
2	10	10	8 ± 4	0.06 ± 0.04
3	10	10	36 ± 17	0.5 ± 0.2
4	10	10	295 ± 67	2 ± 0.8
5	10	10	1709 ± 639	14 ± 6
6	3	2	5445 ± 1841	47 ± 17

[a] Exposed in 1976 and 1977

[b] Estimated from external thoracic counts at 2 weeks post-exposure and estimated lung weights (0.011 x body weight)

[c] Mean ± standard deviation

The earliest observed biological effect was on the hematopoietic system: lymphopenia occurred at the two highest dose levels at 4 wk after exposure to $^{239}Pu(NO_3)_4$. The results of these continuing evaluations are shown in Figure 1. Total leukocyte concentrations were reduced significantly in the two highest dose groups, i.e., Group 5 (mean initial alveolar deposition, ~1700 nCi), and Group 6 (~5500 nCi). The reduction in white cells in Groups 5 and 6 is due to an effect on most leukocyte types

TABLE 2. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{239}Pu(NO_3)_4$.

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	
1359M	0.1	0.080	90.50	0.15	0.06	2.46	3.20	Sacrifice
1375F	0.1	0.073	89.61	0.14	0.01	0.97	4.68	Sacrifice
1407F	0.1	0.092	51.87	0.41	0.13	10.99	18.70	Sacrifice
1389M	0.5	0.053	24.07	0.38	0.08	41.28	26.21	Sacrifice
1390M	0.5	0.051	24.62	0.32	0.11	20.05	44.45	Sacrifice
1445F	0.5	0.057	26.42	0.32	0.11	21.28	44.73	Sacrifice
1329F	1	0.485	70.05	0.16	0.04	8.28	18.79	Sacrifice
1346M	1	0.902	76.81	0.32	0.03	10.45	10.30	Sacrifice
1347F	1	0.699	71.71	0.36	0.08	9.33	14.09	Sacrifice
1336M	1	0.032	71.38	0.22	0.05	5.72	19.73	Sacrifice
1341F	1	0.022	64.43	0.29	0.10	12.92	18.63	Sacrifice
1344F	1	0.052	58.68	0.25	0.04	21.87	16.09	Sacrifice
1335M	1	0.003	19.52	0.07	0.06	6.68	25.04	Sacrifice
1339F	1	0.001	19.08	0.13	0.08	20.92	45.47	Sacrifice
1351M	1	0.002	40.68	1.22	0.09	17.09	28.89	Sacrifice
1522F	3	0.059	54.68	0.57	0.10	11.52	28.24	Sacrifice
1529F	3	0.049	51.68	0.40	0.07	18.48	23.74	Sacrifice
1539M	3	0.072	52.45	0.31	0.05	18.58	25.03	Sacrifice
1564F	12	0.037	18.00	1.27	0.11	33.53	42.63	Sacrifice
1571F	12	0.053	22.37	1.47	0.11	28.76	42.91	Sacrifice
1588M	12	0.053	13.14	0.40	0.12	35.85	46.18	Sacrifice
1424M	14	4.625	33.10	1.43	0.16	26.49	36.88	Radiation Pneumonitis
1517F	16	4.025	18.99	0.94	0.18	29.51	47.88	Radiation Pneumonitis
1510F	17	4.048	22.00	1.15	0.05	20.71	52.00	Radiation Pneumonitis
1420M	25	1.616	16.51	0.86	0.20	7.77	70.06	Radiation Pneumonitis
1471M	34	1.375	9.25	0.73	0.12	26.92	58.34	Radiation Pneumonitis
1518M	42	1.880	6.87	0.24	0.07	21.34	67.51	Radiation Pneumonitis + Lung Tumor
1512M	42	2.136	4.31	0.60	0.08	49.93	42.66	Bone Tumor
1508M	43	1.730	3.24	0.62	0.08	41.53	52.70	Bone Tumor
1459F	51	1.567	4.40	0.15	0.12	30.86	61.41	Radiation Pneumonitis + Lung Tumor
1492F	52	1.202	2.81	0.20	0.17	27.02	66.38	Bone Tumor
1502F	54	3.113	0.80	0.39	0.09	33.33	62.51	Bone Tumor, Lung Tumor
1485F	55	1.052	0.82	0.35	0.07	31.13	63.94	Bone Tumor
1387F	55	0.167	1.41	0.22	0.12	45.43	49.10	Bone Tumor
1429M	59	1.159	4.13	0.35	0.10	37.05	54.70	Bone Tumor, Lung Tumor
1598F	60	0.058	0.90	0.14	0.17	24.44	31.62	Sacrifice
1576M	60	0.065	1.54	0.36	0.13	46.23	39.15	Sacrifice
1605F	60	0.025	1.87	0.11	0.12	52.32	39.37	Sacrifice
1498F	69	0.845	0.59	0.29	0.13	26.65	53.40	Bone Tumor, Lung Tumor

[a] Includes tracheobronchial, mediastinal and sternal lymph nodes
[b] Includes hepatic, splenic and mesenteric lymph nodes

(neutrophils, lymphocytes, monocytes and eosinophils). This is in contrast to the effects of both $^{239}PuO_2$ and $^{238}PuO_2$, which significantly depressed lymphocyte concentrations by 21 mo after exposure to initial lung burdens of ~80 nCi or more. The lymphocytopenia at lower dose levels of plutonium oxides may be related to the more-extensive translocation of plutonium oxide to the tracheobronchial lymph nodes.

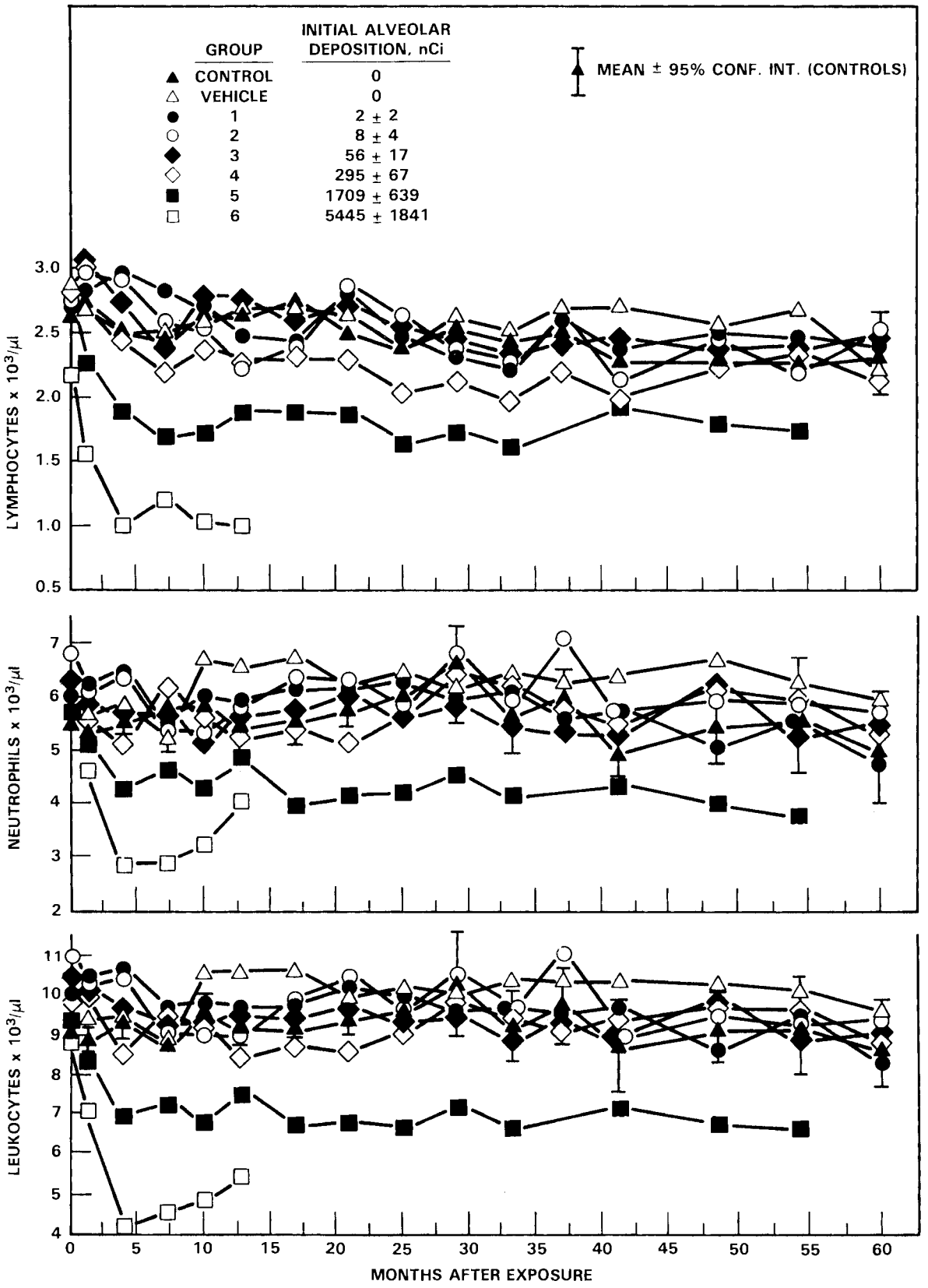

FIGURE 1. Mean Leukocyte, Neutrophil and Lymphocyte Values in Dogs After Inhalation of $^{239}Pu(NO_3)_4$.

All five dogs at the highest dose level and two of 20 dogs at the medium-high dose level died from radiation pneumonitis 14 to 51 mo after exposure. 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.

Small, multiple, bronchioloalveolar carcinomas occurred in two dogs with radiation pneumonitis and in three additional dogs euthanized because of osteosarcomas. Typically, these arose in subpleural areas in proximity to areas of interstitial fibrosis or small cavities communicating with bronchioles. They were composed of irregular proliferations of cuboidal epithelial cells, forming aggregates of epithelial cells extending into adjacent alveoli. A similar small, solitary lung tumor was observed in a dog from the Group 3 dose level sacrificed 62 mo after exposure. No metastases or invasions of nonpulmonary parenchyma were observed.

Osteosarcomas were present in eight dogs euthanized 42 to 69 mo after exposure: seven dogs from the Group 5 dose level and one dog at the Group 4 dose level. The osteosarcomas occurred singly, in humerus, pelvis, sacrum, cranium, and in cervical, thoracic, and lumbar vertebrae. These dogs also had radiation pneumonitis, as described previously, as well as radiation osteosis. The osteosis was generally characterized by peritrabecular fibrosis, composed of relatively hypocellular collagen fibers, and was observed partially surrounding trabeculae in vertebrae, femora, and ribs.

Autoradiographs of liver sections from dogs euthanized 3 to 5 yr after inhalation exposure to the higher dose levels of $^{239}Pu(NO_3)_4$ were compared with liver sections from dogs exposed to levels of $^{239}PuO_2$ that yielded similar concentrations of plutonium in the liver at similar intervals after exposure. The autoradiographs showed that the nitrate-exposed dogs had >99% of plutonium activity in diffusely distributed single tracks (only rarely in alpha stars), whereas the oxide-exposed dogs had >99% of the plutonium activity concentrated in alpha stars (only rarely in single tracks). The difference in microdistribution and character of the alpha activity probably influenced the biological effect.

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 by 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 in these values. The periodic excursions in mean values were usually because of high values in one or two dogs at a particular sampling period, and occurred in all treatment groups, including controls.

These studies will continue in the future.

• Inhaled Transuranics in Rodents

Principal Investigator: C. L. Sanders

Other Investigators: J. Mahaffey, J. M. Morris, and K. Rhoads

This project examines the interactions of external and internal radiation from mixtures of radionuclides present within the nuclear fuel inventory. The objective of the project is to evaluate the effects of mixed radiation insults, using "key" radiation sources as indicative of overall processes that may occur following release of nuclear fuel into the air. Previously initiated studies of immunological effects of plutonium inhalation are also being completed as part of this project.

The literature was reviewed, and consideration was given to technical aspects of administering mixed radiation insults in order to develop the experimental design. Animals will be exposed to three inhaled beta/gamma emitters of differing energies, or to two inhaled alpha emitters (one that is insoluble in the lung and one that is soluble), in a regimen that will result in approximately uniform, whole-body exposure. The regimens are:

- External whole-body exposure to ^{60}Co gamma rays
- Internal, nearly whole-body exposure to a low-energy beta ray given as tritiated water
- $^{85,90}Sr$ as a high-fired oxide, by inhalation
- ^{144}Ce as a high-fired oxide, by inhalation
- ^{239}Pu as a high-fired oxide by inhalation
- ^{244}Cm as a high-fired oxide by inhalation.

This mixture of radionuclides will provide differing radiation doses in space and time and differing energies and LETs. Emphasis is on tumor formation in the lung, liver, bone and bone marrow following mixed radiation insults.

The first phase of the study determined the $LD_{50(30)}$ dose following acute, whole-body exposure to gamma rays from ^{60}Co (Figure 1). The dose was found to be 800 rad. In future studies, Wistar rats will be exposed to external gamma irradiation so as to receive one-half the $LD_{50(30)}$ dose.

In the second phase of the study we examined the influence of mixed radiation exposures on the metabolic fate of individual radionuclides. In the first experiment, two groups of 35 female Wistar rats

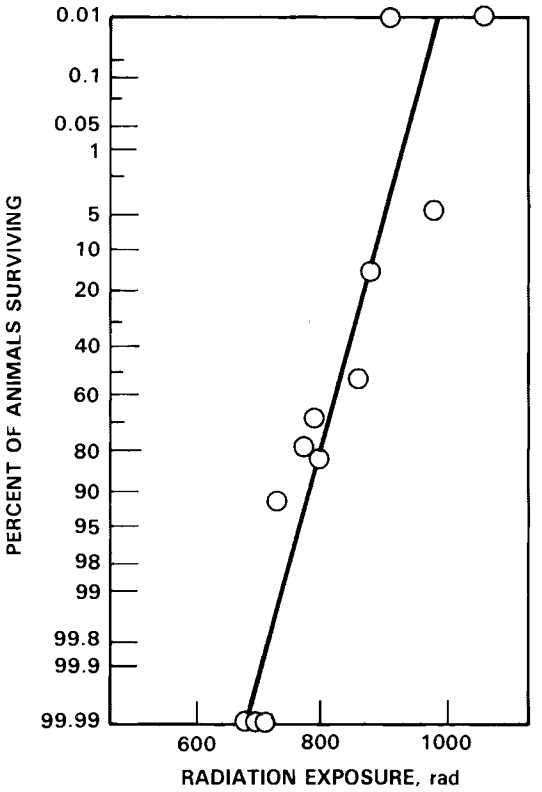

FIGURE 1. $LD_{50(30)}$ Curve for Wistar Rats Exposed to ^{60}Co Gamma Radiation.

that were previously unexposed or had received a whole-body gamma dose of 400 rad/day previously were exposed by inhalation to a high-fired aerosol of $^{239}PuO_2$ (tagged with ^{169}Yb to facilitate external counting). A second study was identical to the first except rats were exposed to an aerosol of high-fired $^{244}CmO_2$.

In a third study of this phase, two groups of 35 rats each were given a single inhalation exposure to a mixture of $^{239}PuO_2$ and $^{244}CmO_2$, calcined together in a ratio of 1:1 by activity, with no ^{169}Yb-tagged ^{239}Pu. For each study group, five rats

were whole-body-counted and killed at 0, 3, 7, 14, 35, 70 and 120 days after inhalation. Animals were also killed for tissue analysis of radionuclide content.

Body burdens at 7 days after inhalation were 37 nCi for plutonium only, 39 nCi for plutonium + external gamma, 68 nCi for curium only, 71 nCi for curium + external gamma and 43 nCi for plutonium + curium. By 120 days after exposure, the body burdens had decreased to 18, 26, 43, 45 and 43%, respectively, of the 7-day body burdens. Based only on whole-body-counting data, it appeared that external whole-body gamma irradiation resulted in a higher retention of plutonium (Figure 2). However, whole-body gamma irradiation did not appear to significantly alter the whole-body clearance of the much more soluble transuranic, curium (Figure 3). The clearance of curium from the lung following inhalation of curium-plutonium particles was similar to the clearance of curium inhaled alone (Figure 4), indicating that the curium separated from plutonium, behaving in the lung as if the plutonium were not present. Scintillation counts of curium and plutonium levels in tissues are not yet available.

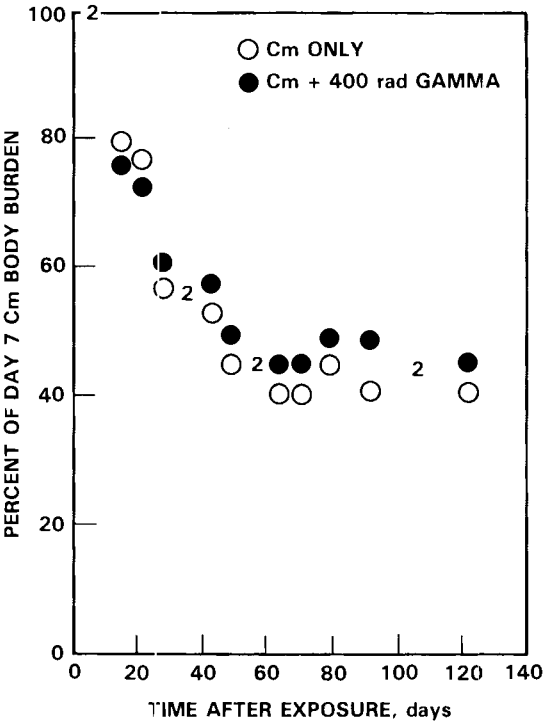

FIGURE 3. Curium Body Burden vs. Time as Percent of 7-Day Values. Experiments 3 (Curium Only) and 4 (Curium + Gamma).

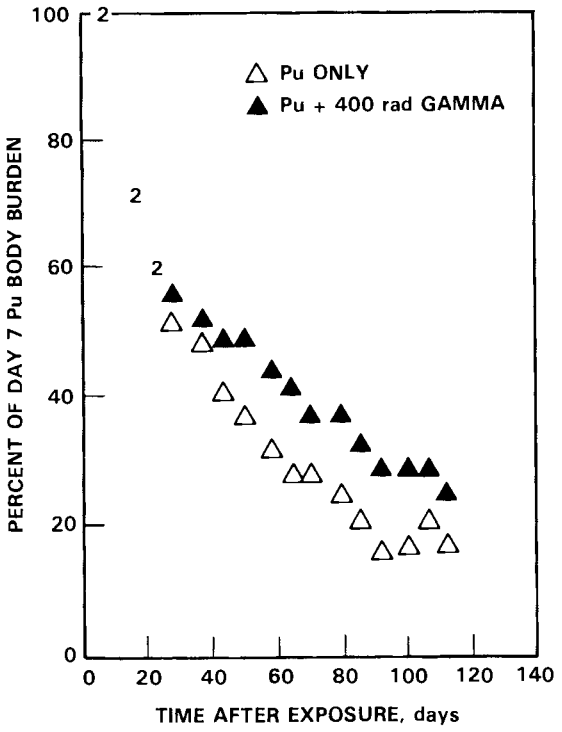

FIGURE 2. Plutonium Body Burden vs. Time as Percent of 7-Day Values. Experiments 1 (Plutonium Only) and Experiments 2 (Plutonium + Gamma).

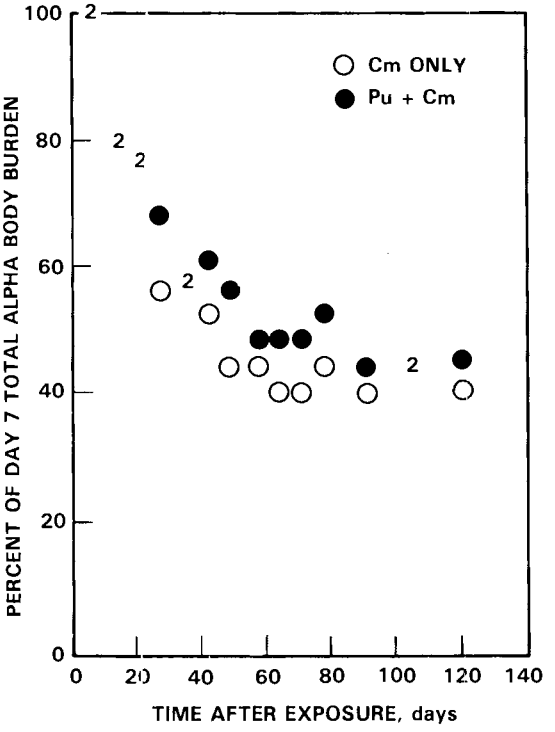

FIGURE 4. Total Alpha Body Burden vs. Time as Percent of 7-Day Values. Experiments 3 (Curium Only) and 5 (Plutonium + Curium).

In the immunology studies, cell-mediated immunity and distribution of T-lymphocytes were measured in rats after exposure to high-fired $^{239}PuO_2$. Cellular immunity was measured with mitogen-induced lymphocyte activation assays, using cells from peripheral blood and spleen stimulated with T- or B-cell-specific mitogens. The mitogens used were concanavalin-A (Con-A) and phytohemagglutinin (PHA), T-cell-specific plant lectins; pokeweed mitogen (PWM), mainly B-cell-specific; and bacterial lipopolysaccharide (LPS), a B-cell-specific agent. The distribution of T-lymphocytes in spleen-cell preparations of control and exposed animals was based on the uptake of ^{3}H-uridine.

Rats were divided into three groups: 1) unexposed controls; 2) low-dose ^{239}Pu inhalation; and 3) high-dose ^{239}Pu inhalation groups (Table 1). Measurements were made at 28 days, 105 days and 253 days after exposure. The initial alveolar deposition and the calculated rad dose to lungs (based on ^{169}Yb whole-body counts) and ^{239}Pu lung counts for each group of animals are shown in Table 1. At 30 days, no significant changes in exposed rats, when compared to controls, were seen in: body or spleen weights, mitogen responses of spleen or peripheral-blood lymphocytes, or in uptake of ^{3}H-uridine by spleen-cell preparations. At 105 days, a statistically significant reduction in responses of spleen cells to PWM and LPS were observed in both exposed groups. Also, the uptake of ^{3}H-uridine by spleen-cell preparations from the high-dose group was significantly reduced when compared to controls. The peripheral-blood lymphocyte responses of both exposure groups to PHA and PWM was significantly increased when compared to control values. At 253 days after exposure, we continue to observe a statistically significant reduction in the response of spleen cells to PWM and LPS. However, the response of peripheral-blood lymphocytes from exposed animals was not significantly different from values observed for controls.

These observations are consistent with a direct effect on the humoral immunity of plutonium-exposed animals that would lead to a reduced capacity for antibody responsiveness to new antigenic challenges. At none of the exposure periods was lymphopenia observed in exposed rats, nor were there any significant differences in body or spleen weights for the exposed groups when compared to controls.

TABLE 1. Immunological Effects of Inhaled $^{239}PuO_2$ in Rats.

N	Initial Alveolar Deposition, nCi	Time of Sacrifice After Exposure, days	Rad Dose to Lung on Day of Sacrifice	Statistically Significant Immunological Changes in Lymphocytes: Spleen Cell Mitogen Response, %(a,b)	Peripheral Blood Mitogen Response, %(a,b)	T-Lymphocyte Levels
10	--	28	--	0	0	0
11	11.0 ± 6.8	28	26.2 ± 15.7	0	0	0
8	126.5 ± 36.6	28	287.3 ± 79.8	0	0	0
10	--	105	--	0	0	0
11	9.9 ± 5.8	105	48.7 ± 28.7	PWM ↓(c) 21 LPS ↓ 26	PHA ↑(c) 44 PWM ↑ 111	0 0
8	125.6 ± 33.5	105	609.9 ± 163.7	PWM ↓ 23 LPS ↓ 27	PHA ↑ 16 PWM ↑ 74	0 0
10	--	253	--	0	0	0
10	5.8 ± 3.7	253	42.7 ± 27.4	PWM ↓ 26 LPS ↓ 15	0 0	0 0
9	145.6 ± 30.6	253	833.0 ± 125.1	PWM ↓ 26 LPS ↓ 29	0	0

(a) Percent change from control
(b) $P<0.05$
(c) Arrows indicate direction of change

• Low-Level $^{239}PuO_2$ Lifespan Studies

Principal Investigator: C. L. Sanders

Other Investigators: J. A. Mahaffey and K. E. McDonald

The overall purpose of this project is to determine the dose-response curve for lung-tumor incidence in rats following inhalation of $^{239}PuO_2$ at levels producing a lifespan radiation dose of from about 1 to over 1000 rad. About 40% of all lifespan exposed and sham-exposed rats are now on experiment. We have demonstrated the usefulness of ^{169}Yb as a tag to estimate initial alveolar deposition of ^{239}Pu at all dose levels in the lifespan portion of the study.

A master $^{239}PuO_2$ lung clearance curve is being constructed to calculate radiation dose to the lung at death for individual rats. This curve is based on initial alveolar depositions as measured by ^{169}Yb counts at 7 and 14 days after exposure. For this metabolism study, a group of 70 rats was exposed to an aerosol of ^{169}Yb-$^{239}PuO_2$, resulting in a mean initial alveolar deposition of about 10 nCi ^{239}Pu. Groups of five rats were killed at intervals for up to 1 yr after exposure. Whole-body counts for ^{169}Yb were performed periodically, and all excreta and tissues were analyzed for ^{169}Yb and ^{239}Pu contents.

A preliminary analysis of these data shows that ^{169}Yb whole-body counting is a good indicator of ^{239}Pu contents in the lung up to about 15 days after exposure; after this period it appears that ^{169}Yb is retained in the body to a greater degree than is ^{239}Pu (Figure 1). Even so, statistical correlations of ^{169}Yb whole-body counts and actual ^{239}Pu found in the lung using liquid scintillation counting indicate continuing significant correlations up to 64 days after exposure.

Nearly 40% of all lifespan-exposed and sham-exposed rats have been placed on the

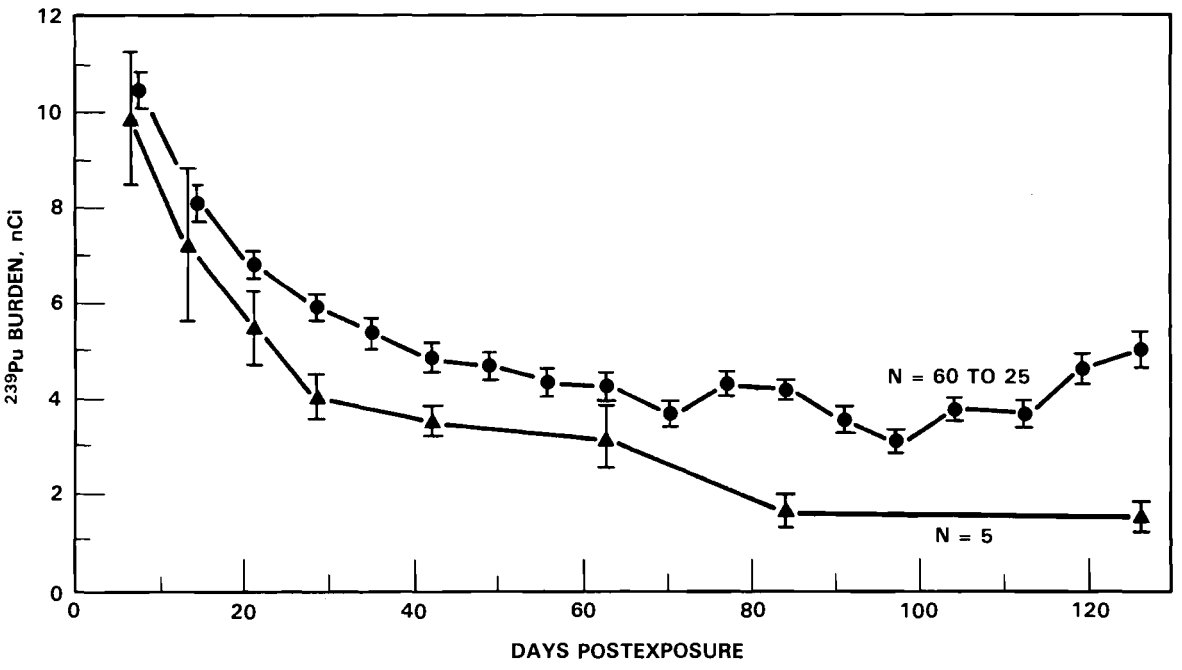

FIGURE 1. Lung Clearance of Inhaled ^{169}Yb-$^{239}PuO_2$ by Rats Given a Single, 30-min Exposure Resulting in an Initial Alveolar Deposition of About 10 nCi ^{239}Pu. Actual ^{239}Pu levels in the lung from periodic sacrifices are shown in the bottom curve (n = 5). Estimated ^{239}Pu levels in the lung from whole-body counts of ^{169}Yb are shown on the top curve (n = 25 to 65); bars represent standard errors around the mean.

study as of October 1982 (Table 1). All rats given target initial alveolar deposi-tions of 2, 4, 8, 16, 32, 64 or 128 nCi ^{239}Pu are on study; about one-third of the 1-nCi ^{239}Pu group has also been exposed. Only four exposed rats and one control rat have died; three deaths, at the highest exposure level, were due to radiation pneu-monitis.

TABLE 1. Protocol for Low-Level $^{239}PuO_2$ Study.

Initial Alveolar Deposition, nCi ^{239}Pu	No. Rats on Lifespan Study			No. Rats as Controls			No. Exposed Rats[a] Killed at 14 Days After Exposure
	Projected	Current	Dead	Projected	Current	Dead	
0.5	1020	--	--	510	--	--	170
1	510	180	0	255	90	0	85
2	210	210	0	105	105	0	34
4	100	100	0	45	45	0	15
8	60	60	0	30	30	0	10
16	60	60	1	30	30	0	10
32	60	60	0	30	30	0	10
64	60	60	0	30	30	0	9
128	60	60	3	30	30	1	8

[a] About 5 rats are routinely killed at 14 days after exposure in each group of 35 exposed rats; the ^{239}Pu contents of the lung as measured by liquid scintillation are compared with estimated ^{239}Pu contents based upon whole-body counts for ^{169}Yb, providing a continuing check of the accuracy of our estimation procedures for ^{239}Pu initial alveolar deposition.

• Cigarette Smoke and Plutonium

Principal Investigator: R. E. Filipy

Other Investigators: W. J. Bair, R. L. Buschbom, J. L. Pappin, and D. L. Stevens

Technical Assistance: S. D. Harris, S. G. Irby, and J. F. McShane

The major objective of this project is to obtain experimental data that are directly applicable to resolving the question of whether cigarette smokers are at greater risk than nonsmokers to potential health effects of inhaled plutonium. Because cigarette smokers constitute a large fraction of the population, a synergistic effect of plutonium and cigarette smoke might influence estimates of the health risk for plutonium and other transuranics released to the environment.

An autoradiographic technique developed for detection of plutonium on the interior surface of pulmonary airways (Annual Report, 1978) was used on the lungs of rats from an earlier experiment. The data demonstrated the effect of cigarette-smoke exposure on the mucociliary clearance mechanism. A similar technique, using cellulose nitrate track-etch film, is being applied to the pulmonary airways of dogs.

Distribution and Retention of $^{239}PuO_2$ in Pulmonary Airways of Rats

Results of a previously reported experiment with rats showed a marked cigarette-smoke-induced reduction in pulmonary clearance of inhaled $^{239}PuO_2$. A group of 35 rats was exposed to cigarette smoke for 7 mo; another group of 35 rats was sham-exposed for the same period of time. Both groups were exposed, by inhalation, to $^{239}PuO_2$ (approximately 80 nCi initial lung burden [ILB]), and smoke and sham exposures were resumed 7 days afterward. In vivo (whole-body) counts indicated that smoke- and sham-exposed rats retained means of 64 and 39% of their ILBs, respectively, 6 wk after inhalation of plutonium (Table 1); the difference was highly significant ($P < 0.0002$).

The pulmonary airways of these rats were examined using an autoradiographic technique developed for quantifying the distribution of plutonium particles. The technique involved isolation of large airways from lung parenchyma, separation of the mucosa from cartilage and adventitia, and mounting the sheet of epithelium on a glass microscope slide so that the inside surface of the airway faced outward. This surface was coated with liquid photographic emulsion, exposed, developed, and coverslipped. The final preparation consists of epithelium from approximately 2.5 cm of trachea and 2.0 cm of both the right and left bronchi. A transparent grid was superimposed over the slide, and the alpha "stars" within each grid square (area, ~1 mm^2) were counted. Figure 1 is a photomicrograph of the preparation as it appears during evaluation. Data recorded include the location of each grid square with respect to the total airway surface and the number of alpha stars each grid square contains.

A summary of the data collected from rats killed at 1, 7, and 42 days after inhalation is given in Table 1. In sham-exposed rats, the concentration of plutonium particles in pulmonary airways on day 42 was less than that on day 1 by a factor of almost five. Corresponding data from smoke-exposed rats show a concentration reduced by a factor of 14. Smoke-exposed rats had significantly ($P < 0.01$) lower concentrations of plutonium in their airways than did sham-exposed rats regardless of when they were killed.

Microscopically, it is impossible to distinguish whether the plutonium particles causing the autoradiographic star are located within or beneath the mucosal blanket. The concentrations of plutonium particles in the airways of smoke-exposed rats were consistently low, compared to those of sham-exposed rats, even though in vivo counts verify approximately equal amounts of plutonium inhaled by both groups. This indicates that particle concentrations reflect clearance rate at the time of death. This observation is also indicated by the inverse relationship between airway particle concentration and in vivo counts; apparently, plutonium particles in the airways have little effect on total body count.

Two observations made during the evaluation of the airway preparation are noteworthy. First, aggregations of plutonium particles were not necessarily associated with bifurcation in the airways. Many of the bifurcations are clearly visible in the bronchial preparations; there was no readily apparent selective deposition or retention

TABLE 1. The Effect of Cigarette Smoke Exposure on Clearance of $^{239}PuO_2$ From the Lungs of Rats (Mean ± SD)

Exposure Group	Sacrifice Time Postexposure, days	In Vivo Counts			Pu Particles per mm²			
		Day 4 Postexposure[a], cpm	1 Day Before Sacrifice		Trachea	Right Bronchus	Left Bronchus	Airway Average
			cpm	% of day 4				
Pu Only	1				9.2 ± 6.6 (6)	15.2 ± 8.4 (6)	9.5 ± 6.0 (10)	11.2 ± 4.9 (22)
Smoke + Pu	1				4.4 ± 1.4 (9)	4.6 ± 3.1 (10)	4.4 ± 3.3 (9)	4.2 ± 1.8 (28)
Pu Only	7	169 ± 32 (10)[b]	147 ± 27 (10)	88	6.8 ± 3.6 (6)	9.4 ± 4.4 (8)	11.8 ± 7.6 (8)	10.4 ± 6.4 (22)
Smoke + Pu	7	159 ± 30 (10)	147 ± 25 (10)	94	0.8 ± 0.6 (8)	1.6 ± 0.7 (9)	2.2 ± 1.2 (7)	1.4 ± 0.6 (24)
Pu Only	42	163 ± 22 (10)	64 ± 11 (9)	39	2.1 ± 1.6 (7)	2.6 ± 1.7 (8)	2.2 ± 1.4 (6)	2.3 ± 1.5 (21)
Smoke + Pu	42	161 ± 47 (9)	103 ± 24 (9)	67	0.3 ± 0.1 (9)	0.3 ± 0.2 (9)	0.3 ± 0.1 (8)	0.3 ± 0.1 (26)

(a) The count at 4 days postexposure was considered the initial lung burden.
(b) Number of observations upon which each mean is based

FIGURE 1. Alpha "Stars" in an Autoradiograph of the Inside Surface of a Rat Trachea

at these sites. However, there were distinct regional differences in particle concentration in the tracheal preparations. Higher particle concentrations were frequently noted at the dorsal wall of the trachea. Since, at necropsy, the lungs were inflated with air and fixed by vascular perfusion with 2% glutaraldehyde, plutonium in the airways at the time could not have been translocated by the fixative. Subsequent scanning electron micrographs of the tracheal wall of a rat revealed that the dorsal wall was much more ciliated than the ventral wall. The micrographs (Figures 2 and 3) show the trachea of an unex-

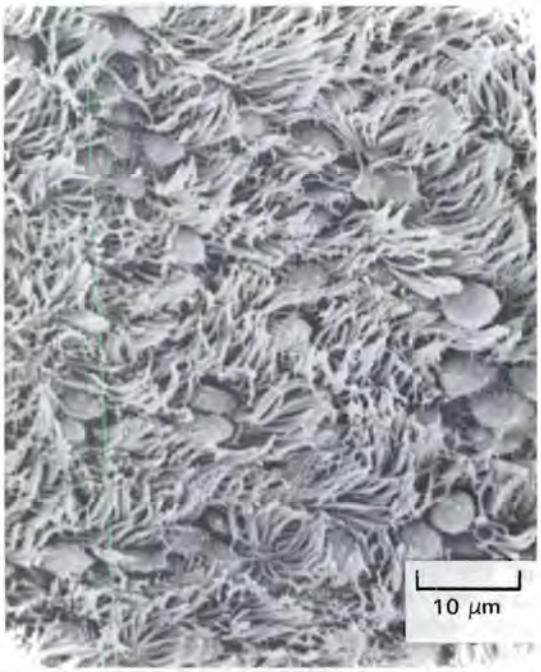

FIGURE 2. Scanning Electron Micrograph of the Dorsal, Inside Surface of a Rat Trachea.

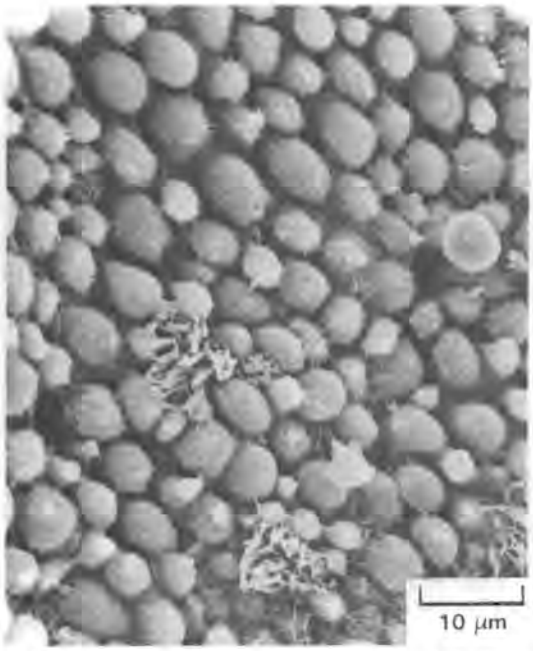

FIGURE 3. Scanning Electron Micrograph of the Ventral, Inside Surface of a Rat Trachea.

posed, control rat. Although this difference has been alluded to in light microscopic studies of the trachea, the magnitude of the difference shown by scanning electron microscopy was quite surprising. Further investigation of airway anatomy will include microscopy of rat bronchi and of the airway of at least one other species.

When this program began, one hypothesis considered was that areas of pulmonary airway mucosa would be denuded of cilia by cigarette smoke, resulting in selective retention of plutonium in those areas. The data collected thus far do not indicate such selective retention. We conclude, rather, that the greater pulmonary retention of plutonium by smoke-exposed rats (compared to sham-exposed rats) demonstrated by whole-body counting is a direct result of the diminished mucociliary clearance induced by cigarette smoke. More data will be available in the future from autoradiographs of lung sections from these rats and from radiochemical analysis of remaining lung tissue.

Distribution and Retention of $^{239}PuO_2$ in Pulmonary Airways of Dogs

Cigarette-smoke-exposed and sham-exposed dogs were killed approximately 60 wk after inhalation exposure to a $^{239}PuO_2$ aerosol. Lungs were inflated with air and fixed by vascular perfusion. Retention and distribution of plutonium in pulmonary airways will be characterized by a form of autoradiography, using cellulose nitrate track-etch film. The refined technique for this purpose is as follows: lung parenchyma is stripped from the tracheobronchial tree down to approximately the level of the secondary bronchi of each lung lobe. These are split, flattened, and presented so that track-etch film is in contact with the internal surface. After an empirically determined exposure time, the film is removed and etched with NaOH solution, which results in the appearance of alpha "stars" where the film was in contact with a plutonium particle. A disadvantage of this technique is that the film is no longer in contact with the tissue at evaluation; however, photographs taken of the tissue preparation during exposure are expected to compensate. On the other hand, a marked advantage to this technique over conventional autoradiography is that several films may be made of the same tissue, if necessary. Because this work is in its initial stages, no results are available at this time.

• Toxicity of Sodium-Lithium

Principal Investigator: R. H. Busch

Other Investigator: J. E. Morris

The objective of this project is to determine possible toxic effects of aerosolized reaction products of sodium and lithium that might be released from accidental breaks in reactor coolant systems employing these metals as heat transfer agents. In current studies, rats were injected intravenously with lithium chloride (1 mg) to determine blood clearance characteristics. Measurements over a 4-hr test period revealed that levels dropped rapidly after injection to values 10-15 times higher than those observed in rats prior to intravenous injection.

Previous studies have concentrated on chemical and physical characterization of sodium coolant aerosols and the biological effects of various aerosol concentrations (1500 to 3600 μg/L) on rats, mice and guinea pigs. Clinical observations in exposed animals included respiratory distress, with dyspnea and wheezing. Pathological lesions, primarily involving the pharynx and larynx, included accumulations of mucus, vesiculation, and edematous mucosa. In additional studies in rats, bacteremia occurred at 1 to 14 days after exposure. In subsequent immunological studies on rats exposed to a single aerosol concentration (2600 μg/ml) and killed 3 to 4 days after exposure, a potential suppression of cellular immunity was reflected by the reduced response of spleen cells to mitogens.

Initial studies for assessing effects in rats exposed to lithium have concentrated on generating aerosols of lithium combustion products. Because technical problems have been encountered in producing aerosols of sufficiently high concentration to permit preliminary dose-effect studies, efforts have been directed at developing definitive information on blood concentration and clearance.

For these studies, we have explored methods of cannulating the right jugular vein in rats for serial serum sampling and for the intravenous instillation of lithium (salt) solutions. To determine the clearance of lithium from the blood stream, Sprague-Dawley rats were anesthetized, cannulated, and injected intravenously with 1 mg of lithium as LiCl in 5% dextrose solution. Serum samples were taken 10 min prior to the lithium injection and at various periods (2 min to 4 hr) after injection.

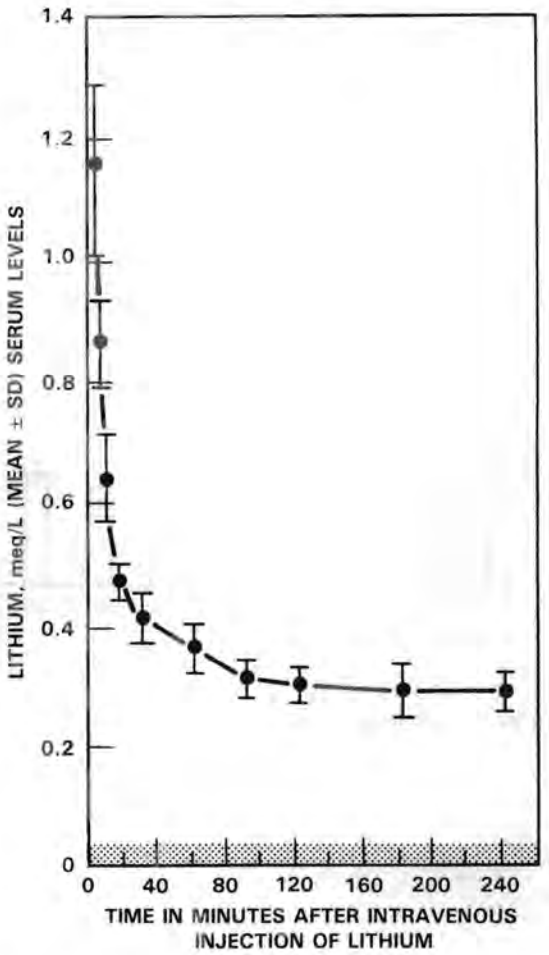

FIGURE 1. Lithium Serum Levels in Rats 0 to 4 hr After Injecting Lithium Chloride. Values are mean ± SD; N = 6. Shaded area across bottom represents the mean ± SD of pre-injection lithium levels.

In the preliminary study (Figure 1), the maximum mean levels (1.15 meq/L) were observed at 2 min after intravenous injection. The meq/L of lithium in serum continued to drop thereafter: the mean value at 8 min was 0.64 meq/L, and by 16 min, 0.47 meq/L. By 90 min after injection, the value had dropped to approximately 0.3 meq/L and remained at this level for the next 2.5 hr. Pre-injection values observed

for lithium were approximately 0.02 meq/L, approximately 10 to 15 times less than the values observed at 4 hr after injection.

In the second experiment, rats were anesthetized, cannulated and injected intravenously 1 day after surgery with 1 mg of lithium as LiCl in 5% dextrose solution. Serum samples were taken on the day of surgery, 10 min prior to the lithium injection, and at 4, 12, 24, 36, 48, 72, 84, 96, 108 and 168 hr after injection. The mean lithium levels (Figure 2) at 4 hr after injection were 0.31 meq/L, similar to values observed in the first study. At 12 hr after injection, the mean values had dropped to approximately 0.18 meq/L. The values continued to decrease: serum levels at 24 hr were 0.13 meq lithium/L; at 36 hr after injection, the mean values were 0.10 meq/L. Over the next few days the levels were from 0.09 to 0.07 meq/L, which was in the range (0.07 meq/L) observed for these rats prior to lithium injection.

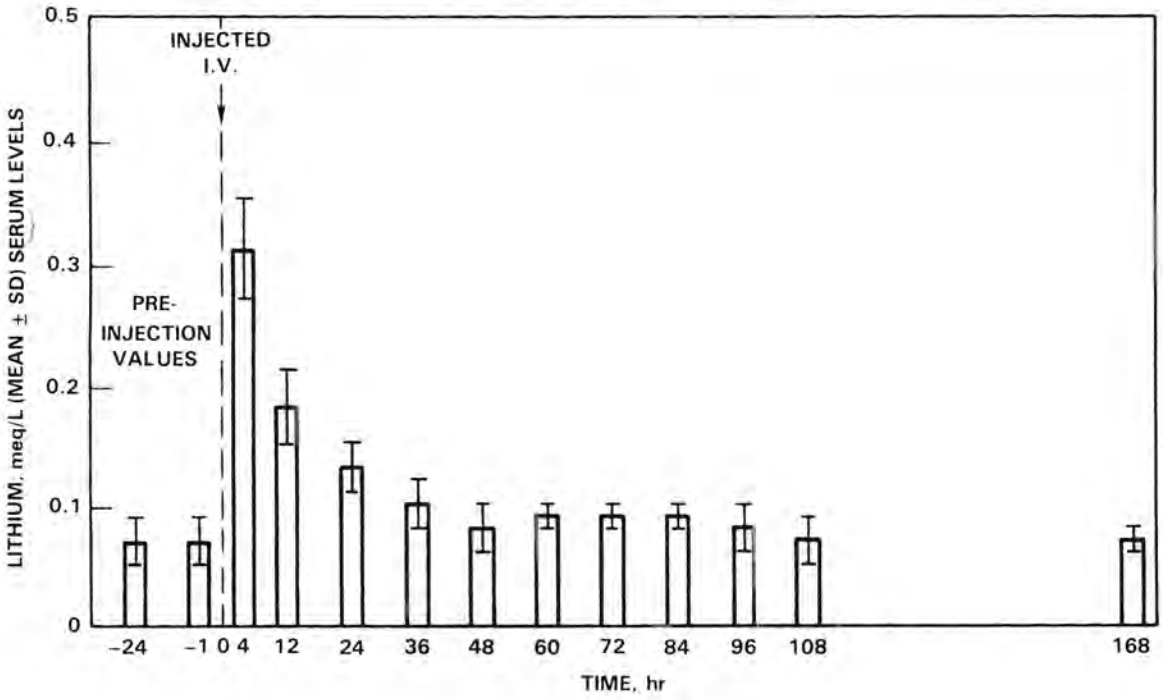

FIGURE 2. Long-Term Lithium Serum Levels in Rats Injected With Lithium Chloride. Values are mean ± SD; N = 6.

• Inhalation Hazards to Uranium Miners

Principal Investigator: F. T. Cross

Other Investigators: R. L. Buschbom, G. E. Dagle, R. E. Filipy, P. O. Jackson, S. M. Loscutoff, and R. F. Palmer

Technical Assistance: G. Brodaczynski, C. R. Petty, and W. L. Skinner

This project is investigating levels of uranium mine air contaminants, using both large and small experimental animals to model human respiratory system 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.

Small-Animal Studies

Approximately 2000 male, specific-pathogen-free Wistar rats are currently on study; the 4000 and 5000 Series experiments (Tables 1 and 2) are designed to clarify the roles of unattached RaA daughters, and the degree of radon daughter disequilibrium, in the development of respiratory system disease. The 6000 and 7000 Series experiments (Table 3) are designed to develop the relationships between response and exposure to radon daughters (at two rates of exposure) and carnotite uranium ore dust. The 8000 Series experiments (Table 4) are designed to extend the exposure-response relationships to levels appropriate to current exposure conditions in the mines and to lifetime environmental exposures. The exposures of 7000 and 8000 Series animals are currently in progress; the exposures of 4000, 5000 and 6000 Series animals are completed; a few of the 6000 Series animals are still living.

TABLE 1. Radon-Daughter Unattachment Fraction Study in Rats (4000 Series experiments).

Exposure Regimen[a]	Total Exposure, WLM[b]
500 WL Radon Daughters Low RaA Unattachment (~2%) ~15 mg/m^3 Uranium Ore Dust	5120
500 WL Radon Daughters Intermediate RaA Unattachment (~10%) ~0.7 mg/m^3 Uranium Ore Dust	5120
500 WL Radon Daughters High RaA Unattachment (~24%) ~0.4 mg/m^3 Uranium Ore Dust	5120
Controls	

[a] 32 animals in each group, exposed 90 hr/wk

[b] Working level (WL) is defined as any combination of the short-lived radon daughters in 1 liter of air that will result in the utlimate emission of 1.3×10^5 MeV of potential α-energy. Working Level Month (WLM) is an exposure equivalent to 170 hr at a 1-WL concentration.

TABLE 2. Radon-Daughter Disequilibrium Study in Rats (5000 Series experiments).

Exposure Regimen[a] Daughter Equilibrium Ratios				Total Exposure, WLM[b]
Rn	RaA	RaB	RaC-C'	
1	0.9	0.4	0.2	5120
1	0.5	0.07	0.01	5120
Controls				

[a] 32 animals in each group, exposed 90 hr/wk at 500 WL and 15 mg/m^3 uranium ore dust

[b] Working Level (WL) is defined as any combination of the short-lived radon daughters in 1 liter of air that will result in the ultimate emission of 1.3×10^5 MeV of potential α-energy. Working Level Month (WLM) is an exposure equivalent to 170 hr at a 1-WL concentration.

We have concluded that the most significant lesions related to radon daughter and carnotite ore-dust exposures in the 4000 and 5000 Series experiments are neoplastic and non-neoplastic lesions of the respiratory tract. Histopathologic data for these lesions and survival times are shown in Table 5.

The data in Table 5 suggest that high disequilibrium of radon daughters (column 5) is associated with a higher risk than moderate disequilibrium (column 2). Furthermore, the risk increases with RaA unattachment level (columns 2 and 3) but appears to

TABLE 3. Exposure-Response Relationship Study for Radon-Daughter Carcinogenesis in Rats (6000 Series experiments).

Number of Animals[a]	Exposure Regimen[b,c]	Total Exposure, WLM[d]
32	1000 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	10,240
32	1000 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	5120
32	1000 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	2560
32	1000 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	1280
64	1000 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	640
128	1000 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	320
32	Controls	

[a] Number of animals is sufficient to detect the predicted incidence of lung tumors at the 0.05 to 0.1 level of significance, assuming linearity of response between 0 and 9200 WLM (see footnote d), and 0.13% spontaneous incidence.
[b] Exposure rate, 90 hr/wk; planned periodic sacrifice.
[c] Study will be repeated @ 100 WL rate (without periodic sacrifice) to augment previous limited exposure-rate data (7000 series experiments).
[d] Working Level (WL) is defined as any combination of the short-lived radon daughters in 1 liter of air that will result in the ultimate emission of 1.3 x 10^5 MeV of potential α-energy. Working Level Month (WLM) is an exposure equivalent to 170 hr at a 1-WL concentration. Previous exposure at 900 WL for 84 hr/wk to 9200 WLM produced a 60% incidence of carcinoma.

TABLE 4. Low Exposure-Response Relationship Study for Radon-Daughter Carcinogenesis in Rats.

Number of Animals[a]	Exposure Regimen[b]	Total Exposure, WLM[c]
64	100 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	640[d]
64	100 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	320[d]
160	100 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	160
352	100 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	80
448	100 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	40
512	100 WL Radon Daughters 15 mg/m^3 Uranium Ore Dust	20
160	Controls	

[a] Number of animals is sufficient to detect lung tumors at the 0.05 to 0.1 level of significance, assuming linearity of response between 0 and 640 WLM (see footnote c), and 0.13% spontaneous incidence.
[b] Exposure rate, 90 hr/wk; planned periodic sacrifice of 32 animals from group
[c] Recent exposures indicate a tumor incidence of 16% at 640 WLM. Working Level (WL) is defined as any combination of the short-lived radon daughters in 1 liter of air that will result in the ultimate emission of 1.3 x 10^5 MeV of potential α-energy. Working Level Month (WLM) is an exposure equivalent to 170 hr at a 1-WL concentration.
[d] Repeat exposure is for normalization with Table 3 data.

decrease at the highest RaA unattachment level (column 4). In view of the significant decrease in survival time, the decrease at the highest RaA unattachment level may be more apparent than real. The high incidence of non-neoplastic lesions in this group of animals is supportive evidence that a competing risk analysis should be performed on the data.

Large-Animal Studies

Thirty-five beagle dogs are currently on study to determine the pathogenic role of inhalation exposure to carnotite uranium ore dust. We are particularly interested in clarifying the role of the ore dust in the production of the massive pulmonary fibrosis observed in an earlier study, in which beagle dogs were exposed to radon daughters and mixtures of uranium ore dust and cigarette smoke. The present study (chronic, head-only exposures) began when the dogs were about 2½ yr old. Along with routine physical examinations and periodic hematologic and clinical chemistry measurements, histopathologic, radiometric, morphometric and pulmonary function evaluations were conducted on these dogs.

The only significant pulmonary-function change attributed to carnotite uranium-ore-dust exposure (at ~15 mg/m^3, for 4 hr/day, 5 days/wk) is an increased slope of the single-breath N_2 washout curve, suggesting an uneven distribution of ventilation. This change was observed in dogs exposed for less than 1 yr and continued without increase through 5 yr of exposure. Measurements of pulmonary resistance, made through 5 yr exposure, showed slight age-related changes and increasing difference between control and exposed animals with duration of exposure. These two changes are suggestive of bronchitis, similar to the "industrial" bronchitis of mine workers. Along with a change in the dynamic pulmonary compliance values, these findings indicate changes in the distal airways of the dogs' lungs.

The most notable pulmonary lesions observed in dogs exposed for up to 4 yr are vesicular emphysema, peribronchiolitis and focal

TABLE 5. Summary of Respiratory Tract Lesions and Survival Times (4000 & 5000 Series experiments).

	RaA Unattachment Groups				
Neoplastic Lesions	2%	10%	24%	High Disequilibrium	Controls
Lung					
Epidermoid Carcinoma	9	7	2	7	0
Adenocarcinoma	8	17	5	17	1
Adenosquamous Carcinoma	0	2	0	4	0
Mesothelioma	1	1	0	0	0
Adenoma	1	2	1	2	0
Total Number of Rats with Lung Tumors	16/32	25/32	8/32	22/32	1/64
Nose					
Nasal Carcinoma	1	4	2	0	0
Total Number of Rats with Respiratory Tract Tumors	17/32	25/32	9/32	22/32	1/64
Non-Neoplastic Lesions					
Lung					
Adenomatous Hyperplasia	20	21	12	28	2
Squamous Metaplasia	1/32	4/32	4/32	3/32	0/64
Nose					
Adenomatous Hyperplasia	0	1	0	0	0
Squamous Metaplasia	9/26	15/30	20/29	11/31	4/60
Survival Time (days)	451 ± 210	524 ± 148	355 ± 186	526 ± 160	671 ± 178

pneumoconiosis. These lesions, described in the 1981 Annual Report (PNL-4100, PT 1), were contrasted with the lesions observed in the earlier study, in which beagle dogs were exposed to mixtures of radon daughters, uranium ore dust and cigarette smoke. No animals were killed following 5 yr of exposure to determine any further progression of pulmonary lesions.

Radiometric analyses of lung tissues were performed on two of three animals killed each year (through 4 yr) following the start of uranium-ore-dust exposures. Previous inhalation studies in our laboratory involving three species of animals (rat, hamster and beagle dog) and two varieties of uranium ore dust in secular equilibrium (carnotite and pitchblende) revealed consistent separation of uranium and thorium isotopes in tissues of animals at necropsy. The pattern of higher retention of thorium than uranium was noted shortly after exposures began and suggested that uranium ore, when present as an airborne contaminant, should be regulated on the basis of its constituent radionuclides, making the ^{230}Th level the determining factor in maximum permissible air concentrations. Because the carnotite ore experiments were confounded by the presence of other air contaminants, such as radon daughters, diesel engine exhaust fumes and cigarette smoke, and because subsequent measurements of miners' lungs at the University of Utah revealed that uranium and thorium isotopes remain in near-equilibrium, it was decided to repeat some of these measurements in the lungs of beagle dogs exposed to carnotite ore dust alone. The data are shown in Table 6.

The current data are qualitatively consistent with the previous data but show even higher disequilibrium of uranium and thorium isotopes. Approximately 80 g of lung tissue were randomly selected for radiometric analyses. The spread in data with each exposure period may reflect individual animal differences as well as differences in the distribution of the isotopes in the lungs. The previous data showed disequilibrium ratios of ^{230}Th to ^{238}U ranging from 5.6 to 7.4 in the lungs of beagle dogs exposed 4 hr/day, 5 days/wk for about 4 yr. Similar exposure protocols to carnotite ore

TABLE 6. Uranium and Thorium Isotope Concentrations and Ratios in Beagle Dogs' Lungs Following Exposure to Carnotite Uranium Ore Dust.

Dog Number	Duration of Exposure, yr	Wet Tissue Weight, nCi/kg					
		^{238}U	^{234}U	^{230}Th	$^{238}U/^{234}U$	$^{230}Th/^{238}U$	$^{230}Th/^{234}U$
1675	1	4.51 ± 0.12	4.27 ± 0.11	31.6 ± 0.8	1.06	7.00	7.39
1679	1	6.25 ± 0.10	6.42 ± 0.10	39.2 ± 0.8	0.97	6.28	6.11
1462	2	4.26 ± 0.05	4.38 ± 0.05	25.4 ± 0.6	0.97	5.97	5.80
1469	2	8.26 ± 0.14	8.53 ± 0.14	73.0 ± 1.4	0.97	8.84	8.56
1468	3	5.22 ± 0.09	5.40 ± 0.09	24.8 ± 0.7	0.97	4.74	4.59
1499	3	7.49 ± 0.10	7.70 ± 0.10	112.6 ± 3.1	0.97	15.0	14.6
1473	4	7.95 ± 0.19	8.26 ± 0.19	106.4 ± 1.9	0.96	13.4	12.9
1481	4	8.33 ± 0.23	8.63 ± 0.23	179.4 ± 3.8	0.97	21.5	20.8

dust alone resulted in a ratio ranging from about 5 to 22, steadily increasing with duration of exposure. Subsequent analyses of lungs of sacrificed animals will reveal whether this disequilibrium continues to increase.

• Toxicology of Krypton-85

Principal Investigator: J. E. Ballou

Other Investigators: G. E. Dagle, H. S. DeFord, D. W. Murphy, M. R. Sikov, H. D. Tolley, and D. H. Willard

Technical Assistance: A. W. Endres

The purpose of this research 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. We have also included dose-effect studies in rats exposed acutely as newborns or chronically for most of their life span to identify tissues at risk and determine tumorigenic potency.

This report presents progress in two ongoing dose-effect studies employing male and female Wistar rats exposed to graded dose levels of ^{85}Kr. The first study involves adult rats exposed continuously (24 hr/day, 7 days/wk) for 808 days to ^{85}Kr atmospheres equivalent to 10^2, 10^3 or 10^4 times the maximum permissible concentration (MPC) in air. ($MPC_a = 3 \times 10^{-7}$ μCi ^{85}Kr/ml for areas of uncontrolled access or residential areas.) In the second study, newborn rats were exposed to ^{85}Kr <48 hr after birth to investigate the tumorigenic effect of skin-surface doses of approximately 958, 2328 or 4738 rad. Effects on life span, weight gain and other preliminary observations of toxicity, including tumorigenesis, were presented for both studies in Annual Reports for 1975 through 1981.

In the chronic study, ^{85}Kr exposures were discontinued after 808 days when approximately 90% of the original 400 rats (100 rats per group, including a group of room-air controls) were dead. The surviving rats were transferred from the exposure chambers to standard animal quarters, where they were weighed each month, radiographed, necropsied, and otherwise processed in the same manner as during the exposure period. Malignant lesions observed in tissues are summarized in Table 1.

The incidence of malignant lesions was not treatment-related in tissues receiving the greatest radiation dose (skin > lung > adrenal > gonads), nor in the other organ systems where they were found. The chi-square test for homogeneity, which compared tumor incidence in ^{85}Kr-exposed groups and controls, was not significant at $P > 0.1$. Nor were differences in tumor incidence among ^{85}Kr-exposed groups significant at $P > 0.1$. The results demonstrate that continuous exposure of rats to ^{85}Kr concentrations equivalent to 100 to 10,000 times the MPC_a for the general population did not significantly affect life span or malignant tumor incidence. Although the skin dose was reduced by ~50% from the theoretical infinite beta cloud dose because of cage shielding, the beta dose to other organs and tissues should be relatively unaffected. The relative ineffectiveness of ^{85}Kr exposure therefore supports the conservatism of the established limits for occupational and population exposure to ^{85}Kr.

Newborn rats were exposed to ^{85}Kr in two replicate studies, 1 yr apart. Since the dose groups for the two studies were quite similar (skin dose was estimated by thermoluminescent dosimetry), the results were combined for presentation in Table 2. The data suggest a clear association between ^{85}Kr exposure and skin cancer. Malignant lesions of the epidermis appear to be a specific result of ^{85}Kr radiation, as would be expected from the range of the 0.67-MeV beta emission and the proximity of the target cells, presumably located in the basal-cell layer. In our study, a dose of about 1000 rad produced four basal cell carcinomas, a lesion which is extremely rare in the Wistar rat. (None were found in the 399 rats examined in the chronic exposure study.)

Benign lesions, e.g., skin papillomas, trichoepitheliomas (involving the hair follicles) and calcifying epitheliomas, also appear to be dose-related. They were found in six rats from the high dose group, three rats from the intermediate dose group, and one rat in the low dose group. None of these lesions were observed in the control rats.

TABLE 1. Cumulative Radiation Dose and Malignant Tumor Response in Target Organs of Rats Exposed Chronically to ^{85}Kr Atmospheres.

	Number of Tumors			
Chamber Concentration, μCi/ml	3×10^{-3}	3×10^{-4}	3×10^{-5}	Control
Skin	(1465)[a]	(141)	(18)	(0.11)
Squamous Cell Carcinoma	2	0	0	1
Neurofibrosarcoma	1	0	0	1
Lung	(28)	(3)	(0.3)	--
Adenocarcinoma	0	1	0	0
Malignant Mesothelioma	0	1	0	0
Malignant Histiocytoma	1	0	1	1
Malignant Lymphoma	1	1	0	0
Adrenal	(2.3)	(0.2)	(0.02)	--
Adenocarcinoma	0	0	0	1
Cortical Carcinoma	1	2	1	2
Malignant Pheochromocytoma	0	1	2	2
Gonads	(1.0)	(0.1)	(0.01)	--
Ovarian Carcinoma	1	0	0	0

[a] Cumulative radiation dose in rad/yr

TABLE 2. Malignant Tumors in Rats Exposed for 6 hr as Newborns to ^{85}Kr Atmospheres.

Mean Skin Dose, rad ± SD	Mean Exposure Conc., μCi/ml ± SD	Number of Rats	Number of Rats with Skin Carcinoma			
			Basal Cell	Squamous	Sebaceous	Undifferentiated
958 ± 100	5.2 ± 0.5	32	4	0	0	0
2328 ± 240	12.7 ± 1.3	33	14	3	6	0
4738 ± 490	25.9 ± 2.7	25	15	5	2	2
Control	--	33	0	0	0	0

• Toxicity of Thorium Cycle Nuclides

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Technical Assistance: R. A. Gies

The purpose of this project is to investigate the biological hazards associated with uranium-thorium breeder fuels and fuel recycle process solutions. Initial studies emphasize the metabolism and long-term biological effects of inhaled ^{233}U-^{232}U nitrate and oxide fuel materials and of ^{231}Pa, a major, long-lived, radioactive waste product.

This report presents progress in two ongoing studies, also discussed in previous Annual Reports, entitled "Disposition and Late Effects of Inhaled $^{233}UO_2(NO_3)_2$ and $^{232}UO_2(NO_3)_2$ in Rats" and "Early Disposition of Inhaled ^{231}Pa Citrate."

Long-term retention of graded doses of inhaled $^{233}UO_2(NO_3)_2$ and $^{232}UO_2(NO_3)_2$ was determined in male Wistar rats to estimate radiation-dose/biological-effect relationships. Retention in liver and kidney is shown in Figure 1 for the highest-dose group for both isotopes, i.e., 35.9 and 53 nCi initial lung burden (ILB) for ^{233}U and ^{232}U, respectively. Uranium-232 results for liver are not yet complete. Results are shown for rats sacrificed in groups of five each at 7, 30, 60, 100, 150 and 200 days after exposure and for individual rats that died during the lifespan study of biological effects. Parameters of retention determined from these curves are shown in Table 1.

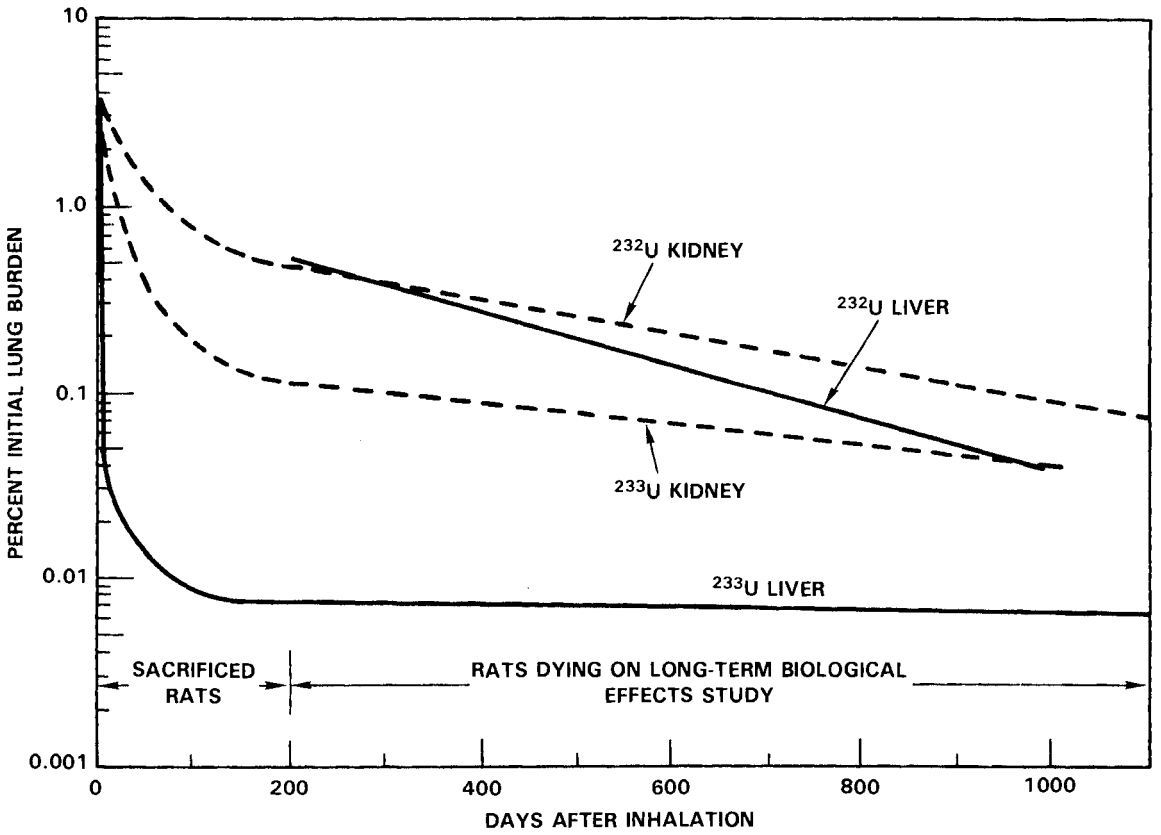

FIGURE 1. Retention of Inhaled Uranyl Nitrate (^{233}U and ^{232}U) in Rat Kidney and Liver.

TABLE 1. Retention Parameters and Estimated Radiation Dose for ^{233}U and ^{232}U in Rat Kidney and Liver.[a]

	Retention Interval, days[b]				
	0 - 200		200 - 1000		Cumulative Dose
	$T_{1/2}$, days	% ILB	$T_{1/2}$, days	% ILB	(rad) for 750 Days
Kidney					
^{233}U	38	1.5	520	0.2	5 (130; 17)[c]
^{232}U	39	3.9	320	0.7	23 (440; 50)[c]
Liver					
^{233}U	68	0.02	3800	0.01	0.04
^{232}U	NA[d]	NA[d]	215	1.0	2[e]

(a) Values are for the high-level (35.9 and 53 nCi ILB) ^{233}U and ^{232}U exposure groups, respectively.
(b) Retention parameters were determined from single exponentials fit to data collected between 0-200 days and 200-1000 days.
(c) Values in parentheses are the corresponding dose estimates for lung and skeleton, respectively.
(d) Data not yet available.
(e) The radiation dose estimate included only the 200- to 1000-day retention component. The total dose is expected to be ~twofold greater.

Proportionately greater amounts of ^{232}U were deposited in kidney and liver compared to ^{233}U. Retention kinetics in kidney were fairly similar for the two isotopes; however, in liver, differences in amount deposited and kinetics of retention were more marked. Similar differences in the rates of clearance from lung and retention in skeleton were reported in an earlier Annual Report, i.e., ^{232}U appears to be cleared more rapidly from lung and retained more tenaciously in skeleton than ^{233}U. It is probable that these differences in metabolism are related to the ~2000-fold difference in uranium mass presented by ^{233}U and ^{232}U or to the larger aerosol particle size observed with ^{233}U. Precautions were taken to assure that the chemical/physical forms of the ^{233}U-^{232}U uranyl nitrate solutions were identical, but the aerosol generator solutions were more highly concentrated and more viscous with ^{233}U.

Retention in liver and kidney could be described by two exponential functions; one with half-life ranging from 40 to 70 days, the other with half-life >200 days (Figure 1). The cumulative radiation dose (uranium parent dose only) calculated from these parameters and survival over the average life span (~750 days) are also shown in Table 1, along with comparable values for lung and skeleton. It is apparent that lung and skeleton received the greatest radiation dose and, as shown in the Annual Report for 1980, also exhibited a dose-related tumor response. Tumor incidences in lung, skeleton, kidney and liver were, for ^{233}U, 22, 1.6, 1.6 and 3.1%; for ^{232}U, 58, 3.3, 3.3 and 3.3%. These lesions were absent from control animals, except for lung (4.5%) and kidney (2.6%). The results clearly indicate that the lung is the major target organ for inhaled ^{233}U and ^{232}U uranyl nitrate tumorigenicity.

Male Wistar rats exposed to graded doses of ^{231}Pa citrate aerosols (1, 17 or 56 nCi ILB) have been observed for ~950 days after exposure. Retention in the lungs of rats exposed to the high-dose level (56 nCi ILB) is shown in Figure 2. The curve was constructed using data from both sacrificed rats and long-term rats, as described above for uranium retention. The means and standard deviations are shown for the serially sacrificed rats (five/group) at the indicated time intervals. The data could be described by two exponential functions with half-lives of 30 and 513 days, accounting for 92 and 8%, respectively, of the initial amount of ^{231}Pa deposited in the lung. The short-lived lung clearance component usually associated with absorption and mucociliary processes was not readily extrapolated from these data.

Cumulative radiation dose to the lung was calculated assuming clearance according to the equation:

$$Y = 91.9\, e^{-0.023x} + 8.1\, e^{-0.00135x}$$

and summing over 663 days (the average life span of this exposure group). The estimated lung dose was 430 rad, well within the carcinogenic range, and delivered about equally by the intermediate-lived and long-lived components. Approximately one-half the dose was delivered during the initial 75 days after exposure. Retention data for other tissues and for other dose groups are not yet available, and the pathologic examination of tissues is still in progress.

An analytical method specific for ^{231}Pa in the presence of its decay products has been developed and tested (Table 2). The method employs dry ashing of biological samples, solubilization of the ash in HCl, anion-exchange separation to remove iron and selected ^{231}Pa decay products, and combined Aliquat-336 extraction/terphenyl scintillator-counting to further separate ^{231}Pa from residual daughter (^{227}Th) activity. Recovery of a known amount of ^{231}Pa from a variety of biological samples ranged from 84 to 96%, with an average value of 92% for the 40 samples analyzed. The method is being used to analyze ^{231}Pa in samples from rats exposed to inhaled ^{231}Pa citrate aerosols.

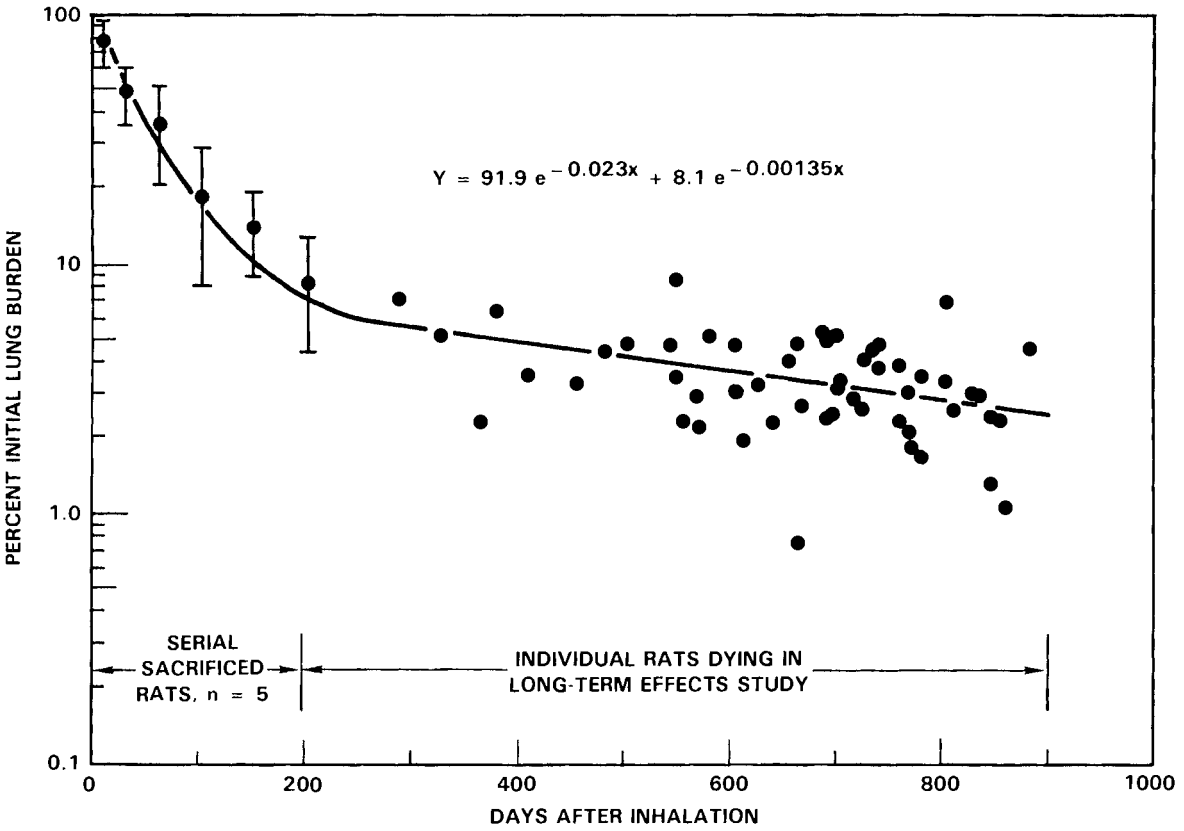

FIGURE 2. Retention of ^{231}Pa in the Lungs of Rats Exposed to ^{231}Pa Citrate Aerosols.

TABLE 2. ^{231}Pa Recovery in Spiked Tissue Samples.

Sample[a]	Recovered Activity, dpm ± SD[b]	% Recovery[c]
Pelt	10,931 ± 570	95.8 ± 5.5
Lung	10,474 ± 695	91.8 ± 6.4
Liver	10,552 ± 871	92.5 ± 7.9
Kidney	10,703 ± 741	93.8 ± 6.8
Skeleton	11,004 ± 314	96.4 ± 3.6
Tissue Residue	9593 ± 341	84.1 ± 3.6
Urine	9832 ± 926	86.2 ± 8.4
Feces	10,967 ± 657	96.1 ± 6.2

[a] Five replicates of each sample were spiked with ^{231}Pa in HCl solution, ashed at ~500 °C and solubilized in HCl ± HF.

[b] Samples were analyzed by scintillation counting after separation of iron on a Dowex-1 anion exchange column. The counting cocktail contained Aliquat-336 extractant and terphenyl scintillator in a toluene solution.

[c] Percent recovery was based on a spike value of 11,409 ± 265 dpm ^{231}Pa determined by gamma spectrometry using an intrinsic germanium detector.

• Modifying Radionuclide Effects

Principal Investigator: L. B. Sasser

Contributor: D. D. Mahlum

Technical Assistance: R. L. Music

This project involves a study of the relationship of physiological and environmental factors to the metabolism and effects of radionuclides. We have studied placental transfer and suckling as pathways of americium entry into the newborn or juvenile rat. Rats were injected intravenously with 5 μCi of ^{241}Am while nulliparous (30 days prior to mating), pregnant (day 19 of gestation), or lactating (1 day after parturition), and subsequent litters were killed to determine ^{241}Am retention. A deficit in reproductive performance was observed in the group injected before mating, as evidenced by reduced number and weight of offspring.

In past studies, we have observed, in female rats injected intravenously with monomeric ^{239}Pu, that the incidence of mammary tumors was higher and the latency period shorter than those in control animals. To determine if these tumors were a direct result of ^{239}Pu deposition in the gland, the relationship between tumor incidence and plutonium deposition was investigated. It soon became clear that the amount of plutonium deposited in the mammary gland and uterus could be influenced by pregnancy and lactation. Data reported previously (Annual Reports, 1979, 1980) demonstrated that the relative amounts of plutonium reaching the offspring via the placenta and by nursing was a function of the temporal relationship between plutonium administration and pregnancy.

We have observed, in other experiments, that americium is deposited in the liver and other soft tissues of the nonpregnant rat and that only a small percentage is retained by bone, whereas plutonium is deposited to a greater extent in bone, where its retention is prolonged. Because of these differences, it seems likely that americium may also behave differently from plutonium under physiological stresses, such as pregnancy and lactation. Therefore, the objective of this study is to determine the effect of pregnancy and lactation on the transfer of ^{241}Am to the offspring of the rat. The experiment was designed so that female rats were dosed while nulliparous (30 days prior to mating), pregnant (day 19 of gestation), or lactating (1 day after parturtion); the subsequent transfer of ^{241}Am to the offspring was then measured as a function of their age.

Three-month-old, pregnant, Sprague-Dawley rats were divided into two groups of 23 each and intravenously dosed with 5 µCi of ^{241}Am on day 19 of gestation (pregnant) or 1 day postpartum (lactating). An additional 30 females were similarly dosed 30 days prior to conception (nulliparous). Females, and their progeny, from the pregnant and lactating groups were killed at 1, 4, 9, 14, or 21 days after injection. The nulliparous group, which were not bred until 30 days after injection, were killed when their progeny were -2, 1, 6, 11, or 18 days of age. Samples of liver, kidney, femur, spleen, mammary gland, and uterus were analyzed for ^{241}Am by gamma counting. Tissues with low concentrations of ^{241}Am (blood, nidation sites, milk, and fetuses) were ashed for analysis by liquid scintillating counting.

Approximately one-half of the ^{241}Am was found in the liver of the pregnant and lactating groups 1 day after injection. Retention by the liver appeared to be slightly greater in the lactating group than in the pregnant group at all times measured, but preliminary analyses (t-test) do not show statistical differences. The half-time of americium in the liver was approximately 22 days in both groups, nearly twice as great as previously observed in nonpregnant rats. Differences between the two groups are not apparent for other tissues measured to date.

An unexpected result of this experiment was a deficit in reproductive performance in the group injected while nulliparous. The weight of the offspring of the nulliparous and pregnant groups, at various ages, is shown in Table 1. Litter weights appeared less in the nulliparous group at all ages, although statistical evaluation (t-test) revealed that differences were significant only at -2, 11, and 18 days of age.

Approximately 40% (12 of 30) of the nulliparous group had small litters ($P < 0.01$), averaging only 2.6 fetuses per litter (Ta-

TABLE 1. The Weight of Offspring of Rats Injected With ^{241}Am When Nulliparous (30 Days Prior to Mating) or Pregnant (19 Days of Gestation).

Age of Offspring, days	Weight of Offspring, g[a]						P
	Nulliparous Group			Pregnant Group			
−2	2.93	0.11	(5)[b]	3.93	0.17	(5)	<0.01
1	6.64	0.21	(5)	6.83	0.18	(3)	NS
6	11.4	0.6	(5)	12.8	0.4	(5)	NS
11	20.3	1.0	(5)	24.0	0.5	(5)	<0.02
18	37.6	0.2	(5)	43.7	1.3	(5)	<0.05

[a] Mean ± SE
[b] The number of litters is shown in parentheses.

TABLE 2. Reproductive Performance of Rats Producing Either Normal or Small Litters After Being Injected With ^{241}Am While Nulliparous.[a]

Size of Litter	Total No. of Litters	No. Fetuses per Litter	No. Nidation Sites	No. Corpora Lutea
Normal	18	>8	10.6 ± 0.4	13.6 ± 0.8
Small[b]	12	2.6 ± 0.6	3.5 ± 0.6	10.8 ± 1.1
P		<0.01	<0.01	NS

[a] Mean ± SE
[b] Litters having five or less pups were arbitrarily classified as small litters.

ble 2). They were therefore arbitrarily divided into two subgroups based on litter size; i.e., normal or a reduced number (five or less per litter). A marked reduction ($P < 0.01$) in the number of nidation sites was also observed, but the number of corpora lutea were only slightly fewer than in the normal subgroup. These results imply that a significant number of preimplantation deaths occurred in a large fraction of the nulliparous group. No evidence of a similar effect was found in the pregnant or lactating groups.

Preliminary estimates indicated that the dose to the ovary, the reproductive organ having the greatest americium concentration, was approximately 1.5 rad per day. Further studies will be required to verify these findings and to accurately determine the dose received by reproductive organs.

• Fetal and Juvenile Radiotoxicity

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Technical Assistance: J. A. Cushing, P. S. Lytz, B. Hogberg, E. K. Lakey, R. L. Music, T. A. Pierce, and S. A. Watson

This project is directed at obtaining detailed comparative information on the deposition, distribution, retention, and toxicity of radionuclides in the prenatal and juvenile mammal. Because quantitative data cannot necessarily be extrapolated to man, our emphasis is directed toward establishing patterns, phenomenologic interactions, and relationships which will be useful in determining appropriate exposure levels for the rapidly growing infant or child and for pregnant women.

Recent results have shown that injection of pregnant rats with ^{239}Pu increases the incidence and severity of adenomatous hyperplasia of the liver in the offspring; the magnitude of these effects is related to dose and prenatal age at exposure. Analysis of combined data from several experiments leads to the conclusion that perinatal rats are more sensitive to bone tumor induction by ^{239}Pu alpha-particle irradiation than are adults. Further histopathologic evaluations of material from earlier experiments have demonstrated that most of the increased incidence of thyroid tumors following ^{131}I exposure is attributable to follicular tumors. An analysis of the literature led to the conclusion that prenatal irradiation can lead to an increased or decreased incidence of tumors, depending on the specific details of the experimental design and system.

The protocol for an experiment to examine the role of stage of gestation in late effects produced by prenatal exposure to ^{239}Pu was described in a previous Annual Report (1978). In this experiment, pregnant rats were intravenously exposed to 0, 0.3, 3, or 30 nCi/g body weight of heavily citrated ^{239}Pu at 9, 15, or 19 days of gestation (dg). The offspring were periodically evaluated and necropsied at death or at 30 mo of age. As described in last year's Annual Report (1981), longevity of the offspring was decreased most markedly in those exposed at 19 dg, less at 15 dg, and least at 9 dg. Histopathological evaluation of tissues has been completed, and data analyses are nearing completion. Bone tumor incidence was increased by the highest dose, 30 nCi/g, and was higher in those exposed at 19 dg than in those exposed earlier in gestation. Since there was a significant interaction between age at exposure and survival, these data are being adjusted to account for differences in survival time. Another particularly interesting finding was an interaction between age at exposure to ^{239}Pu, dose, and the incidences of adenomatous hyperplasia and tumors of the liver; these also require survival data adjustment for full interpretation since the former is a lesion associated with older age and is thought by many to be an early stage of hepatic tumor development. To better examine this relationship, liver sections from rats which survived to or beyond 800 days of age were graded for adenomatous hyperplasia, using a scale of 0 to 5, in which a tumor was assigned the maximum grade (Table 1). There was a clear increase in the percent of animals with liver hyperplasia, and a trend toward increasing severity, with increasing dose. The latter was even more pronounced in the individual age groups; it was most pronounced in the rats exposed at 19 dg.

The bone tumor incidences in the groups exposed at 19 dg were combined with incidences from an earlier study in which rats were exposed to an overlapping dose range at the same stage of gestation. A significant linear relationship between incidence and radiation dose to the femur was found, although incidence in the highest group (60 nCi/g) was affected by maternally mediated effects (Annual Report, 1981). This relationship and estimates of response from the earlier study (Annual Report, 1977), in which newborn, weanling, and adult rats were also exposed, allowed us to analyze bone tumor susceptibility as a function of age. The radiation dose to the femur required to increase bone tumor incidence by 10% above the control level in the prenatally exposed rats was estimated from the regression of tumor incidence on radiation dose. Straight lines were force-fit to the incidences for the lower dose range for the

TABLE 1. Incidence and Median Grade of Adenomatous Hyperplasia in Rats Surviving to or Beyond 800 Days of Age After Intravenous Injection of Their Dams With Citrated ^{239}Pu at 9, 15, or 19 Days of Gestation (dg).

Exposure Dose, nCi/g	No. Survivors	Hyperplastic, %	Median Grade of Hyperplasia			
			All Ages	9 dg	15 dg	19 dg
0	75	8	1	1	--	--
0.3	70	29	1	1	1	1
3.0	74	35	1	1	1	1
30.0	56	46	2	1	2	3

postnatal animals; the corresponding doses to the femur for a 10% increase were estimated and are shown in Table 2.

TABLE 2. Estimation of Average Radiation Doses (rad) to the Skeleton Required to Produce Bone Tumor Incidences 10% Above Control Incidence (Δ 10% Dose) in Rats Exposed to ^{239}Pu as Fetuses, Newborns, Weanlings, and Adults.

	Age at Injection			
	19-dg Fetus	Newborn	Weanling	Adult
Δ 10% Dose to Femur	29	57	157	305
Conversion Factor: Femur Dose to Skeletal Dose	1.5	1.5	0.7	0.9
Δ 10% Dose to Skeleton	43	86	110	275

As described in last year's Annual Report, distribution and dosimetric data from our ongoing studies have been used to explain age-related differences in the anatomic sites at which bone tumors develop. These data are currently being used to estimate the average skeletal radiation doses which result from ^{239}Pu administration at various times of life, and to calculate conversion factors by which skeletal dose estimates can be obtained by extrapolating from previously calculated femur doses. These tentative conversion factors have been used to obtain estimates of the average skeletal doses required to increase bone tumor incidence by an increment of 10% (Table 2). Although the values are linearly related to age at injection, adequate measures of the error of estimation are not available. It is not clear whether the calculated value of 43 rad in the prenatal groups is significantly different from the corresponding 86 and 110 rad in the newborn and weanling groups, but it is likely that all are lower than the calculated value of 275 rad in adults. Since this difference agrees with results from experiments using X-irradiation, we have concluded that the perinatal rat is more sensitive to bone tumor induction by ^{239}Pu alpha-particle irradiation than is the adult.

An ongoing experiment to evaluate the effects of foster-rearing of neonatal rats and the influence of maternal debilitation on ^{239}Pu bone tumor induction was described in the 1981 Annual Report. The growth curves through 9 wk of age were depressed in all groups of offspring nursed by exposed dams, but the curves for prenatally exposed offspring reared by control dams were similar to those of control offspring reared by their own or control foster dams. It was concluded that exposed dams had a reduced ability to rear offspring. Orthogonal polyomial equations have been fit to the weight curves for the period between 9 wk and 1 yr of age, and pairwise t-tests at the 0.003 level of significance were performed for the 15 possible comparisons (Figure 1). The body weights of control offspring fostered to a control dam (C-C) or of exposed offspring fostered to exposed dams (X-X) continue to be the same as for those reared by their own dams (C and X, respectively). Although the curves for the two cross-fostered groups (C-X and X-C) were not significantly different with this conservative analysis, each continued to reflect its postnatal rearing more than its prenatal exposure.

Several years ago we reported the overall results of a study in which graded doses of ^{131}I were administered to prenatal, neonatal, weanling, and adult rats on five successive days, and thyroid tumor incidence was evaluated in skip-serial sections of the thyroid gland (Annual Report, 1972). During subsequent categorization of tumors by histologic type, we found inconsistencies in interpretation and in the classification system used by the various pathologists who had evaluated the microscopic slides. After several unsuccessful attempts to reconcile these inconsistencies, all material was re-evaluated by one path-

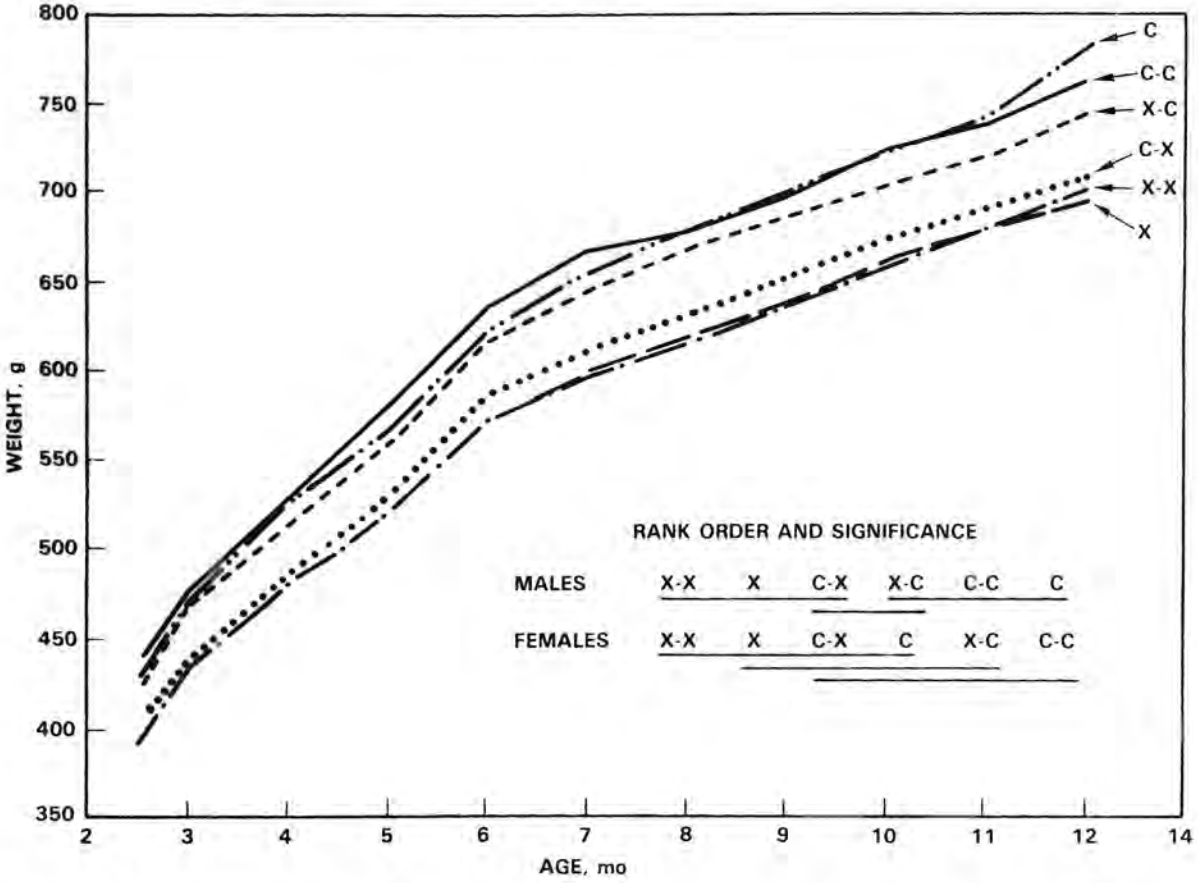

FIGURE 1. Relationships Between Prenatal ^{239}Pu Exposure and Neonatal Fostering on the Weight Curves of Male Offspring. The Ranking of the Curves, in Order of Increasing Weight, is Shown for Male and Female Offspring; Statistical Significance Between Groups is Indicated by Absence of a Common Underline. (The identification of groups is indicated in the text.)

ologist to provide a definitive picture. The previously described general relationship among age, dose, and tumor incidence remains unchanged. Using the classification system of the National Toxicology Program, he categorized thyroid tumors as follicular or C-cell (solid), adenoma or carcinoma. Almost all of the tumors in controls were C-cell, but more than one-half of the tumors in exposed rats were follicular (Figure 2). The fractions of tumors of either type that were malignant were not markedly different. The incidence of follicular tumors increased, then fell with increasing dose, in the adult and weanling groups; it progressively increased with increasing dose in the newborn and prenatal groups. The percentage of rats with C-cell tumors was unaffected at low doses but decreased at the highest dose in all age groups.

Statements in the BEIR III Report gave the impression that the animal fetus is not susceptible to tumor induction by ionizing radiation. Since this impression appeared to be inconsistent with data being obtained in these studies and in other relevant experimental studies, we performed a thorough literature review and analysis, which has been published (Biol. Res. Preg. 2: 167, 1981). We concluded that exposure of prenatal animals to radiation from external sources or internally deposited radionuclides can lead to an increased or a decreased incidence of tumors in adult life. The extent and nature of the effects were related to the conditions under which the experiments were performed, but many of the variations were explainable in terms of phenotypic and ontogenic differences, and non-neoplastic types of biological injury produced by the exposure.

In response to questions arising from NRC Regulatory Guide No. 8.13, a comparable effort was initiated relative to evaluating the contribution to fetal radiation doses from internal deposition of a radionuclide or from a pre-existing maternal burden. A concerted literature review provided an adequate data base on radiopharmaceuticals,

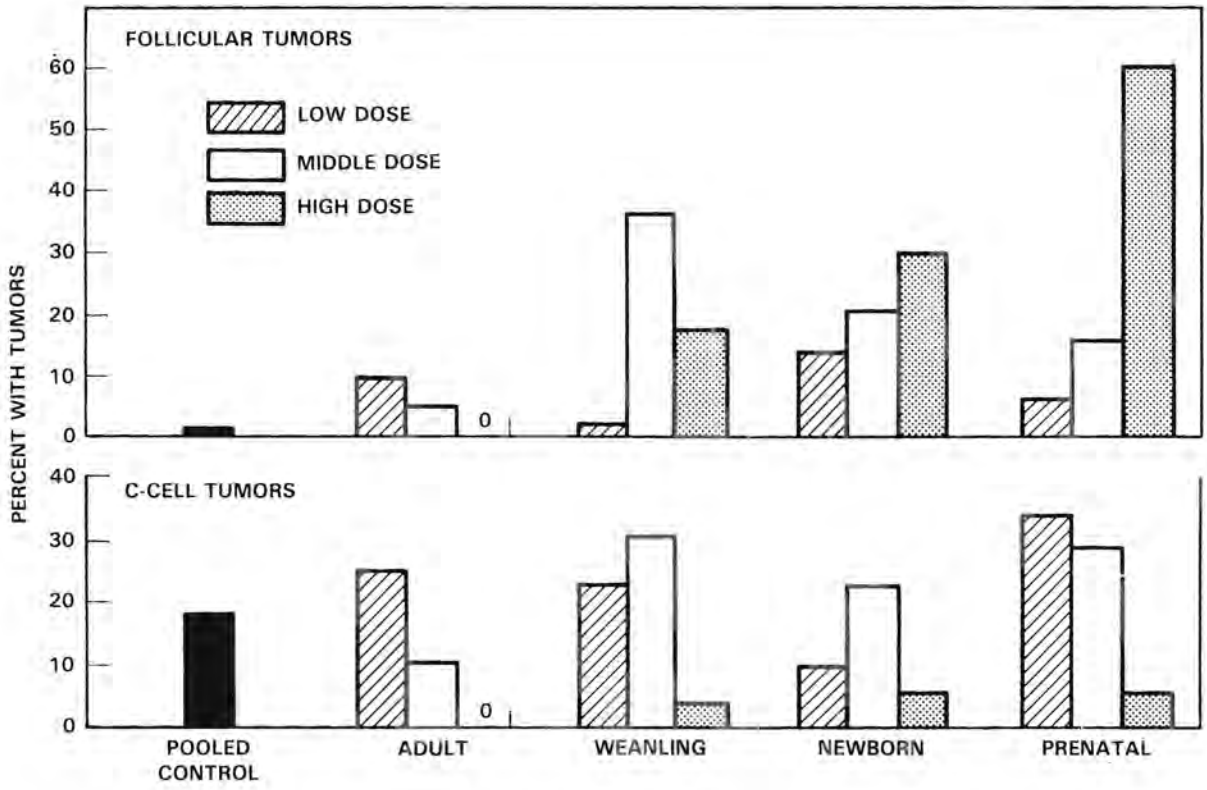

FIGURE 2. Influence of Nominal Exposure Dose and Age at Administration of ^{131}I on the Incidence of Follicular and C-Cell Thyroid Tumors in Rats.

isotopes used as clinical tracers, and elements which are analogs of natural metabolites. Insufficient relevant data are available for the transuranics in the open literature; therefore, an effort is in progress to obtain laboratory reports and other unpublished data and to identify further needs. Although analyses are still in an early stage, a general analytical scheme has been established. First, materials are categorized relative to the degree of "fetal accretion," which involves an interplay between maternal kinetics, placental transfer, and the presence of receptor sites in the conceptus. The quantitative values are influenced by chemical factors, stage of gestation, and associated tissue localization. Dose calculations are straightforward for exposures to materials that are adequately documented and are reasonably well accreted by the conceptus, although the calculations are tedious because of the continuously changing kinetics. For materials for which an adequate data base is not available, we speculated that estimates of maternal blood concentrations, the age-dependent integrals of fetal deposition as a function of maternal blood level, and dilution factors associated with growth could be used to obtain a first-order approximation. This was incorporated into an analytic scheme and was tested using various data bases for the rat. They gave a reasonable approximation to measured values for ^{239}Pu, the transuranic for which most data are available. It is evident that further refinement, based on more definitive values and more relevant data for the human, is required. Although the general scheme would be useful for other nuclides, the present fragmentary data base precludes attempting such calculations.

Studies in the guinea pig to directly measure the clearance of plutonium from dam to fetus were described in last year's Annual Report. Clearance was extremely low and, based on concurrent tritium clearance measurements, we found that blood flow to the placenta decreased soon after exposure at a dose level of 30 μCi/kg body weight. Experiments were initiated to better define this result, and additional studies are in progress using a dose level of 5-10 μCi/kg to establish the dose levels at which the decreased blood-flow effect occurs.

Blood flow appears to decrease at these lower doses, but it will not be possible to conclusively differentiate blood-flow

changes between the two dose levels until the study is complete and the data are fully analyzed. In order to differentiate between blood-flow effects and specific effects on transport of materials across the placenta, experiments are underway which will allow us to develop a means of examining specific transport processes in the placenta. One of the most readily available systems for investigation is the neutral amino acid transport system, which can be examined by measuring the transport of α-aminoisobutyric acid (AIB, a nonmetabolized amino acid). We have now shown that AIB clearance from dam to fetus is the same, both qualitatively and quantitatively, as reported in the literature. In addition, we were able to quantitate a large diffusional component of the clearance (not previously measured), which is present in addition to the active transport mechanism.

• Gut-Related Studies of Radionuclide Toxicity

Principal Investigator: M. F. Sullivan

Other Investigators: B. M. Miller and J. L. Ryan

Technical Assistance: K. D. Fisk, P. S. Ruemmler, and V. D. Tyler

This project is concerned with the behavior of radioactive materials that may be ingested as a consequence of a reactor accident, unavoidable occupational exposure, or after release to the environment and incorporation into the food chain. Current emphasis is on evaluating hazards from ingested actinides as a function of animal age, species, nutrition, and diet, or chemicophysical state of the actinide. We are also concerned with the behavior of actinides that are inhaled and pass through the gastrointestinal (GI) tract after clearance from the lungs.

Recent experiments showed that adult swine absorbed more ^{238}Pu nitrate than had previously been indicated in studies with ^{239}Pu nitrate, three times more than is absorbed by rats. Absorption of ^{238}Pu by rats on a vitamin-D-deficient diet was about 10 times higher than absorption by rats on a balanced diet. Studies on the effect of chemical form on actinide absorption showed that the citrate forms of ^{238}Pu, ^{241}Am and ^{244}Cm were transported in higher quantities than the nitrate forms across the intestine. Citrate had no effect on the ^{237}Np transport, but the mass of isotope administered was found to be important. Absorption by neonates was inversely related to the mass of neptunium gavaged, in constrast to the effect of mass on neptunium absorption by adult rats. Organic binding of ^{238}Pu in liver tissue, *in situ*, resulted in decreased absorption by adult or neonatal rats.

These results demonstrate that animal age, species and nutritional state are important factors in determining GI absorption of actinide compounds. Chemical form and oxidation state also influence transport. These effects vary with animal age and with the actinide in question.

Absorption of Plutonium from the Gastrointestinal (GI) Tract of Adult Swine After Gavage with ^{238}Pu Nitrate

Studies reported from this laboratory about 25 years ago indicated that 0.0022% of gavaged ^{239}Pu nitrate was absorbed from the GI tract of swine. A similar study, a few years later (Annual Report HW-59500 for 1957), indicated that the 30-day retention value was 0.02%, 10 times higher than that reported earlier, with 72% of that quantity retained in bone. Recently, we gavaged four adult male swine with 10 ml of a nitric acid solution containing 500 μCi of ^{238}Pu nitrate. Two of the animals were fasted for 24 hr before gavage; the other two were allowed food ad libitum until they were gavaged. Both groups were fed thereafter. They were maintained in individual metabolism cages for 1 wk, then killed and analyzed for retained ^{238}Pu. Results obtained after measuring the radioactivity in the autoclaved carcasses and in the livers are shown in Table 1.

TABLE 1. Retention of Plutonium by Adult Swine That Were Gavaged With ^{238}Pu Nitrate in Either a Fed or Fasted State and Killed a Week Later.[a]

	Percent of Gavaged ^{238}Pu ± SEM	
Tissue	Fasted	Unfasted
Skeleton	0.012	0.009
Muscle	0.02	0.02
Liver	0.006	0.006
Total Retained[b]	0.038 ± 0.01	0.036 ± 0.004

[a] Two swine per group. See text for details.
[b] Includes all soft tissues except pelt

The data do not indicate a difference in retention due to the feeding regime. The total amount of ^{238}Pu retained (0.037% of the gavaged dose) is higher than either of the values obtained previously, after ^{239}Pu nitrate administration. The distribution of the retained plutonium also differed. In the older study, the skeleton contained 70% of the total ^{239}Pu retained, whereas, in this study, only 30% was in the skeleton, with twice as much present in the skeletal muscle. Urine was collected daily as a measure of excreted ^{238}Pu, but the probability of contamination from fecal material was high. Quantities of ^{238}Pu in urine averaged 0.004 ± 0.002% of the gavaged dose for the first few days, suggesting that contamination had occurred.

The Effect of Dietary Deficiency in Vitamin D or Zinc on Plutonium Absorption from the GI Tract

Previously (Annual Report, 1981), we reported that a deficiency in dietary calcium resulted in an increase in ^{238}Pu absorption from the GI tract. Since vitamin D is involved in calcium absorption and metabolism it seemed likely that it might also influence plutonium absorption. Zinc is known to influence the intestinal transport of many metals because of competition for a common carrier system. Data on the effect of these metabolites could provide information about the mechanism of plutonium absorption and about factors that may influence GI absorption. To test these hypotheses, we performed the experiment described below.

Groups of rats were placed on balanced diets, or diets deficient in either vitamin D or zinc, for 2 wk. They were then gavaged with ^{238}Pu nitrate and continued on the same diets until they were killed 1 wk later. The results (Table 2) indicate that the absence of vitamin D in the diet caused a 10-fold increase in the quantity of ^{238}Pu absorbed and retained. A deficiency in dietary zinc resulted in a threefold increase.

TABLE 2. Gastrointestinal Absorption of Plutonium by Rats Maintained on a Vitamin-D- or Zinc-Deficient Diet for 2 Weeks Before Gavage With ^{238}Pu Nitrate.

	Percent of Gavaged ^{238}Pu ± SEM		
Tissue	Control (8)[a]	Vitamin-D-Deficient (10)	Zinc-Deficient (8)
Carcass	0.007	0.07	0.017
Liver	0.001	0.01	0.002
Urine	0.006	0.007	0.01
Total Absorbed	0.01 ± 0.001	0.1 ± 0.01	0.03 ± 0.004

[a] Number of rats shown in parentheses. See text for details.

The Effect of Organic Binding of Plutonium on Absorption from the GI Tract

Our earlier studies (Annual Reports, 1975 and 1976) indicated that plutonium incorporated in either animal or plant tissue consumed by rats or guinea pigs was absorbed in greater quantities than when gavaged in a nitric acid (pH 2) solution. To learn more about the effect of biological incorporation, 10 μCi of ^{238}Pu citrate (4.5% citrate solution) was mixed with 50 mg of phytic acid, pH 7, and gavaged in a 2-ml volume. To obtain plutonium incorporated in animal tissue, adult rats were injected intravenously with ^{238}Pu citrate and killed 24 hr later; their livers were lyophilized and ground in a mortar; the resulting powder contained 35 μCi of ^{238}Pu/g tissue; 300 mg of this powder, containing 10 μCi of ^{238}Pu, was mixed with 2 ml of 5% Na citrate for gavage. Preparations of nonradioactive powdered liver, mixed with 10 μCi of ^{238}Pu, and a 5% sodium citrate solution containing 10 μCi of ^{238}Pu with no liver powder were administered by gavage to control animals. Results of these experiments (Table 3) indicate that neither the phytate nor the nonradioactive liver tissue influenced ^{238}Pu absorption. Biological incorporation of ^{238}Pu in liver did, however, result in about a twofold reduced retention in both liver and bone.

TABLE 3. Effect of Organic Binding on the Gastrointestinal Absorption of Plutonium-238 Administered to Adult Rats by Gavage.[a]

	Percent of Gavaged ^{238}Pu ± SEM			
Tissue	Phytate	Liver-Incorporated Pu	Liver Control	Citrate Control
Skeleton	0.26	0.13	0.27	0.28
Liver	0.04	0.02	0.05	0.05
Urine	0.01			0.01
Total Retained	0.30 ± 0.05	0.15 ± 0.02	0.32 ± 0.04	0.33 ± 0.08

[a] Six rats per group. See text for details.

The Effect of Garlic Extract on the Absorption of ^{238}Pu from the GI Tract of Rats

Because Kyolic is reported to bind heavy metals and promote excretion, adult rats were intragastrically administered 1.0 ml of a proprietary garlic extract, Kyolic, followed by 1 ml of a nitric acid solution, pH 2, of ^{238}Pu. Measurements of retention and urinary excretion, made a week after gavage (Table 4), show a fourfold increase in retention over the nitrate-gavaged control group.

The Effect of Organic Binding on the Absorption of ^{238}Pu from the GI Tract of Neonatal Rats

Groups of 7-day-old rats were gavaged with either ^{238}Pu citrate, ^{238}Pu citrate and nonradioactive lyophilized liver, or ^{238}Pu that was biologically incorporated in the

TABLE 4. The Effect of Kyolic™ on the Absorption of Plutonium From the Gastrointestinal Tract of Rats Gavaged With ^{238}Pu Nitrate.

	Percent of Gavaged ^{238}Pu ± SEM	
Tissue	Control (4)[b]	Kyolic[a] (5)[b]
Carcass	0.004	0.016
Liver	0.0005	0.003
Total Retained	0.005 ± 0.0002	0.02 ± 0.005
Urine	0.004	0.004
Total Absorbed	0.009 ± 0.01	0.025 ± 0.005

[a] Garlic Extract
[b] Number of rats shown in parentheses.

livers (preparation described above). The results of analyses made a week after gavage (Table 5) show that biological incorporation resulted in slightly decreased retention. The 7-day-old control group given ^{238}Pu citrate retained 10 times as much ^{238}Pu as the adult control group given ^{238}Pu citrate (Table 3). The neonatal group given biologically incorporated ^{238}Pu retained 13 times more than the comparable adult group (Table 3).

TABLE 5. Gastrointestinal Absorption of Organically Bound Plutonium-238 Administered by Gavage to 7-Day-Old Rats That were Killed a Week Later.[a]

	Percent of Gavaged ^{238}Pu ± SEM		
Tissue	Citrate Control	Citrate + Liver Control	Liver-Incorporated ^{238}Pu
Carcass	2.9	2.1	1.8
Liver	0.3	0.2	0.2
Total Retained	3.3 ± 0.3	2.4 ± 0.2	2.0 ± 0.1

[a] Six rats per group. See text for details.

The Effect of Citrate on the Absorption of the Trivalent Actinides Americium and Curium from the GI Tract of Adult or Neonatal Rats

It is well known that the citrate forms of some actinide elements (e.g., plutonium) are absorbed from the GI tract in greater quantities than the nitrate forms (compare control groups in Tables 2-4), presumably because of the tendency of nitrate forms to polymerize. To determine the influence of citrate on ^{241}Am and ^{244}Cm absorption, groups of rats were gavaged with either a nitric acid solution (pH 2) or solutions of the nitrate that were brought to incipient dryness, then diluted with 5% sodium citrate to a concentration of 10 μCi/ml, pH 6. Data obtained from animals gavaged with nitrate or citrate solutions of ^{241}Am and ^{244}Cm (Table 6) indicate that, compared with controls, retention and urinary excretion increased to the same extent for both trivalent actinides. Absorption by adult rats that received both radionuclides in citrate form increased about fivefold over that of controls.

TABLE 6. Influence of Solubility of the Absorption of ^{241}Am and ^{244}Cm From the Gastrointestinal Tract of Rats.[a]

	Percent of Gavaged Dose ± SEM			
	^{241}Am		^{244}Cm	
Tissue	Nitrate	Citrate	Nitrate	Citrate
Carcass	0.004	0.037	0.006	0.03
Liver	0.001	0.02	0.002	0.02
Total Retention[b]	0.006	0.05	0.008	0.05
Urine	0.006	0.01	0.008	0.01
Total Absorption	0.012 ± 0.001	0.06 ± 0.02	0.016 ± 0.002	0.06 ± 0.01

[a] Six rats per group. See text for details.
[b] Includes muscle and lung

In neonatal rats gavaged at 2 days after birth (Table 7), there was no difference between GI absorption of ^{241}Am and ^{244}Cm citrate and that of ^{241}Am and ^{244}Cm nitrate; retention of both trivalent actinides increased over that observed in adults. For ^{241}Am, the increase was about 1000-fold; for ^{244}Cm, 400-fold. For the citrate, comparable increases were 100-fold for ^{241}Am and about half that for ^{244}Cm. These results indicate that animal age and solubility are important factors for predicting absorption from the GI tract.

TABLE 7. Retention of Americium and Curium by Neonatal Rats Gavaged With Nitrate or Citrate Solutions at 2 Days After Birth and Killed a Week Later.[a]

	Percent of Gavaged Dose ± SEM			
	^{241}Am		^{244}Cm	
Tissue	Nitrate (9)[a]	Citrate (8)	Nitrate (6)	Citrate (8)
Carcass	5.1	5.2	2.7	2.5
Liver	0.6	0.6	0.2	0.2
Total Retention	5.8 ± 0.6	5.9 ± 0.5	3.0 ± 0.3	2.8 ± 0.1
GI Tract	3.3	3.3	2.7	3.2

[a] Number of rats shown in parentheses. See text for details.

Effect of Oxidation State and Solubility on the Absorption of Neptunium

Adult rats were gavaged with either nitrate or citrate solutions of ^{237}Np(V) or ^{237}Np(VI). Results (Table 8) indicate that citrate had no appreciable effect on absorption of either the pentavalent or hexavalent oxidation states of neptunium.

TABLE 8. The Effect of Oxidation State and Solubility on the Retention of ^{237}Np in Adult Rats Gavaged With 13.7 mg/kg and Killed a Week Later.[a]

	Percent of Gavaged Dose ± SEM			
	^{237}Np (V)		^{237}Np (VI)	
Tissue	Nitrate	Citrate	Nitrate	Citrate
Carcass	0.19	0.18	0.18	0.16
Liver	0.01	0.01	0.01	0.01
Total Retained	0.2 ± 0.04	0.2 ± 0.004	0.2 ± 0.06	0.18 ± 0.04

[a] Six rats per group. See text for details.

The Effect of Mass on the Absorption of Neptunium from the GI Tracts of Adult or Neonatal Rats

Our earlier results (Annual Report, 1981) indicated that the long-lived isotope ^{237}Np ($T_{\frac{1}{2}}$ = 2.1 x 10^6 yr) is absorbed by rats in higher quantities (>1.0% of the gavaged dose) than the higher-specific-activity isotopes ^{235}Np ($T_{\frac{1}{2}}$ = 396 days) and ^{239}Np ($T_{\frac{1}{2}}$ = 2.3 days). The latter were absorbed in quantities between 25 and 50 times less (0.05%) when a similar amount of radioactivity, but not mass, was administered. To determine if this "mass effect" on GI absorption occurred with ^{237}Np, groups of rats were gavaged with various doses and killed a week later for analysis. The results (Table 9) demonstrate that at 24 mg/kg, absorption was 17 times higher than when the dose was one-fifth as high. It appears from the data that the major effect of dose occurs between 10 and 24 mg/kg. Our earlier results (Annual Report, 1981) showed that, at more than 43 mg/kg, absorption was 60% higher than at the highest dose (24 mg/kg) used in the present study. The effect of neptunium mass on absorption may be due to: 1) an effect of the GI tract contents on the absorption characteristics of neptunium (oxidation state) or 2) an increased permeability of the intestinal mucosa resulting from damaging effects of the high neptunium dose.

To determine if mass of neptunium administered had a similar effect on newborn or juvenile rats, litters of 1-, 2- and 9-day-old rats were gavaged with either ^{237}Np, ^{235}Np or ^{239}Np. The percentage retained (Table 10) in the 1- and 2-day-old rats was inversely related to the mass: more ^{235}Np was retained than ^{239}Np, probably because

TABLE 9. Effect of Mass on Absorption of ^{237}Np From the Gastrointestinal Tract of Adult Rats Administered the Nitrate (pH 1.5) by Gavage.

Dose, μCi/kg:	17	7.1	3.6
Dose, mg/kg:	24	10	5
Number of Rats:	6	6	6
Tissue	Percent of Gavaged Dose ± SEM		
Skeleton[a]	0.87 ± 0.16	0.09 ± 0.01	0.04 ± 0.01
Liver	0.12 ± 0.02	0.01 ± 0.004	0.005 ± 0.001
Urine	0.7 ± 0.06	0.09 ± 0.02	0.05 ± 0.007
Total Retention (minus skin and urine)	1.0 ± 0.2	0.1 ± 0.02	0.05 ± 0.006
Total Absorption (minus skin)	1.7 ± 0.2	0.2 ± 0.03	0.1 ± 0.01

[a] Calculated according to the formula, femur x 24 = skeletal ^{237}Np. The factor of 24 is based on prior total skeleton measurements, divided by the radioactivity of the femur.

TABLE 10. Effect of Mass on Retention by Neonatal Rats Gavaged With ^{237}Np or ^{237}Np Nitrate (pH 2).

Isotope:	^{237}Np	^{235}Np	^{239}Np	
Dose, μCi/kg:	118	50	185	100
Dose, μg/kg:	1.68 x 10^5	3.5 x 10^{-2}	8 x 10^{-4}	4.3 x 10^{-4}
Number of Animals:	6	9	9	11
Age at Gavage, days:	1	1	2	9
Age at Necropsy, days:	8	8	6	13
Tissue	Percent of Gavaged Dose ± SEM			
Carcass	0.43 ± 0.07	3.4 ± 0.2	1.24 ± 0.01	0.87 ± 0.09
Liver	0.03 ± 0.003	0.09 ± 0.006	0.03 ± 0.003	0.026 ± 0.002
Total GI Tract	6.1	56	73 ± 8	59.6 ± 1.3
Total (minus skin and GI tract)[a]	0.43	3.5	1.27	0.90

[a] Total retention includes lung, liver and carcass.

of the 1-day age difference. The 9-day-old rats retained less ^{239}Np than the 2-day-old animals, in contrast to our results for ^{237}Np (Annual Report, 1975). Much more radioactivity was measured in the GI tract after ^{235}Np and ^{239}Np administration than after ^{237}Np. This finding is consistent with the effect of mass found for plutonium retention (Annual Report, 1976) in the neonatal rat intestine.

The Influence of Mass on GI Absorption of ^{233}U

Absorption of uranium by humans in industrial contamination accidents has been reported to be as high as 10%. However, our data with ^{233}U- and ^{232}U-gavaged rats indicated a much lower value of ~0.01% (Annual Report, 1974). We hypothesized that the difference between the human and animal absorption factors might be due to an effect of dose; and that, like neptunium, uranium GI absorption is directly related to dose. To test this hypothesis, we gavaged rats with various doses of uranium. Results (Table 11) over a fivefold range of doses are in reasonable agreement with our previous results, and show no marked mass effect.

TABLE 11. The Effect of Mass on Absorption From the Gastrointestinal Tract of Rats Gavaged With ^{233}U Nitrate.

Dose, μCi/kg:	48	120	240
Dose, mg/kg:	5.1	12.6	25.3
Tissue	Percent of Gavaged Dose ± SEM		
Skeleton	0.01	0.01	0.02
Liver	0.0003	0.0003	0.0001
Kidneys	0.002	0.009	0.005
Total Retained	0.013 ± 0.003	0.013 ± 0.003	0.02 ± 0.004

[a] Five rats per group. See text for details.

• Development of Blood Irradiators

Principal Investigator: F. P. Hungate

Other Investigators: T. Marchioro*, R. Bunnell, W. Riemath, and R. Weller

The fully portable, vitreous-carbon/thulium-170 (VCTm) irradiators were previously developed and tested in goats, sheep and dogs for effects on circulating lymphocytes and on skin graft rejection. This past year the testing was extended to include studies of effects on kidney transplants in dogs. Six pairs of beagle dogs were tested. One of each pair was treated with an activated VCTm (i.e., containing ^{170}Tm); the other was treated comparably, but had an inactive unit (containing ^{169}Tm). Kidney donors were selected for maximum disparity in cellular immune (DLA) type between donor and host. The host's own kidneys were removed so that survival depended on the functioning of the transplanted kidney. The untreated dogs survived 9 to 23 days (mean = 15) after transplant; treated dogs survived 16 to 45 days (mean = 27 days). Histological examination showed that there was a distinct depletion of cells in all lymphoid tissues and a reduced cellular involvement in kidney tissues of treated animals.

The vitreous-carbon/thulium-170 (VCTm), fully portable blood irradiators developed in previous years were tested in beagle dogs from the PNL colony for their effects on kidney allotransplants. Prior to testing, all animals to be used were DLA-typed** and, on this basis, were grouped so that the donor's type differed as much as possible from that of the paired recipient.

After obtaining at least one pretreatment blood sample for hematology, the treatment regimen was initiated by placing an A-V shunt (carotid to jugular) in host animals and inserting an active irradiator in one and an inactive unit in the shunt of the other animal. A thermoplastic vest held the irradiator to protect it and the shunt tubing from damage. Animals were individually caged. Blood flow was monitored at least twice a day. If flow stopped, the shunt was aspirated to remove clots, flow was re-established, and the irradiator was cleaned, sterilized and replaced in the shunt. This was repeated as needed until clots could not be removed and flow could no longer be re-established.

The first animal tested (1841) received only 7 days of pretransplant irradiation and lived only 5 days longer than the control. Subsequently, animals received 2 wk or more of VCTm treatment prior to transplant (Figure 1). Numbers of all white cells were diminished; the effect on lymphocytes was most pronounced.

Figure 2 shows a typical response of the lymphocytes and red cells to irradiation. The drop in red cell count at 29 to 39 days was primarily due to direct blood loss during repeated attempts to remove thrombi from the shunt. The prolonged radiation damage to lymphocyctes is also reflected in all lymphoid tissues, which show severe depletion in germinal centers.

At death (or sacrifice; when death was imminent, BUN exceeded 200, urine output fell sharply and emesis occurred), the kidney and typical lymphoid tissues were removed and preserved for histologic examination. Kidneys from untreated animals were uniformly enlarged and had an appearance typical of rejection. Several, but not all, of the kidneys from treated animals were normal in size and gross appearance. Kidney sections of treated animals showed significantly less cellular invasion than those of untreated animals (Figure 3). No effects on liver or other nonlymphoid tissues were noted.

The VCTm treatment nearly doubled the mean post-transplant survival time of treated animals (24 days) over that in untreated animals (15 days) (Figure 1). It was more difficult to maintain flow through the shunts of treated than of untreated animals, but there was no evidence of deleterious effects in irradiated animals. We experienced no greater problem controlling infection in the treated animals. Results indicate that chronic irradiation of blood permits early resumption of function by transplanted kidneys following irradiation.

*Department of Surgery, University of Washington, Seattle, WA

**DLA typing was provided by the Fred Hutchinson Cancer Center, Seattle, WA.

Irradiation of blood suppresses early rejection without side effects deleterious to wound healing and kidney function. Thus, it complements chemotherapy required for chronic suppression of rejection.

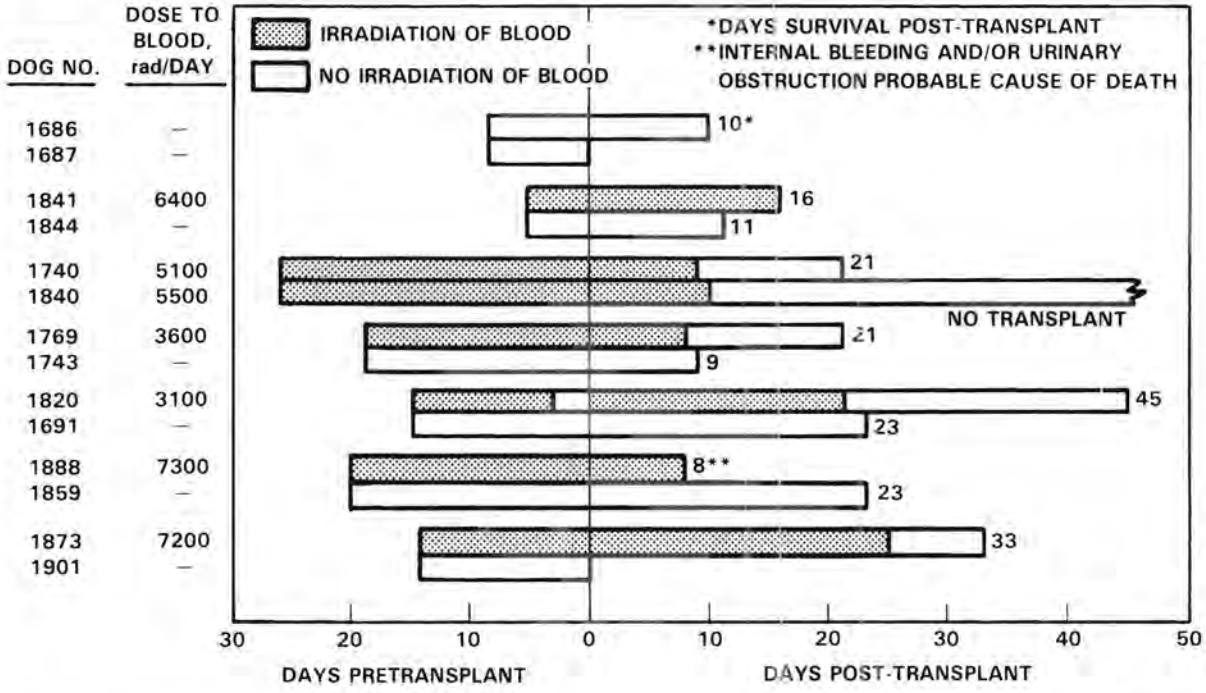

FIGURE 1. Summary of Exposure and Survival History of Dogs With Kidney Allotransplants That Were Treated With Portable Blood Irradiators.

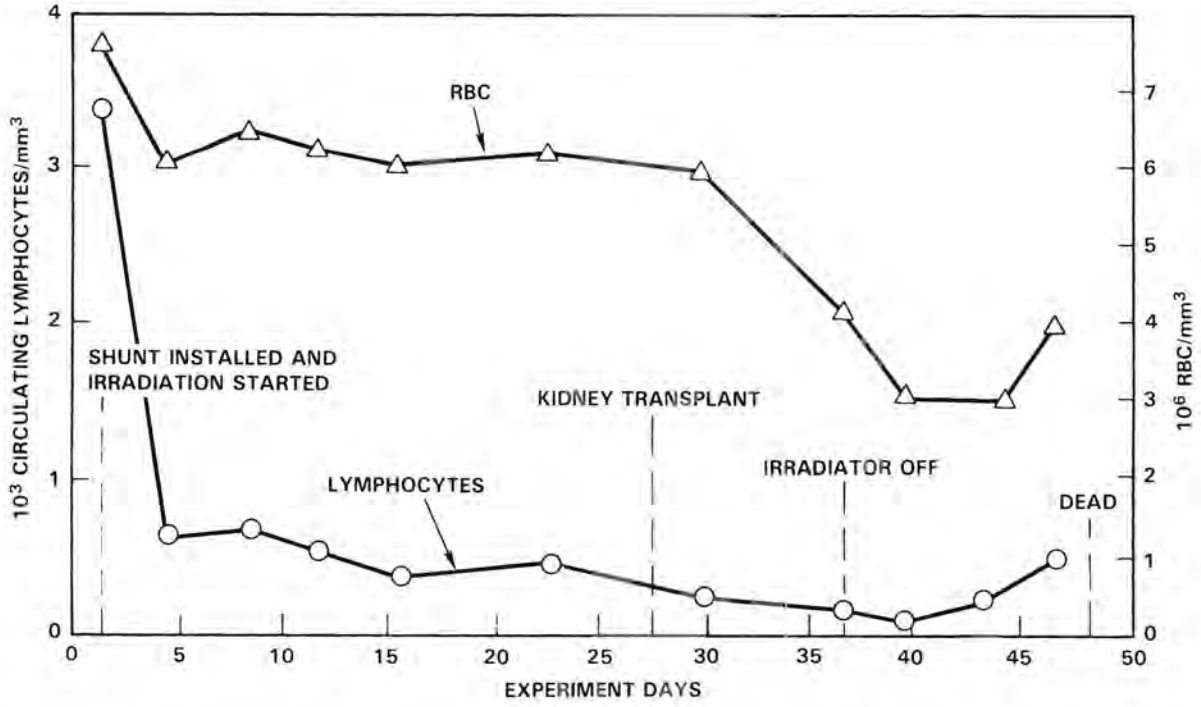

FIGURE 2. Typical Lymphocyte and Red Cell Response in Dogs With Chronic Irradiation of Blood.

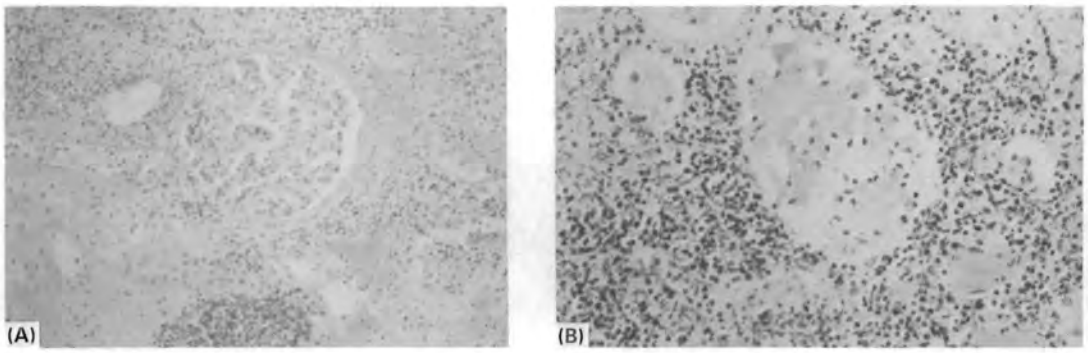

FIGURE 3. Photomicrographs of Transplanted Kidneys From Dogs With (A) Chronically Irradiated Blood and (B) Control.

• Statistical Health-Effects Study

Principal Investigator: E. S. Gilbert and L. E. Sever

Other Investigators: J. A. Buchanan, S. Marks, and H. D. Tolley

A principal objective of this program is to determine if there are demonstrable effects of radiation exposure to the Hanford worker by analyzing mortality records of this population. A secondary purpose is to improve methodology for assessing health effects of chronic low-level exposure to harmful agents or substances, particularly in an occupational setting. In the past year we have updated our analyses and initiated new areas of analysis. Complete documentation was provided for our computer program for the mortality study, and a user's manual is under development. A case-control study of birth defects was started in FY 1982.

Statistical Health-Effects Study

The primary objective of this program is to analyze the mortality of Hanford workers and, particularly, to assess whether there are demonstrable effects of radiation exposure in this population. An analysis of the newly updated mortality file has not been completed as yet. Results of that analysis, when complete, will be published in the journal Radiation Research, where our principal report of the study and an update were published previously.

In addition to the update of the mortality analysis, the following analyses of the data were undertaken:

1. Examination of the effects of confounding factors on the analysis of the relationship of radiation to mortality

2. Investigation of the impact that a suspected change in the Social Security Administration's (SSA) reporting system might have on the Hanford experience

3. Examination of possible lung cancer risk for workers exposed to over 2 rem of cumulative dose.

Task 1 was motivated by an interest in determining if employment cohort and year of death were possible confounding factors in determining an effect due to radiation. The results for this year examined mortality due to all cancers collectively as the outcome variable. In this analysis, which will appear in the Proceedings of the SIAM Institute for Mathematics and Society Conference, the conclusions were that these factors did have a confounding influence on measured radiation effects.

Task 2 was undertaken because of an apparent decrease in reported noncancer deaths by SSA. There is no reason to believe that the SSA system of reporting mortality is biased in favor of death by cancer. Upon closer examination, we discovered a decrease in the deaths reported by SSA from what we expected, actuarially, for the latter part of 1977 and 1978. This reduction was offset for reported cancer deaths by an increase in cancer mortality rates in our cohort. (This increase is similar to that observed in the U.S. population between 1966 and 1978.) The cause and potential impact of the apparent temporary decrease in SSA ascertainment of death are not yet apparent. When the resources of the National Death Index are made available to Hanford Environmental Health Foundation, that source will be used for death ascertainment for deaths dating from 1979.

Task 3, that of investigating lung cancer risks, is still under way. Since smoking history is generally unavailable, the cohorts have been classified into four broad occupational categories. The results of this comparison will be used in support of a case-control study of lung cancer in which smoking history and other confounding risk factors will be investigated.

In response to a need in the Department of Energy research community for a comprehensive data analysis tool to use in large occupational cohort studies, a generalized version of the primary computer program used in this project has been developed. The program, MOX (Mortality and Occupational Exposure Analysis), uses a dynamic, internal-population-based methodology to analyze the relationships between chronic environmental exposure and mortality, tabulated by specific causes of death. (Environmental exposure refers here to exposure to radiation, chemical substances or other quantifiable, potentially hazardous agents.) Documentation of the program has been developed, and a detailed user's manual should be completed early in 1983. The program and manual will be made available to other research investigators.

During FY 1982, a case-control study of birth defects was started. Cases of birth defects in babies born in the three hospitals in the Tri-Cities area are being identified through hospital records, birth and death certificates. We plan to develop information on the prevalence at birth of selected malformations for the period 1956-1980. In addition, two controls are being selected for each case. We will compare cases and controls on the basis of history of employment at Hanford, occupational radiation exposure, and exposure to other hazardous substances. Collection of case and control data is currently under way.

In addition to the above, the paper titled, "Some Confounding Factors in the Study of Mortality and Occupational Exposure," by E. S. Gilbert, was published in the July 1982 issue of the American Journal of Epidemiology. A second paper by E. S. Gilbert, "An Evaluation of Several Methods for Assessing the Effects of Occupational Exposure to Radiation," is "in press" in the journal Biometrics.

• Radioisotope Customer List

Principal Investigator: J. S. Burlison

The purpose of this program is to prepare and distribute the annual document entitled List of DOE Radioisotope Customers with Summary of Radioisotope Shipments. This document lists the FY-1981 commercial radioisotope production and distribution activities of DOE facilities at Argonne National Laboratory, Pacific Northwest Laboratory, Brookhaven National Laboratory, Hanford Engineering Development Laboratory, Idaho Operations Office, Los Alamos Scientific Laboratory, Oak Ridge National Laboratory, Savannah River Plant, and UNC Nuclear Industries. The report (PNL-4177) was published in September 1982.

Fusion

FUSION

Environmental, health and safety problems associated with the fusion technology cannot be precisely identified because of the early stage of development of this technology. Of probable major concern are problems associated with high-strength magnetic fields, with tritium and tritium-breeding materials, with heat transfer materials, with energetic neutrons and the radioactive activation products resulting from interactions with these neutrons. Some of these problems are common to fission as well as fusion technologies and we have considered, in the Fission section, our studies involving the toxicology of sodium and lithium, which may serve as heat transfer and breeding materials in a fusion reactor. In this section, we consider only studies relating to magnetic-field effects.

• Biological Effects of Magnetic Fields

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The objective of this project is to evaluate the effects of magnetic fields on a variety of biological systems. In the past year, these systems have included dominant lethal studies in mice, prenatal development in rats, fertilization and embryonic development in trout, synthetic membranes, and long-term exposure of mice. Although reports continue to appear in the literature describing biological effects of magnetic fields, the current data base is extremely weak. A major weakness in much of the work reported in the literature is the lack of appropriate controls; this is especially true of whole-animal studies. We have therefore constructed a facility which permits exposure and sham exposure of animals under nearly identical conditions. In addition to studies on the effects of magnetic fields on whole-animal systems, another phase of this project has been the investigation of possible mechanisms by which magnetic fields couple to biological systems.

Facilities

As previously reported, a 1200-ft^2 metal building has been renovated to house two identical beam-bending magnets (Type 18D36), which were obtained on loan from the Stanford Linear Accelerator Center (SLAC). These magnets, which we previously used at SLAC, have poles that measure 45.7 x 91.4 cm, with a gap of 17.8 cm. This provides a relatively large cavity with a uniform vertical field. In addition, the space on either side of the poles can be used for exposure to gradient fields.

An environmentally controlled unit, described in a previous Annual Report (1980), is placed in each magnet to house the animals. The environmental units are matched in size, lighting, temperature and humidity. The mode of operation is such that one magnet is energized (to provide the exposure field) while the other is not. This permits control animals to be maintained under conditions which are as similar as possible to those encountered by the exposed groups. Either magnet can be energized, thereby allowing the locations of control and exposed groups to be exchanged between magnets, minimizing potential differences between units.

Trout Egg Fertilization

Studies conducted in FY 1981 and reported in last year's Annual Report have shown that exposure of rainbow trout ova and sperm to a magnetic field strength of 1.0 Tesla (T) for 1 hr prior to fertilization resulted in enchanced fertilization when compared to sham-exposed and combined with nonexposed gametes. An effect was observed on both egg and sperm. The mechanism involved is unknown at this time.

During the past year, we have concentrated our efforts on using this sensitive system to evaluate the effects of exposure duration and field strength on the fertilization process. Ova and sperm from rainbow trout were exposed in a beam-bending magnet (Type 18D36). Four field strengths (0.0, 0.1, 0.5, or 1.0 T) at four durations (5, 15, 30, or 60 min) were used to determine if there was a dose-time response. Because of the large number of ova needed (over 2000/group), and time constraints, a randomized, incomplete, block experimental design was used. In this design, eggs and sperm were extruded from mature rainbow trout. Each group of gametes was divided into four equal aliquots, then sequentially exposed to 4 of the 16 possible treatment that comprise a complete block. After exposure, the eggs and sperm were mixed, incubated for 7 days, preserved, and scored in the blind as either fertile or infertile. Four of these exposure series complete one block of data. Three complete blocks are needed for statistical evaluation.

Data presented in Table 1 show the results of the first completed block. Although no statistical evaluation can be made until the last two blocks have been completed, there is a possible trend toward higher fertilization in groups exposed to the higher field strengths, increasing with length of exposure.

TABLE 1. Effect of Field Strength and Exposure Duration on the Fertilization Success (%) of Rainbow Trout Gamates.

Duration of Exposure, min	Fertility, % Strength of Field (Tesla) 0	0.1 T	0.5 T	1.0 T	Mean of Duration
5	87.90	85.21	90.91	80.02	88.01
15	86.51	89.53	89.16	90.64	88.96
30	84.62	91.67	88.80	91.44	89.13
60	93.02	87.80	90.30	93.36	91.12
Mean Exposure Strength	88.01	88.55	89.79	90.87	

Prenatal Development

We previously exposed pregnant mice to magnetic fields to study the effects on development; no adverse effects were found. Since different species of mammals may react differently to an agent, we have extended our studies to include rats.

Charles River CD female rats were caged nightly with males of the same strain. The presence of sperm in vaginal lavages on the following morning was taken as evidence that copulation had occurred. The morning that sperm were found was designated as 0 day of gestation (dg). Pregnant rats were randomized and assigned to one of three groups. Group I was exposed to a 1-T, static, homogenous field and Group II was exposed to a 2-T/m, static gradient field. Group III was placed in a nonenergized magnet and served as the control. From 0 to 20 dg, all groups were maintained under conditions previously described (Annual Report, 1981).

The rats were killed with CO_2 at 20 dg, and necropsied. Liver, lungs, and kidneys from all animals were weighed; ovaries, uterus, liver, lungs, and kidneys from about one-fourth of the pregnant animals, selected at random from each experimental group, were examined microscopically. The uterus, with ovaries, was removed from each animal immediately at sacrifice. The total number of implantation sites and corpora lutea, living and dead fetuses, and resorption sites were counted. The fetuses and placentas were removed, blotted, and weighed; crown-rump lengths were measured. Each fetus was examined under an illuminated magnifier for gross external abnormalities and assigned, randomly, into one of two groups for more detailed teratologic examination.

In one group, the heads were removed and fixed in Bouin's fluid for subsequent examination of thin, serial, razor-blade-cut sections. Fetuses were then dissected and examined for internal abnormalities. Slightly more than one-half of the fetuses were examined in this manner since we established a requirement that a minimum of two fetuses/horn and four fetuses/litter would be examined, except when fewer fetuses were present. The fetuses of the second group were eviscerated and, together with the decapitated carcasses, were fixed in alcohol, stained with Alizarin red S, and examined for skeletal defects under low-power magnification.

Maternal weight gain during pregnancy, and fetal and placental weights, are shown in Table 2. No significant effects of magnetic field exposure were found, although there is a suggestion of enhanced weight gain in the dams exposed to the homogenous field.

TABLE 2. Effect of Magnetic Field Exposure on Maternal Weight Gain and on Fetal and Placental Weights in Rats.

Group	Maternal Weight Gain, g	Fetal Weights, g	Placental Weights, g
Sham-Exposed	80.1 ± 17.4	3.14 ± 0.36	0.44 ± 0.07
Homogenous	94.5 ± 21.1	3.30 ± 0.32	0.45 ± 0.06
Gradient	80.8 ± 14.9	3.25 ± 0.38	0.45 ± 0.08

Table 3 shows the effect of magnetic field exposure on the average number of fetuses/litter and on the incidence of fetal deaths. Litter size was unaffected by treatment; however, there was an increased incidence of fetal deaths in the group exposed to the gradient field. Teratologic evaluation did not show any effect of magnetic field exposure on the incidence of malformations.

Dominant Lethal Effects

In an earlier experiment, we obtained evidence that the dominant lethal effects of a 300-mg/kg dose of ethylmethane sulfonate (EMS) in male mice were mitigated by exposure of the mice to a 1-T, static, homogenous magnetic field. Since the number of male mice studied in that experiment was small, we repeated the experiment using larger numbers.

Charles River CD-1 male mice were injected i.p. with 0, 200, or 300 mg/kg of EMS.

TABLE 3. Fetal Mortality in Rats as Affected by Magnetic Field Exposure.

Group	No. Corpora Lutea	Total No. Fetuses	Average No. Fetuses/Litter	No. Fetal Deaths	Affected Litters/ Total Litters
Sham-Exposed	15.8 ± 2.3	292	13.3 ± 2.6	17	12/22
Homogenous	14.6 ± 2.4	228	13.4 ± 2.0	23	11/17
Gradient	15.1 ± 4.4	96	12.0 ± 3.2	13	5/8

Three subgroups were formed from each treatment, and were exposed for 24 hr to: a) no magnetic field, b) a 1-T, static, homogenous magnetic field, or c) a 2-T/m, static gradient field. Three days later, each male was caged with three unexposed female mice. After 7 days, the females were removed and replaced with three new females. This procedure was repeated until the males had experienced four successive weeks of breeding. Ten days after separation from the males, the females were killed, and the uteri were removed. The number of implants, viable fetuses, and resorptions was determined. The incidence of early resorptions was used as a measure of dominant lethality.

Figure 1 shows the total number of viable implants for each treatment group throughout the 4 wk of breeding. The smallest number of implants during the first week of breeding was in the groups of males that were exposed to 300 mg/kg of EMS. This persisted throughout the 4 wk of breeding for those not exposed to magnetic fields. The effect was less pronounced when the males were exposed to either the gradient or homogenous magnetic field. Much of the apparent effect of the magnetic fields appears to be due to a higher pregnancy rate among females bred to males from the exposed group (Figure 2).

Figure 3 shows the incidence of early resorptions for the various experimental groups for breeding wk 1-4. There appeared to be no consistent effect of exposure to magnetic fields alone, although during the first week of breeding, the males exposed to the homogenous field produced a significantly higher incidence of early resorptions than did nonexposed males. This effect was not seen during subsequent breedings.

The incidence of early resorptions was, as expected, highest during the first 2 wk of breeding for animals exposed to 300 mg/kg of EMS. Exposure of mice that received 300 mg/kg EMS to the homogenous field significantly decreased the incidence of early resorptions during breeding wk 1 and 2; this effect was not seen in the 200-mg/kg EMS group.

Molecular Studies

A system was constructed to test the physical stability of phospholipid bilayer membranes to sudden changes in magnetic-flux density. Using a published procedure developed at this laboratory, planar bilayer membranes of sphingomyelin were prepared across a hole, 2 mm^2 in area, in a polyethylene support separating two compartments of the same buffered aqueous solution. This support had been placed between the pole faces of a large electromagnet. The magnetic-flux density at the membrane was varied suddenly from 0 to 1 T while the integrity of the membrane was monitored visually with the aid of a low-power microscope. Although the membrane in the magnetic field occasionally ruptured, no correlation was observed between this phenomenon and the sudden imposition of the high-flux density.

Long-Term Exposure to Magnetic Fields

Long-term exposures of female CD-1 mice to homogenous 1-T and 2-T/m fields were initiated. Twenty-five mice were placed in the gradient field, 50 mice were placed in the homogenous field, and 75 mice were placed in the control magnet. No remarkable, field-related changes were observed in the first 5 mo of the study in mortality, morbidity, and body weights. In addition to these variables, we plan to obtain terminal clinical chemistry and hematological measurements as well as organ weights and full pathological examination of any lesions. Exposure of these animals is scheduled to last 18 mo, or until funding is withdrawn from the project.

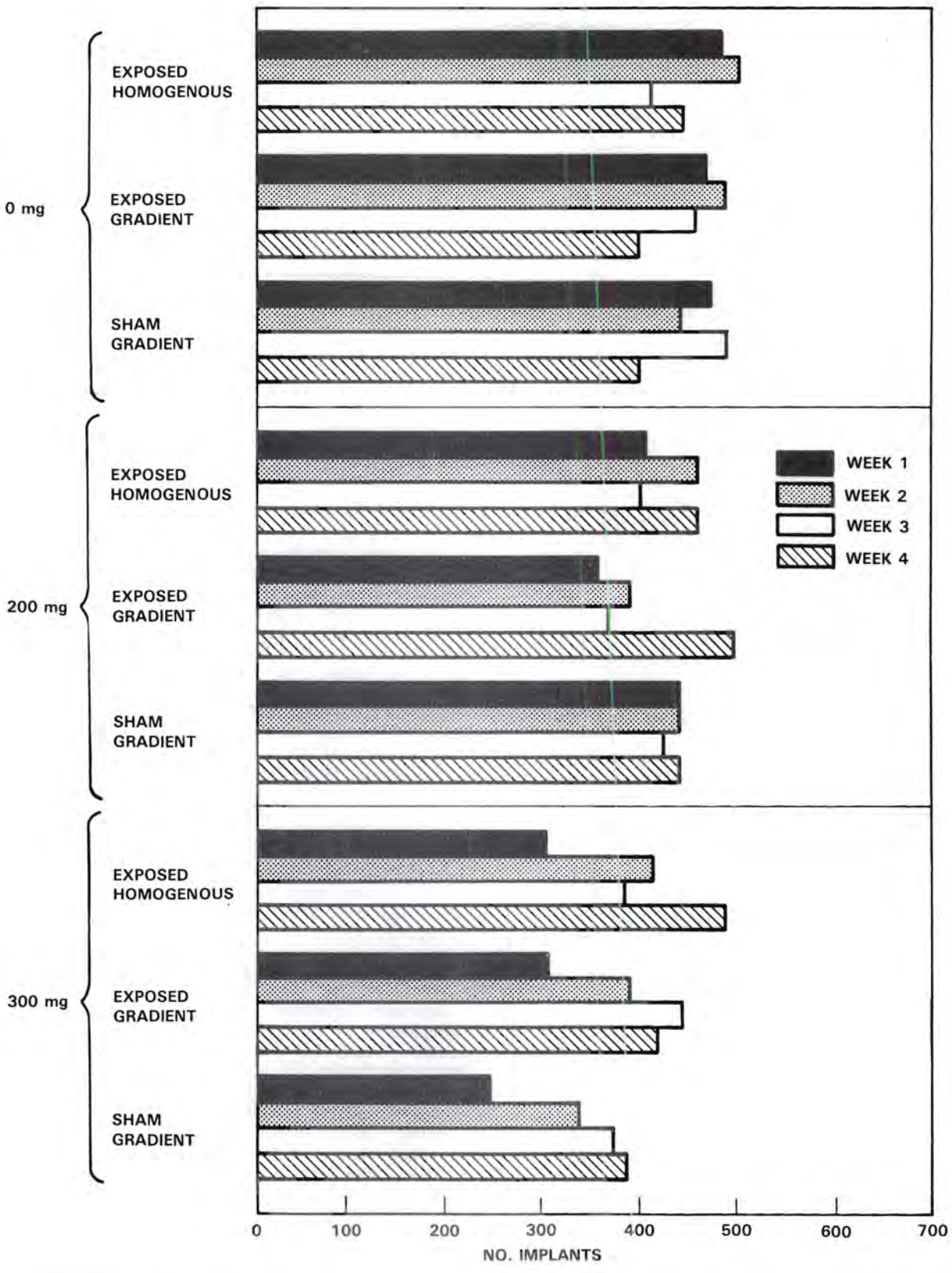

FIGURE 1. Total Number of Implants/Group for Each Week of Breeding. Implants observed following breeding of males exposed to ethylmethane sulfonate and/or a 1-Tesla or 2-Tesla/m static magnetic field.

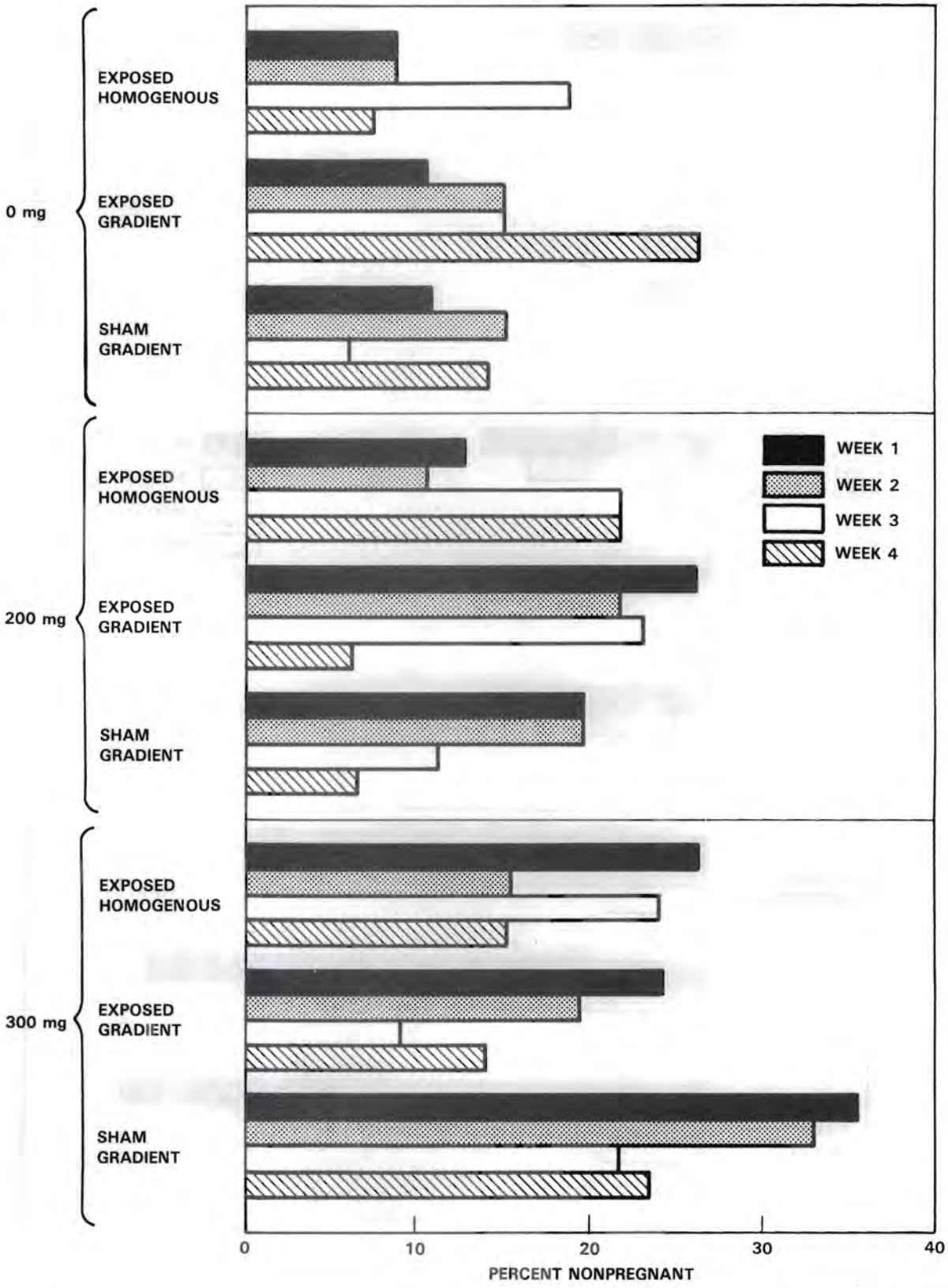

FIGURE 2. Percentage of Nonpregnant Females Following Breeding of Males Exposed to Ethylmethane Sulfonate and/or a 1-Tesla or 2-Tesla/m Static Magnetic Field.

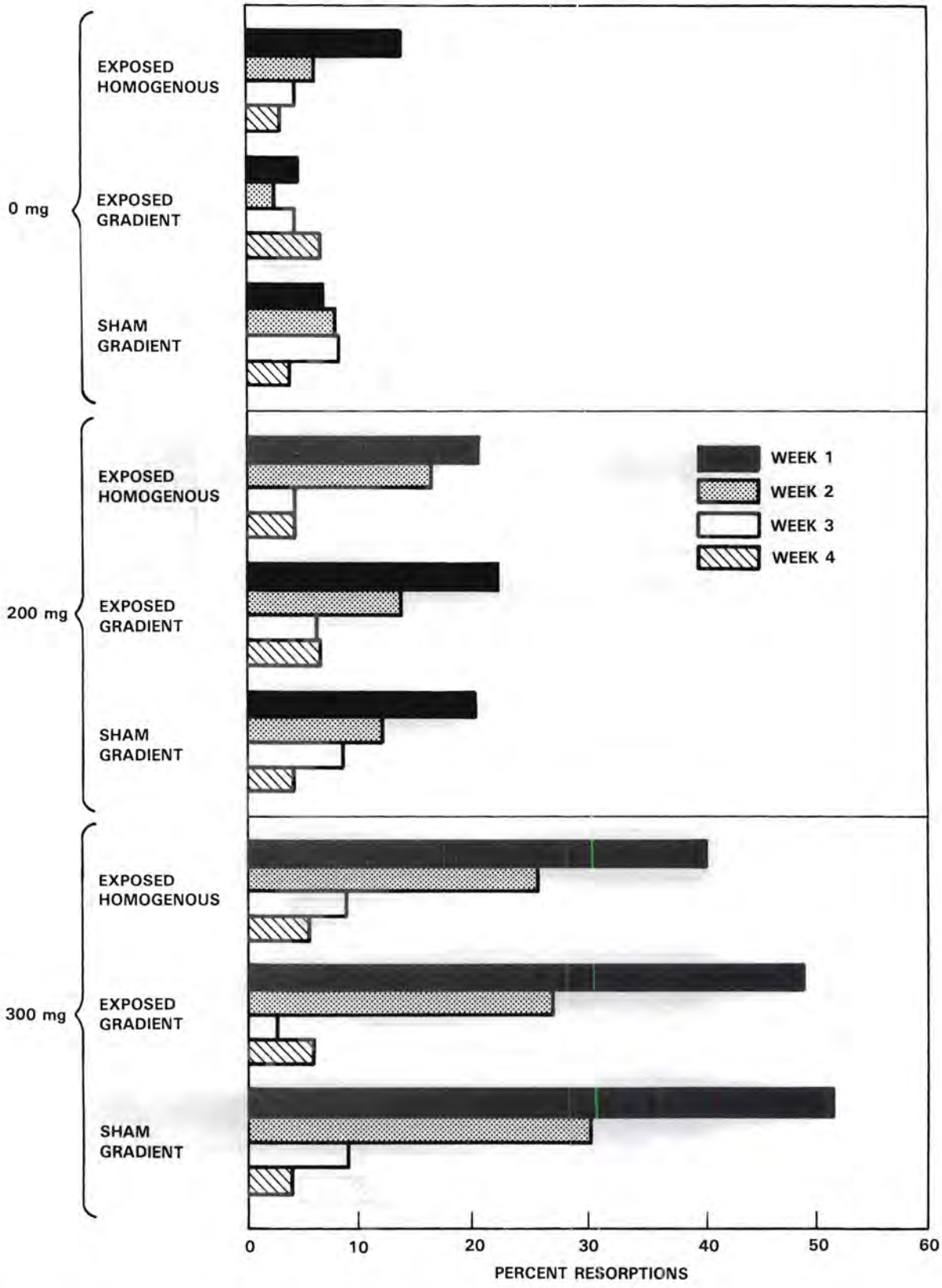

FIGURE 3. Total Resorptions Versus Total Implants in Females Bred to Males Exposed to Ethylmethane Sulfonate and/or a 1-Tesla or 2-Tesla/m Static Magnetic Field.

Multitechnology

• Metal-Membrane Interactions

Principal Investigator: R. P. Schneider

Other Investigators: H. Drucker and R. A. Lindberg

Technical Assistance: L. M. Butcher

Using different model systems in *Neurospora crassa*, two studies within this project examine the role of membranes in regulation of metabolism and entry of metals in cells. Ribonuclease, secreted by *Neurospora* in response to deprivation of carbon, nitrogen or phosphorus and the presence of extracellular protein, was purified and characterized. Amino acid and substrate specificity analyses revealed that the enzyme secreted in response to nitrogen deprivation differed from enzymes produced in response to lack of phosphorus or carbon. Investigation of the zinc uptake system of *Neurospora* showed that the K_m of uptake was about 1 nM if Zn^{2+} is the substrate. The uptake system is irreversibly inactivated by zinc but is stable in the presence of an inhibitor of protein synthesis. These data suggest specific destruction of the system, preventing accumulation of toxic concentrations of the metal.

In spite of the ubiquitous, increasing presence of toxic metals derived from the production of energy in the environment, little is known of their interactions with membrane uptake and regulatory systems. The ionic forms of most toxic metals penetrate cell membranes slowly; it therefore seems likely that many of their effects are exerted at the membrane level or are determined by membrane-regulated entry into cells. Information on the interaction of metals with defined membrane functions may therefore be expected to aid in predicting potential effects of trace metals from fossil-fuel utilization and processing.

Using different model systems, two studies within this project examine the role of membranes in regulation of metabolism and their role in regulating entry of toxic metals into cells.

Regulation of Extracellular Enzymes in N. crassa

The synthesis of extracellular degradative enzymes by N. crassa are regulated at the level of transcription by at least four different regulatory molecules. Three are products of regulatory genes involved in obtaining nutrient elements (C, N and S, or P). A fourth is a membrane-to-nucleus signal, mediating information about impermeant extracellular components. In the Annual Report for 1981, we reported the detection of a new ribonuclease from N. crassa and described its regulation. In the current study we have further examined regulatory phenomena, and purified and characterized the ribonucleases from cultures grown under each of the three states of derepression.

Extracellular ribonuclease activity is derepressible by limiting any of three elements derivable from ribonucleic acid: P, N, or C, and requires a protein inducer. The Nit-2 mutant strain was tested for ribonuclease production. This strain lacks an active regulatory protein that initiates transcription of nitrogen-utilizing enzymes in wild-type strains. The mutant responded to manipulation of media components similarly to the wild type (Annual Report, 1981), except that it was incapable of nitrogen derepression of ribonuclease synthesis. An analogous mutant for phophorus derepression (Nuc-1) was unable to produce the enzyme when phosphorus was limiting. P-reg, which contains a mutation in a regulatory protein that interacts with the Nuc-1 gene product, was constitutive for ribonculease production. Data obtained from experiments with these mutants indicate that regulation of the ribonuclease gene product is at the level of transcription.

To ascertain if the ribonucleases produced under the different regimes of derepression were the same, we purified the enzyme from cultures that were deprived of C, N, or P and contained gelatin. The procedure consisted of an ultrafiltration step to reduce volume, adsorption to a cation exchange column with subsequent elution by a salt gradient, and two successive chromatographic separations on a Sephadex G-75 column. The procedure was the same for all cultures except that thermolysin was added to the -C cultures, and the -P culture filtrates were treated with N. crassa alkaline protease to digest the gelatin.

The three enzymes were physically and enzymatically characterized and compared. All three cross-reacted with goat antibody against the -N enzyme in Ouchterlony's and interfacial tests. Molecular weights were identical for the three enzymes by gel filtration (Figure 1) and SDS gel electrophor-

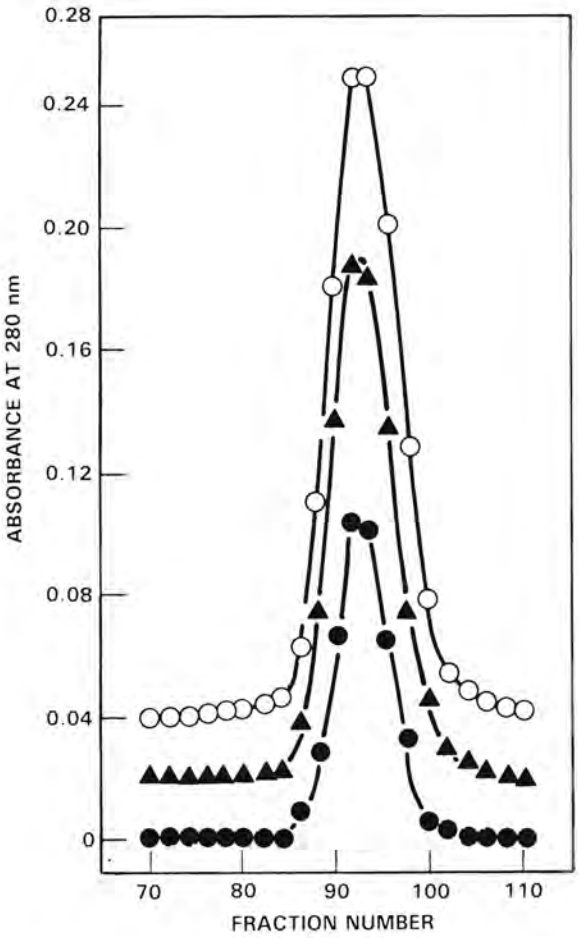

FIGURE 1. Sephadex G75 Gel Filtration of Ribonuclease From N. crassa. Ribonuclease purified from C-starved (●), N-starved (▲), and P-starved (○) cultures were chromatographed under identical conditions. The profiles for -N and -P are offset 0.02 and 0.04 (O.D.) units, respectively.

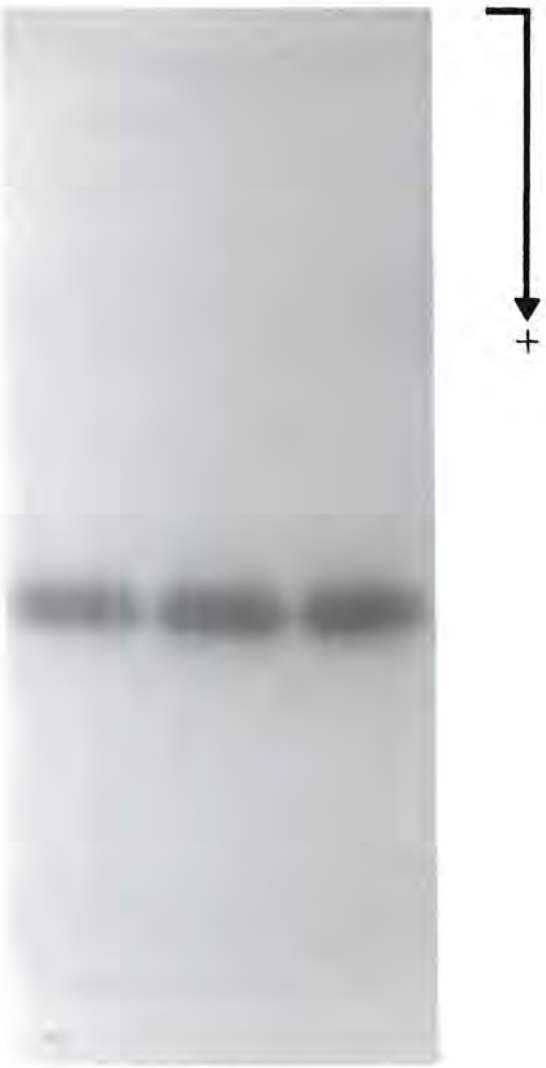

FIGURE 2. SDS Poly Acrylamide Slab Gel Electrophoresis of Ribonuclease From: A, N-Starved Cultures; B, C-Starved Cultures; and C, P-Starved Cultures.

esis (Figure 2). This indicates that effective molecular sizes are similar when in native conformation and under denaturing conditions. Tests for thermal stability, often used as a sensitive criterion for single amino acid substitutions, gave identical results for the three ribonucleases. However, when digestion of homopolyribonucleotides was investigated, the -N enzyme was found to differ from the other two (Figure 3). None of the ribonucleases cleaved poly A or poly U at the enzyme levels used. Amino acid analyses also showed differences: The -N enzyme had a methionine residue, and the -P or -C enzyme did not. There were also significant differences in the amounts of proline, glycine, serine, and tyrosine.

The data described here show that the three ribonucleases appeared identical by the usual criteria, but that some differences exist in primary structure. These differences may imply that there are three different genes for the three enzymes; however, a one-gene model may also explain the differences. The latter alternative can be explained by postulating that three different promoters initiate transcription, but not at the same initiation sites; or that editing of the messenger RNA depends on which nutrilite is limiting. Genetic analysis and isolation of the gene and/or message will be required to elucidate the molecular mechanisms involved in regulation of an enzyme by multiple regulatory molecules.

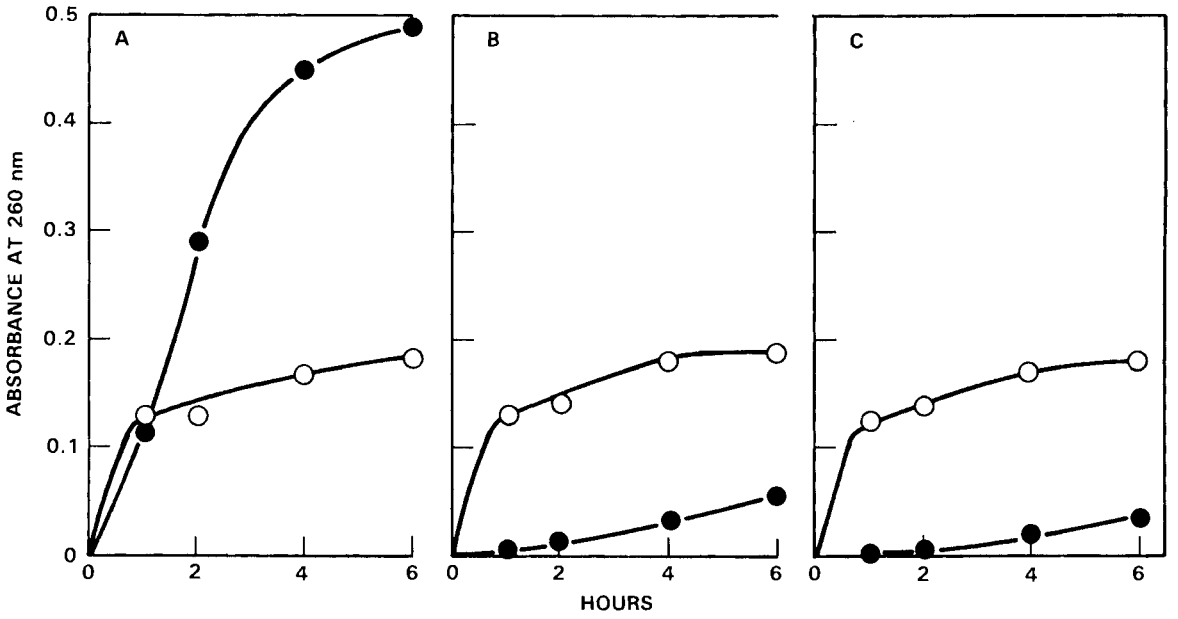

FIGURE 3. Substrate Specificity of Purified Ribonucleases. Ribonuclease from N-, C-, and P-starved cultures (A, B, and C, respectively) were reacted with polyguanylic acid (○), and polycytidylic acid (●), and acid-soluble products were read at A_{260}.

Uptake of Zinc by N. crassa

Previous studies (Annual Report, 1981) have shown that the measurable ability of N. crassa to take up zinc from the medium is dependent on prior deprivation for the metal. The data imply that the starvation for zinc derepresses the synthesis of a zinc-specific uptake system. The time course of uptake is biphasic, with an initial rapid rate of about 1 min duration, followed by a longer (>10-min) phase with a rate about 40% that of the first.

Studies this year have characterized the uptake system in cells that have been starved for zinc for 4 hr. The dependence of uptake rate on zinc concentration is hyperbolic, typical of the dependence of reaction rate of enzymes on substrate concentration (Figure 4). Throughout the range of zinc concentrations used, the rate of the slow phase is about 40% that of the fast phase. This suggests that the biphasic nature of the uptake is not the result of initial loading of a compartment and subsequent rate-limiting transfer from this compartment. The concentration of zinc that supports the one-half-maximal rate is about 1 μM. However, the uptake experiments are conducted in 8 mM citrate, which complexes (chelates) 99.9% of the zinc. If the substrate for uptake is zinc citrate, the K_m of the system for this complex is about 1 μM; if free zinc is the

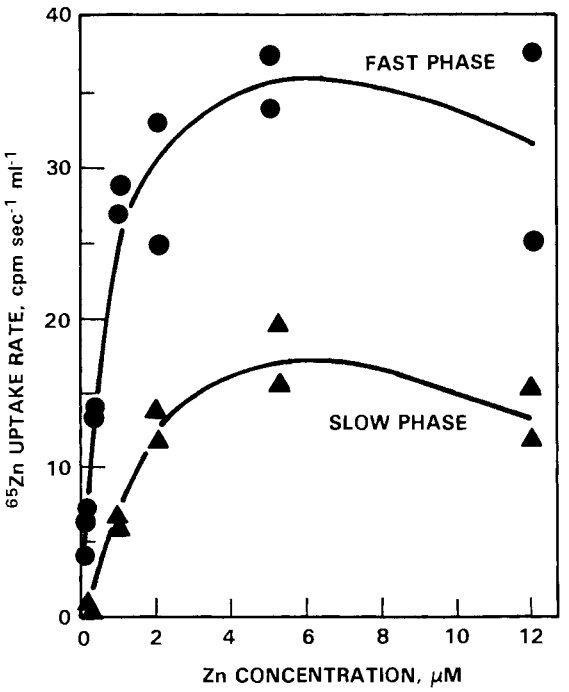

FIGURE 4. Rate of Uptake of Zinc-65 by Zn-Stained Neurospora as a Function of Zn Concentration.

substrate, the K_m is about 1 nM. Experiments have shown that ^{14}C-citrate is not taken up in amounts equivalent to that of zinc, thus zinc-citrate complex is not transported. Furthermore, increasing the concentration of citrate reduces ^{65}Zn uptake. This also suggests that the zinc-citrate is not the substrate, because additional citrate would not substantially affect the concentration of zinc-citrate but would reduce the concentration of free zinc. Zinc-65 uptake is not inhibited by concentrations of Fe^{2+}, Ca^{2+} or Mg^{2+} 100-fold higher than that of zinc, demonstrating that it is specific for zinc.

The uptake system is inhibited by low concentrations of EDTA (1×10^{-4} M). Inhibition is time-dependent (half-time, 20 min) and is irreversible. Addition of an inhibitor of protein synthesis prevents recovery from inhibition. These data suggest that EDTA causes the release of an exterior protein required for zinc transport, possibly the zinc-binding protein. However, attempts to isolate an extracellular zinc-binding protein from EDTA-exposed cultures have not been successful.

One would expect the activity of the zinc uptake system to be closely regulated because zinc is a nutrient, however, high concentrations are toxic. When derepressed, the system must be negatively regulated to avoid the accumulation of toxic cellular concentrations of the metal. In order to investigate negative control of the uptake system, zinc-starved cells were split into three subcultures: in one (control), the medium contained no zinc; in another, the medium contained an inhibitor of protein synthesis (cycloheximide) and no zinc; in the third the medium contained 10 μM zinc. At appropriate times, samples of the cultures were removed, washed and assayed for their ability to transport zinc. The culture incubated in the absence of zinc continued to synthesize the uptake system, as expected (Figure 5). The uptake

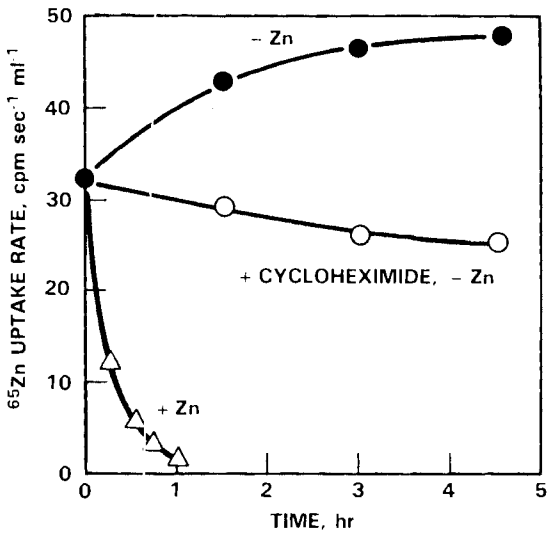

FIGURE 5. Rate of Uptake of Zn as a Function of Time of Previously Zn-Depressed Neurospora Incubated in: Zn-Free Medium Containing Cycloheximide, and Medium Containing 10 μM Zn.

rate of the cells incubated with cycloheximide remained about constant for 4.5 hr, showing that the system does not turn over at a high rate. The cells incubated with zinc, however, lost the ability to take up zinc with a half-life of about 10 min. The data suggest that the transport system of zinc is specifically inactivated, possibly by a protease, in response to adequate extra- or intracellular levels of zinc. Substrate-specific degradation of uptake systems and enzymes is an unusual and interesting form of regulation.

During the next year we will examine uptake of toxic trace metals by zinc-depressed N. crassa and ascertain whether other metals cause the same loss of the uptake system as zinc.

• Alveolar Clearance of Inhaled Metal Oxides

Principal Investigator: C. L. Sanders

Technical Assistance: K. Rhoads

Metal oxides produced during the combustion of fossil fuels constitute a potential hazard to man. The objectives of this project are to determine the fate of metals and ash deposited in the lung and their acute and chronic toxicity.

Carcinogenicity of Single and Multiple Intratracheal Instillations of Cadmium Oxide in the Rat

One hundred and ninety male, Fischer-344 rats were divided into four groups: Group 1 (46 rats) received an instillation of saline; group 2 (48 rats) received an instillation of 25 μg cadmium oxide (CdO) in saline at 70 days of age; group 3 (46 rats) received an instillation of 25 μg CdO at 70 and 100 days of age; and group 4 (50 rats) received an instillation of 25 μg CdO at 70, 100 and 130 days of age. Animals were held for lifespan study.

No difference in survival time was noted between control and any CdO-treated group (Figure 1). There was a marginal increase in mammary tumors; otherwise, instilled CdO did not result in increased incidence of tumors in the lung, testis, liver, kidney or prostate gland (Table 1). There was, however, a significant difference ($P < 0.05$) when all but Leydig cell tumors in the testis were included in the analysis.

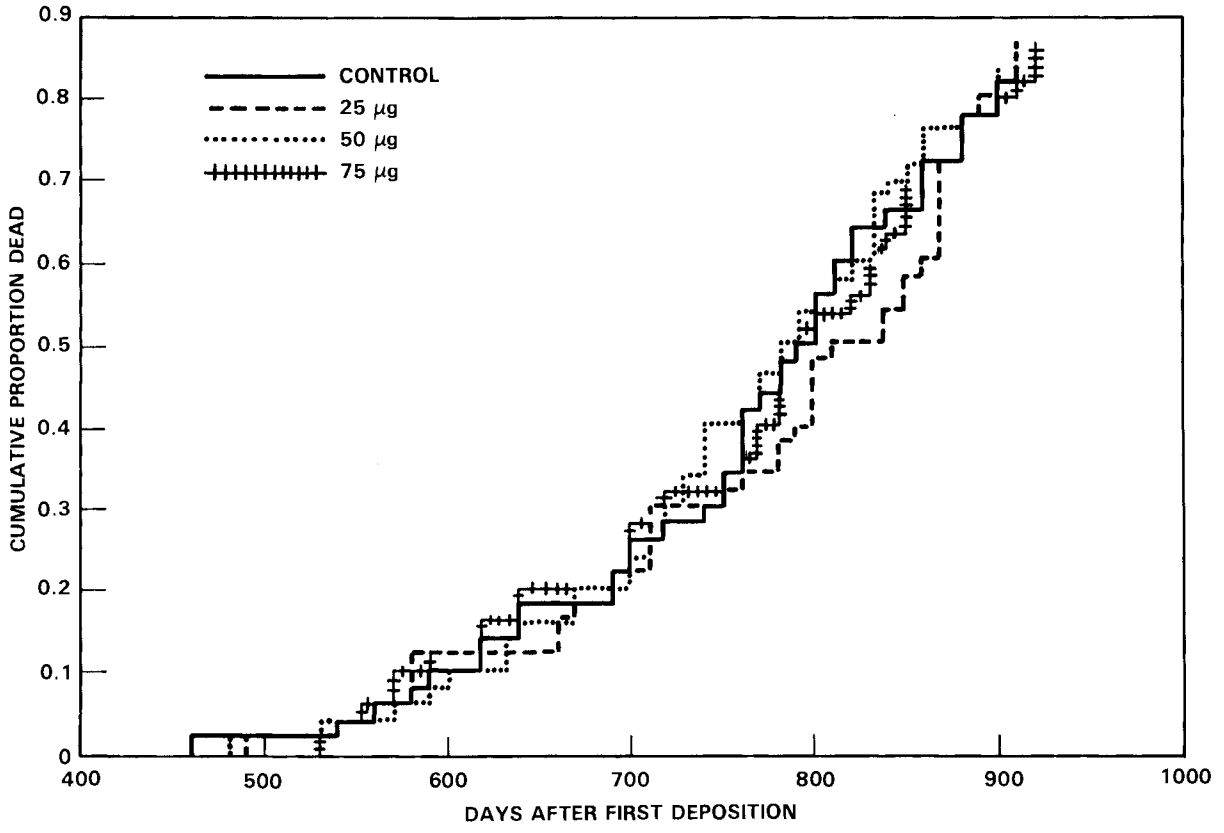

FIGURE 1. Mortality Curves for F-344 Rats Given Single or Multiple Intratracheal Instillations of Cadmium Oxide in Doses of 25 μg.

TABLE 1. Number (Percent) of Lesions, by Exposure Group, in Rats Given Intratracheal Instillations of Cadmium Oxide.

Lesion Location/Type	Control		Exposed 25 μg		50 μg		75 μg	
Number of Rats at Risk	45		44		41		48	
Abdomen/Stomach								
Tumors[a]	1	(2%)	2	(5%)	3	(7%)	3	(6%)
Adrenal Glands								
Pheochromocytoma	4	(9%)	1	(2%)	2	(5%)	5	(10%)
Blood								
Leukemia	17	(38%)	14	(32%)	15	(37%)	18	(38%)
Lung								
Metaplastic Lesions[b]	0		1	(2%)	1[c]	(2%)	2	(4%)
Adenocarcinoma	0		0		2[c]	(5%)	0	
Mammary Glands								
Fibroadenoma	3	(7%)	7	(16%)	5	(12%)	11	(23%)
Skin								
Fibroma	3	(7%)	4	(9%)	3	(7%)	5	(10%)
Malignant Tumors[d]	2	(4%)	0		0		4	(8%)
Spleen								
Tumors[e]	1	(2%)	0		0		1	(2%)
Testes								
Leydig Cell Tumor	31	(69%)	37	(84%)	34	(83%)	38	(79%)
All Tissues								
Lesions[i]	38	(84%)	42[f]	(95%)	38[g]	(93%)	46[h]	(96%)

(a) Fibrosarcoma (1), lipoma (1), mesothelioma (3), leiomyoma (2), undifferentiated sarcoma (1), undifferentiated carcinoma (1)
(b) Adenomatosis (3), squamous metaplasia (1)
(c) One rat had both an adenocarcinoma and a squamous metaplasia.
(d) Melanoma (1), squamous carcinoma (4), endothelioma (1)
(e) Hemangioendothelioma (1), hemangiosarcoma (1)
(f) Includes undifferentiated sarcoma (1) of unknown tissue origin
(g) Includes squamous cell carcinoma (1) of unknown tissue origin
(h) Includes undifferentiated sarcoma (1) and lymphoma (2) of unknown tissue origin
(i) Each rat with multiple lesions counted only once.

The number of tumors per rat (excluding Leydig cell tumors) was 0.69, 0.66, 0.76 and 1.0 for 0, 25, 50 and 75 μg CdO groups, respectively. The 75-μg CdO group had significantly greater numbers of tumors than controls.

We concluded that single or multiple subacute exposures of the lung to cadmium did not significantly increase tumor formation at any anatomical site except, possibly, the male mammary gland. Overall, CdO had no significant impact on survival of rats.

Long-Term Reactivity of Lung and Mediastinal Lymph Nodes Following Intratracheal Instillation of Sandy Loam Soil or Mount St. Helen's Volcanic Ash

Eighty-two female, Fischer-344 rats were instilled with either saline, or a respirable-sized sandy loam soil (Ritzville sandy loam obtained from the Hanford Reservation in Washington), or with a respirable-sized volcanic ash sample obtained from Pullman, Washington. Rats were given either two or seven consecutive,

weekly instillations of 11 mg soil or ash suspended in saline per instillation for total doses of 22 mg or 77 mg, respectively. Rats were killed at about 400 days after initial instillation.

Significantly elevated levels of lipid-phosphorus and of protein were found in lung lavages of rats given ash compared to levels in those given soil particles. Microscopic examination revealed a significantly ($P < 0.05$) greater degree of pulmonary lipoproteinosis and fibrosis, and incidence of bronchiolar hyperplasia, in ash-exposed rats (Tables 2 and 3).

Lymph nodes of ash-exposed rats were 8-18 times larger than in soil-exposed rats due to an abundant cellular microgranuloma formation and an early fibrosis in nodes of ash-exposed rats (Table 4). Mount St. Helens volcanic ash is apparently more biologically reactive than soil particles commonly found in eastern Washington.

TABLE 2. Pathological Response of Lung and Mediastinal Lymph Nodes to Intratracheally Instilled Soil or Ash Particles.

Treatment	No. Rats	Lifespan, days[b]		Pulmonary Pathology[a]		
		Median	Mean	Lipo-proteinosis	Fibrosis	Bronchiolar Hyperplasia, %
Control (X 7)[c]	20	532	533 ± 11	0.0 ± 0.0	0.0	0.0
Soil (X 2)	6	398	398 ± 1	1.7 ± 0.5	0.0 - 1.0	0.0
Ash (X 2)	4	407	407 ± 1	2.8 ± 1.0	1.0 - 2.0	75
Soil (X 7)	21	425	398 ± 67	2.0 ± 0.6	0.0 - 1.0	9.5
Ash (X 7)	14	400	396 ± 15	3.4 ± 0.7[d]	1.0 - 2.0	43
Ash (X 7)	17	408	396 ± 62	2.7 ± 0.7	1.0 - 2.0	53

[a] Lipoproteinosis and fibrosis of lung sections are graded on a scale of increasing severity from 0 - 5; bronchiolar hyperplasia values are incidences.
[b] After first intratracheal instillation
[c] ( ) = number of intratracheal instillations of 11 mg/instillation for soil- or ash-treated animals.
[d] All but this group underwent lung lavage.

TABLE 3. Amount of Protein, Lipid-Phosphorus and Nucleated Cells in Lung Lavages Following Intratracheal Instillation of Soil or Ash Particles.

Treatment	Protein, μg/ml	Lipid-Phosphorus, μg/ml	Nucleated Cells, x 10^3/ml
Control (X 7)[a]	270 ± 120 (20)	5.6 ± 1.3 (20)	780 ± 300 (12)
Soil (X 2	520 ± 210 (6)[b]	16 ± 2.2 (6)[b]	1500 ± 500 (6)
Ash (X 2)	530 ± 55 (4)[b]	16 ± 3.3 (4)[b]	1000 ± 240 (4)
Soil (X 7)	490 ± 130 (17)[b]	18 ± 4.2 (17)[b]	930 ± 190 (9)
Ash (X 7)	860 ± 140 (14)[b,c]	22 ± 3.1 (14)[b,c]	1100 ± 360 (6)

[a] ( ) = number of intratracheal instillations of 11 mg/instillation for soil- or ash-treated animals.
[b] Significantly greater than control group at $P < 0.001$
[c] Significantly greater than soil (X 7) group at $P < 0.05$

TABLE 4. Reactivity of Mediastinal Lymph Nodes Following Intratracheal Instillation of Soil or Ash Particles.

Treatment		Mass of Largest Lymph Node, mg	
Control	(X 7)[a]	6.4 ± 4.1	(15)[b]
Soil	(X 2)	13 ± 5.2	(6)
Ash	(X 2)	240 ± 82	(4)
Soil	(X 7)	31 ± 15	(17)
Ash	(X 7)	240 ± 77	(15)

[a] ( ) = number of intratracheal instillations of 11 mg/instillation for soil- or ash-treated animals.
[b] Number of rats examined

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 $^{239}PuO_2$, $^{238}PuO_2$, 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 x 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 NCI	NCI/G LUNG	NCI/ KG	INHALATION EXPOSURE WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	MONTHS SINCE INHALATION 9/30/82	DEATH	COMMENTS ON DEAD DOGS
CONTROL	738	F	0	0.00	0.00					161.2*		
CONTROL	740	F	0	0.00	0.00					161.2*		
CONTROL	749	F	0	0.00	0.00					159.9*		
CONTROL	755	M	0	0.00	0.00					159.8*		
CONTROL	766	M	0	0.00	0.00					159.5*		
CONTROL	775	F	0	0.00	0.00				10/05/81		147.3*	Pul. Thromboembolism
CONTROL	785	M	0	0.00	0.00					158.4*		
CONTROL	789	M	0	0.00	0.00					158.1*		
CONTROL	792	M	0	0.00	0.00				04/28/76		79.5*	Oral Tumor
CONTROL	800	F	0	0.00	0.00					155.3*		
CONTROL	801	M	0	0.00	0.00				02/23/82		148.1*	Lung Tumor
CONTROL	811	F	0	0.00	0.00					154.2*		
CONTROL	846	M	0	0.00	0.00					153.4*		
CONTROL	861	M	0	0.00	0.00					153.0*		
CONTROL	868	F	0	0.00	0.00					151.7*		
CONTROL	872	F	0	0.00	0.00					151.6*		
CONTROL	878	M	0	0.00	0.00					149.7*		
CONTROL	882	M	0	0.00	0.00				11/06/81		138.7*	Hemangiosarcoma, Liver
CONTROL	885	F	0	0.00	0.00					148.9*		
CONTROL	903	F	0	0.00	0.00					146.6*		
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		140.4		
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		134.8		
D-1 LOWEST	858	M	0	0.00	0.00	13.5	18.2	07/06/71		134.8		
D-1 LOWEST	865	F	0	0.00	0.00	9.0	17.4	07/06/71		134.8		
D-1 LOWEST	879	M	0	0.00	0.00	14.5	17.9	10/07/71		131.8		
D-1 LOWEST	886	F	0	0.00	0.00	10.5	18.2	11/10/71		130.7		
D-1 LOWEST	907	F	0	0.00	0.00	11.5	15.9	11/10/71		130.7		
D-1 LOWEST	825	F	1	0.01	0.12	11.5	18.1	06/08/71		135.8		
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		130.7		
D-1 LOWEST	832	F	2	0.02	0.22	9.0	16.5	04/26/71		137.2		
D-1 LOWEST	900	M	3	0.02	0.22	13.0	16.0	11/10/71	05/21/82		126.3	Round Cell Sarcoma
D-1 LOWEST	870	F	4	0.03	0.32	12.0	16.9	07/06/71		134.8		
D-1 LOWEST	899	F	4	0.03	0.31	11.5	16.0	11/10/71	03/29/81		112.6	Hemangiosarcoma, Heart
D-1 LOWEST	867	M	5	0.04	0.41	11.5	17.4	07/06/71		134.8		
D-1 LOWEST	891	M	6	0.04	0.41	14.0	16.0	11/10/71	06/26/81		115.5	Septicemia
D-1 LOWEST	850	F	5	0.05	0.59	8.5	21.3	10/07/71		131.8		
D-1 LOWEST	853	M	8	0.05	0.51	15.0	21.3	10/07/71		131.8		
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 NCI	NCI/G LUNG	NCI/ KG	INHALATION EXPOSURE WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	MONTHS SINCE INHALATION 9/30/82	DEATH	COMMENTS ON DEAD DOGS
D-1 LOWEST	770	F	6	0.06	0.63	9.5	19.1	01/19/71		140.4		
D-1 LOWEST	788	M	8	0.06	0.62	13.0	18.7	02/09/71		139.7		
D-1 LOWEST	893	M	9	0.06	0.61	14.0	14.9	10/07/71		131.8		
D-1 LOWEST	807	F	8	0.07	0.73	11.0	14.6	02/09/71	07/24/81		125.4	Putitary Tumor, Cushing's
D-1 LOWEST	841	F	6	0.07	0.75	8.0	17.7	06/08/71		135.8		
D-1 LOWEST	908	M	9	0.07	0.77	11.0	15.9	11/10/71	04/01/80		100.7	Unknown, Pul. Hyalinosis
D-2 LOW	776	M	10	0.07	0.74	13.5	20.2	03/04/71		138.9		
D-2 LOW	842	M	10	0.07	0.77	13.5	18.6	07/06/71		134.8		
D-2 LOW	767	M	10	0.08	0.83	12.0	18.2	12/21/70		141.3		
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		135.8		
D-2 LOW	871	M	13	0.09	0.96	13.5	16.9	07/06/71		134.8		
D-2 LOW	874	M	16	0.11	1.24	13.0	16.8	07/06/71		134.8		
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		135.8		
D-2 LOW	748	F	14	0.16	1.75	8.0	19.5	01/19/71	08/19/81		127.0	Unknown Cause
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	0.17	1.90	10.0	19.1	07/06/71		134.8		
D-2 LOW	831	F	21	0.18	2.00	10.5	17.9	06/08/71		135.8		
D-2 LOW	881	F	19	0.19	2.09	9.0	17.7	10/07/71		131.8		
D-2 LOW	780	F	24	0.22	2.40	10.0	18.2	01/19/71	04/08/82		134.6	Processing
D-2 LOW	859	M	35	0.22	2.41	14.5	18.2	07/06/71		134.8		
D-2 LOW	757	M	36	0.23	2.57	14.0	18.5	12/21/70		141.3		
D-2 LOW	876	F	19	0.24	2.69	7.0	17.9	10/07/71		131.8		
D-2 LOW	806	F	26	0.25	2.74	9.5	15.3	03/04/71		138.9		
D-2 LOW	813	F	32	0.29	3.20	10.0	15.1	03/04/71		138.9		
D-2 LOW	877	F	34	0.29	3.24	10.5	17.9	10/07/71		131.8		
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		137.2		
D-3 MED-LOW	781	F	48	0.38	4.17	11.5	17.3	12/21/70	02/20/81		122.0	Lung Tumor, Kidney Tumor
D-3 MED-LOW	771	F	44	0.40	4.40	10.0	19.2	01/20/71		140.3		
D-3 MED-LOW	782	M	62	0.42	4.59	13.5	19.0	02/10/71		139.6		
D-3 MED-LOW	786	M	62	0.42	4.59	13.5	19.5	03/04/71		138.9		
D-3 MED-LOW	752	M	62	0.43	4.77	13.0	18.6	12/21/70	02/22/79		98.1	Lung Tumor, Adrenal Tumor
D-3 MED-LOW	823	M	65	0.44	4.81	13.5	16.8	04/26/71		137.2		
D-3 MED-LOW	883	M	63	0.44	4.85	13.0	17.7	10/07/71		131.8		
D-3 MED-LOW	778	M	74	0.46	5.10	14.5	20.2	03/04/71	08/26/79		101.7	Pul. Thromboembolism
D-3 MED-LOW	838	M	56	0.46	5.09	11.0	17.8	06/08/71		135.8		
D-3 MED-LOW	795	F	54	0.52	5.68	9.5	15.0	01/20/71		140.3		
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		131.8		
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	0.68	7.44	9.0	17.8	06/08/71	07/05/79		96.9	Pyometra

* 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 NCI	NCI/G LUNG	NCI/ KG	INHALATION EXPOSURE WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	MONTHS SINCE INHALATION 9/30/82	DEATH	COMMENTS ON DEAD DOGS
D-3 MED-LOW	797	F	85	0.70	7.73	11.0	16.4	03/04/71		138.9		
D-3 MED-LOW	848	F	75	0.72	7.94	9.5	21.3	10/07/71		131.8		
D-3 MED-LOW	827	F	89	0.74	8.09	11.0	16.7	04/26/71		137.2		
D-3 MED-LOW	697	M	140	0.85	9.33	15.0	19.5	10/30/70	05/08/80		114.3	Card. Valve insufficiency
D-3 MED-LOW	750	M	118	0.93	10.26	11.5	19.6	01/20/71		140.3		
D-3 MED-LOW	884	M	123	1.12	12.30	10.0	17.8	10/08/71		131.7		
D-3 MED-LOW	844	F	135	1.17	12.86	10.5	17.6	06/08/71		135.8		
D-3 MED-LOW	905	F	127	1.36	14.94	8.5	15.9	11/10/71		130.7		
D-4 MEDIUM	866	M	200	1.35	14.81	13.5	17.4	07/06/71		134.8		
D-4 MEDIUM	809	F	157	1.36	14.95	10.5	15.3	03/04/71	05/28/81		122.8	Livr Cirrhosis, Thyrd Tmr
D-4 MEDIUM	764	F	158	1.37	15.05	10.5	18.2	12/21/70	07/07/82		138.5	Processing
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		137.2		
D-4 MEDIUM	814	F	140	1.50	16.47	8.5	15.1	03/04/71	10/17/79		103.5	Lng Tmr, Thyroid Adenoma
D-4 MEDIUM	836	M	256	1.66	18.29	14.0	17.8	06/08/71	03/16/81		117.3	Lung Tumor
D-4 MEDIUM	819	F	163	1.74	19.18	8.5	18.2	06/08/71		135.8		
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	01/26/81		115.6	Bronchopneumonia
D-4 MEDIUM	860	M	254	1.85	20.32	12.5	17.3	06/08/71	06/24/82		132.5	Lung Tumor
D-4 MEDIUM	833	F	248	2.37	26.11	9.5	16.5	04/26/71		137.2		
D-4 MEDIUM	810	F	302	2.39	26.26	11.5	15.3	03/04/71	09/09/81		126.2	Lung Tumor
D-4 MEDIUM	794	M	444	2.60	28.65	15.5	17.7	03/04/71	02/17/81		119.5	Pituitary Tmr, bad adrenl
D-4 MEDIUM	854	M	465	2.64	29.06	16.0	21.3	10/08/71	01/25/82		123.6	Lung Tumor
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	2.89	31.76	8.5	14.6	02/10/71	09/09/82		139.0	Processing
D-4 MEDIUM	805	F	257	3.12	34.27	7.5	18.5	06/08/71	07/22/82		133.5	Processing
D-4 MEDIUM	812	M	438	3.19	35.04	12.5	17.1	04/26/71	11/12/79		102.6	Lung Tumor
D-4 MEDIUM	857	M	486	3.40	37.38	13.0	17.3	06/08/71	07/01/80		108.8	Lung Tumor
D-4 MEDIUM	892	M	494	3.59	39.52	12.5	16.0	11/10/71	10/26/81		119.5	Lung Tumor
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	03/26/80		108.7	Lung Tumor
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	04/29/80		106.7	Lung Tumor
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	02/03/81		110.8	Uri Bladr & Lng & Adr Tmr
D-5 MED-HIGH	856	F	818	5.72	62.92	13.0	18.2	07/07/71	05/02/79		93.8	Lung Tumor
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	11/02/79		99.9	Lung Tumor
D-5 MED-HIGH	909	M	737	6.70	73.70	10.0	15.9	11/10/71	06/04/81		114.8	Lung Tumor
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	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 NCI	NCI/G LUNG	NCI/ KG	INHALATION EXPOSURE WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	MONTHS SINCE INHALATION 9/30/82	DEATH	COMMENTS ON DEAD DOGS
D-5 MED-HIGH	820	F	847	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	889	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	804	M	1344	10.18	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	08/15/73		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	19.8	02/09/71	06/26/75		52.5	Lng Tmr, Osteoarthropathy
D-5 MED-HIGH	702	F	1682	15.29	168.20	10.0	19.8	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	890	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	66.85	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

* Indicates age in months since birth, all other ages are in months since exposure.

DOSE-EFFECT STUDIES WITH INHALED PU-238 OXIDE IN BEAGLES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION NCI	NCI/G LUNG	NCI/ KG	INHALATION EXPOSURE WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	MONTHS SINCE INHALATION 9/30/82	DEATH	COMMENTS ON DEAD DOGS
CONTROL	939	M	0	0.00	0.00					136.9*		
CONTROL	949	F	0	0.00	0.00					136.7*		
CONTROL	978	M	0	0.00	0.00					136.5*		
CONTROL	990	F	0	0.00	0.00				07/08/79		97.4*	Pyometra
CONTROL	996	F	0	0.00	0.00					136.0*		
CONTROL	1005	M	0	0.00	0.00					136.0*		
CONTROL	1007	F	0	0.00	0.00					136.0*		
CONTROL	1024	M	0	0.00	0.00					135.5*		
CONTROL	1038	M	0	0.00	0.00					133.4*		
CONTROL	1045	M	0	0.00	0.00					133.4*		
CONTROL	1054	F	0	0.00	0.00					133.1*		
CONTROL	1061	F	0	0.00	0.00				07/07/81		118.2*	Malignant Lymphoma
CONTROL	1093	M	0	0.00	0.00					129.3*		
CONTROL	1097	F	0	0.00	0.00					128.6*		
CONTROL	1112	M	0	0.00	0.00					128.4*		
CONTROL	1116	F	0	0.00	0.00					128.1*		
CONTROL	1186	F	0	0.00	0.00					121.5*		
CONTROL	1197	M	0	0.00	0.00					121.0*		
CONTROL	1209	M	0	0.00	0.00					120.7*		
CONTROL	1225	F	0	0.00	0.00					119.9*		
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		95.8*	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	998	M	0	0.00	0.00	10.5	19.6	01/18/73		116.4		
D-1 LOWEST	1003	M	0	0.00	0.00	14.0	19.6	01/18/73		116.4		
D-1 LOWEST	1023	F	0	0.00	0.00	12.5	19.2	01/18/73		116.4		
D-1 LOWEST	1039	M	0	0.00	0.00	11.0	17.0	01/18/73		116.4		
D-1 LOWEST	1044	F	0	0.00	0.00	11.5	17.0	01/18/73		116.4		
D-1 LOWEST	1055	M	0	0.00	0.00	13.0	16.8	01/18/73		116.4		
D-1 LOWEST	1063	M	0	0.00	0.00	14.5	16.7	01/18/73	11/11/80		93.8	Brain Tumor, Heart Tumor
D-1 LOWEST	1105	F	0	0.00	0.00	10.0	16.4	05/31/73		112.0		
D-1 LOWEST	1194	F	0	0.00	0.00	10.5	19.8	04/18/74		101.4		
D-1 LOWEST	1215	M	0	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		101.4		
D-1 LOWEST	951	M	2	0.01	0.14	14.0	19.3	12/19/72		117.4		
D-1 LOWEST	1008	M	2	0.01	0.15	13.5	19.6	01/18/73		116.4		
D-1 LOWEST	1193	F	2	0.01	0.16	12.5	19.8	04/18/74		101.4		
D-1 LOWEST	959	M	3	0.02	0.22	13.5	19.2	12/19/72		117.4		

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DOSE-EFFECT STUDIES WITH INHALED PU-238 OXIDE IN BEAGLES

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION NCI	NCI/G LUNG	NCI/ KG	INHALATION EXPOSURE WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	MONTHS SINCE INHALATION 9/30/82	DEATH	COMMENTS ON DEAD DOGS
D-1 LOWEST	1069	F	2	0.02	0.24	8.5	18.1	05/31/73		112.0		
D-1 LOWEST	1095	F	2	0.02	0.19	10.5	16.6	05/31/73		112.0		
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	03/05/81		98.5	Bone Tumor, Fibrosarcoma
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		103.1		
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		117.4		
D-1 LOWEST	1106	F	5	0.05	0.50	10.0	16.4	05/31/73		112.0		
D-2 LOW	1065	F	6	0.05	0.60	10.0	18.3	05/31/73		112.0		
D-2 LOW	1082	M	11	0.06	0.69	16.0	18.0	05/31/73	12/04/79		78.1	Paralysis, Spinal Crd Deg
D-2 LOW	1188	M	11	0.06	0.71	15.5	18.4	02/26/74		103.1		
D-2 LOW	1084	M	13	0.07	0.76	17.0	17.5	05/31/73		112.0		
D-2 LOW	1090	F	10	0.08	0.83	12.0	17.3	05/31/73		112.0		
D-2 LOW	1222	M	15	0.10	1.11	13.5	19.0	04/18/74		101.4		
D-2 LOW	971	F	13	0.11	1.24	10.5	19.2	12/19/72		117.4		
D-2 LOW	999	F	11	0.11	1.16	9.5	18.7	12/19/72		117.4		
D-2 LOW	1229	M	16	0.11	1.19	13.5	16.8	02/26/74		103.1		
D-2 LOW	1070	M	22	0.12	1.33	16.5	18.1	05/31/73		112.0		
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		117.4		
D-2 LOW	1033	M	17	0.14	1.55	11.0	19.1	02/22/73		115.2		
D-2 LOW	1036	F	16	0.14	1.52	10.5	18.2	02/22/73		115.2		
D-2 LOW	1216	M	23	0.16	1.77	13.0	19.3	04/18/74		101.4		
D-2 LOW	1060	F	22	0.18	2.00	11.0	17.8	02/22/73		115.2		
D-2 LOW	981	M	30	0.21	2.31	13.0	19.0	12/19/72		117.4		
D-2 LOW	1046	M	27	0.22	2.45	11.0	18.1	02/22/73		115.2		
D-2 LOW	1050	F	22	0.22	2.44	9.0	18.1	02/22/73		115.2		
D-2 LOW	1078	F	29	0.22	2.42	12.0	18.0	05/31/73		112.0		
D-2 LOW	1207	F	22	0.24	2.59	8.5	17.6	02/26/74		103.1		
D-2 LOW	1196	F	28	0.25	2.80	10.0	17.9	02/26/74		103.1		
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		112.0		
D-3 MED-LOW	1089	F	41	0.31	3.42	12.0	17.3	05/31/73		112.0		
D-3 MED-LOW	972	F	40	0.33	3.64	11.0	19.2	12/19/72		117.4		
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		101.4		
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		106.8		

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D-3 MED-LOW	1165	M	76	0.43	4.75	16.0	17.3	11/06/73		106.8		
D-3 MED-LOW	1309	M	60	0.44	4.80	12.5	18.5	03/04/75		90.9		
D-3 MED-LOW	1318	M	67	0.45	4.96	13.5	18.1	03/04/75	03/08/76		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		90.9		
D-3 MED-LOW	960	M	68	0.54	5.91	11.5	19.2	12/19/72	11/07/80		94.6	Malignant Lymphoma
D-3 MED-LOW	1072	M	98	0.54	5.94	16.5	18.1	05/31/73		112.0		
D-3 MED-LOW	1190	F	71	0.54	5.92	12.0	18.1	02/26/74		103.1		
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		117.4		
D-3 MED-LOW	1040	M	84	0.61	6.72	12.5	18.2	02/22/73	03/04/81		96.3	Parathyroid Adenoma
D-3 MED-LOW	1059	F	71	0.65	7.10	10.0	17.8	02/22/73		115.2		
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		112.0		
D-3 MED-LOW	1000	F	70	0.71	7.78	9.0	18.7	12/19/72		117.4		
D-3 MED-LOW	1056	M	97	0.71	7.76	12.5	17.9	02/22/73		115.2		
D-3 MED-LOW	1004	M	116	0.73	8.00	14.5	19.6	01/18/73		116.4		
D-3 MED-LOW	1026	M	116	0.78	8.59	13.5	19.2	01/18/73		116.4		
D-3 MED-LOW	1043	F	98	0.89	9.80	10.0	18.1	02/22/73	09/21/81		102.9	Putitary Tumor, Cushing's
D-3 MED-LOW	1031	F	76	0.92	10.13	7.5	19.1	02/22/73		115.2		
D-3 MED-LOW	1212	F	111	1.19	13.06	8.5	17.6	02/26/74		103.1		
D-4 MEDIUM	1176	M	129	0.87	9.56	13.5	15.5	10/06/73		107.8		
D-4 MEDIUM	1221	F	124	1.13	12.40	10.0	19.0	04/18/74		101.4		
D-4 MEDIUM	1195	M	228	1.38	15.20	15.0	18.1	02/26/74		103.1		
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	148	1.42	15.58	9.5	17.9	02/22/73		115.2		
D-4 MEDIUM	997	M	203	1.60	17.65	11.5	19.6	01/18/73		116.4		
D-4 MEDIUM	991	F	194	1.76	19.40	10.0	18.8	12/19/72		117.4		
D-4 MEDIUM	1177	M	262	1.76	19.41	13.5	16.6	11/06/73		106.8		
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		112.0		
D-4 MEDIUM	973	F	271	2.24	24.64	11.0	19.2	12/19/72		117.4		
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		112.0		
D-4 MEDIUM	1114	M	430	2.70	29.66	14.5	16.4	05/31/73		112.0		
D-4 MEDIUM	1062	M	435	2.93	32.22	13.5	17.8	02/22/73		115.2		
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	01/18/80		79.6	Hemangiosarcoma, Heart
D-4 MEDIUM	1030	F	340	3.25	35.79	9.5	19.1	02/22/73		115.2		
D-4 MEDIUM	1198	M	539	3.50	38.50	14.0	17.9	02/26/74		103.1		
D-4 MEDIUM	952	F	365	3.69	40.56	9.0	19.2	12/19/72		117.4		
D-4 MEDIUM	1166	M	673	4.08	44.87	15.0	17.3	11/06/73		106.8		

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D-4 MEDIUM	1220	F	518	4.28	47.09	11.0	19.0	04/18/74		101.4		
D-4 MEDIUM	992	F	555	4.39	48.26	11.5	18.8	12/19/72		117.4		
D-4 MEDIUM	983	M	617	4.67	51.42	12.0	19.0	12/19/72		117.4		
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		106.8		
D-5 MED-HIGH	1035	F	571	5.46	60.11	9.5	18.2	02/22/73		115.2		
D-5 MED-HIGH	1192	F	754	6.53	71.81	10.5	18.1	02/26/74		103.1		
D-5 MED-HIGH	1140	M	1014	6.58	72.43	14.0	18.2	11/06/73	12/14/81		97.2	Bone Tumor
D-5 MED-HIGH	1071	M	1269	6.79	74.65	17.0	18.1	05/31/73	01/09/81		91.3	Bone Tumor, Lung Tumor
D-5 MED-HIGH	1173	M	1023	7.75	85.25	12.0	17.3	11/06/73	02/09/82		99.1	Bone Tumor
D-5 MED-HIGH	1178	M	1125	8.52	93.75	12.0	16.6	11/06/73		106.8		
D-5 MED-HIGH	1047	M	900	8.61	94.74	9.5	18.1	02/22/73		115.2		
D-5 MED-HIGH	1109	F	1119	8.85	97.30	11.5	16.4	05/31/73	08/06/80		86.2	Bone & Lng Tmr, Addison's
D-5 MED-HIGH	1160	F	1344	10.18	112.00	12.0	17.3	11/06/73	09/22/81		94.5	Bone Tumor, Lung Tumor
D-5 MED-HIGH	1211	M	1764	11.06	121.66	14.5	17.6	02/26/74	05/17/82		98.6	Bone Tumor
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	04/24/81		85.9	Bone Tumor
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	15.88	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	11/01/79		80.3	Bone Tumor, Adrenal Tumor
D-6 HIGH	1002	M	2907	18.88	207.64	14.0	19.6	01/18/73	01/21/80		84.1	Bone Tumor, Lung Tumor
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	Lng 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

* Indicates age in months since birth, all other ages are in months since exposure.

INHALED PLUTONIUM NITRATE IN DOGS

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		9/30/82	DEATH	
CONTROL	1356	M	0	0.00	0.00					100.7*		
CONTROL	1365	M	0	0.00	0.00					100.6*		
CONTROL	1376	F	0	0.00	0.00				05/11/80		70.8*	Pneumonia
CONTROL	1388	M	0	0.00	0.00				09/11/81		86.7*	Sacrificed
CONTROL	1393	M	0	0.00	0.00					99.3*		
CONTROL	1405	M	0	0.00	0.00					98.9*		
CONTROL	1409	M	0	0.00	0.00					98.8*		
CONTROL	1418	M	0	0.00	0.00					98.5*		
CONTROL	1425	M	0	0.00	0.00				08/02/82		96.5*	Status Epilepticus
CONTROL	1450	F	0	0.00	0.00				11/04/81		87.4*	Sacrificed
CONTROL	1455	F	0	0.00	0.00					97.8*		
CONTROL	1483	F	0	0.00	0.00					96.9*		
CONTROL	1509	M	0	0.00	0.00					96.1*		
CONTROL	1516	F	0	0.00	0.00					95.8*		
CONTROL	1525	M	0	0.00	0.00					95.6*		
CONTROL	1526	M	0	0.00	0.00					95.6*		
CONTROL	1528	F	0	0.00	0.00					95.0*		
CONTROL	1543	M	0	0.00	0.00					94.9*		
CONTROL	1563	F	0	0.00	0.00					84.8*		
CONTROL	1572	F	0	0.00	0.00					84.7*		
CONTROL	1577	M	0	0.00	0.00					84.7*		
CONTROL	1584	F	0	0.00	0.00					84.6*		
CONTROL	1594	F	0	0.00	0.00					84.6*		
CONTROL	1608	M	0	0.00	0.00					84.3*		
CONTROL	1633	F	0	0.00	0.00					77.6*		
CONTROL	1638	F	0	0.00	0.00					77.3*		
VEHICLE	1361	M	0	0.00	0.00	8.5	21.0	02/13/76		79.5		
VEHICLE	1381	F	0	0.00	0.00	9.5	19.8	02/13/76		79.5		
VEHICLE	1392	M	0	0.00	0.00	13.0	22.0	04/22/76		77.3		
VEHICLE	1406	M	0	0.00	0.00	13.5	21.6	04/22/76		77.3		
VEHICLE	1412	F	0	0.00	0.00	9.0	19.0	02/13/76		79.5		
VEHICLE	1421	M	0	0.00	0.00	13.0	23.3	06/23/76		75.2		
VEHICLE	1457	F	0	0.00	0.00	12.0	20.6	04/22/76		77.3		
VEHICLE	1491	F	0	0.00	0.00	8.0	21.6	06/23/76		75.2		
VEHICLE	1504	F	0	0.00	0.00	10.0	20.9	06/23/76		75.2		
VEHICLE	1514	M	0	0.00	0.00	14.0	20.9	06/23/76	08/06/82		73.4	Malignant Lymphoma
VEHICLE	1524	M	0	0.00	0.00	12.0	21.5	07/27/76		74.1		
VEHICLE	1531	F	0	0.00	0.00	9.0	20.9	07/27/76		74.1		
VEHICLE	1542	M	0	0.00	0.00	12.0	20.8	07/27/76		74.1		
VEHICLE	1566	M	0	0.00	0.00	14.0	18.3	03/15/77		66.5		
VEHICLE	1578	M	0	0.00	0.00	10.5	18.2	03/15/77		66.5		
VEHICLE	1593	F	0	0.00	0.00	11.0	18.0	03/15/77		66.5		
VEHICLE	1601	F	0	0.00	0.00	8.5	18.0	03/15/77		66.5		

* Indicates age in months since birth, all other ages are in months since exposure.

INHALED PLUTONIUM NITRATE IN DOGS

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION NCI	NCI/G LUNG	NCI/ KG	INHALATION EXPOSURE WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	MONTHS SINCE INHALATION 9/30/82	DEATH	COMMENTS ON DEAD DOGS
VEHICLE	1620	M	0	0.00	0.00	12.0	21.1	12/01/77		58.0		
VEHICLE	1634	F	0	0.00	0.00	10.5	19.6	12/01/77		58.0		
VEHICLE	1651	F	0	0.00	0.00	11.0	19.2	12/01/77		58.0		
D-1 LOWEST	1416	M	0	0.00	0.00	12.0	22.1	05/20/76		76.4		
D-1 LOWEST	1458	F	0	0.00	0.00	10.5	21.5	05/20/76		76.4		
D-1 LOWEST	1489	F	0	0.00	0.00	8.5	20.5	05/20/76		76.4		
D-1 LOWEST	1501	M	0	0.00	0.00	14.0	20.4	05/20/76		76.4		
D-1 LOWEST	1515	M	0	0.00	0.00	13.5	19.8	05/20/76		76.4		
D-1 LOWEST	1573	M	0	0.00	0.00	11.5	19.4	04/19/77		65.4		
D-1 LOWEST	1581	M	0	0.00	0.00	16.5	19.3	04/19/77		65.4		
D-1 LOWEST	1596	M	0	0.00	0.00	14.0	19.2	04/19/77		65.4		
D-1 LOWEST	1600	F	1	0.01	0.11	11.0	19.2	04/19/77		65.4		
D-1 LOWEST	1603	M	2	0.01	0.12	14.0	19.2	04/19/77		65.4		
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		76.4		
D-1 LOWEST	1570	F	2	0.02	0.17	10.5	19.4	04/19/77		65.4		
D-1 LOWEST	1465	F	4	0.03	0.35	12.0	21.0	05/20/76		76.4		
D-1 LOWEST	1470	F	3	0.03	0.29	10.5	21.0	05/20/76		76.4		
D-1 LOWEST	1507	M	4	0.03	0.32	14.0	19.8	05/20/76		76.4		
D-1 LOWEST	1592	F	4	0.03	0.29	13.5	19.2	04/19/77		65.4		
D-1 LOWEST	1607	M	5	0.03	0.35	13.0	19.0	04/19/77		65.4		
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		76.4		
D-1 LOWEST	1583	F	4	0.04	0.40	9.5	19.2	04/19/77		65.4		
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		65.4		
D-2 LOW	1513	M	0	0.00	0.00	11.5	19.8	05/20/76		76.4		
D-2 LOW	1520	M	1	0.01	0.12	10.5	19.5	05/20/76		76.4		
D-2 LOW	1415	M	2	0.02	0.20	11.5	22.2	05/20/76		76.4		
D-2 LOW	1575	M	3	0.02	0.19	14.0	19.4	04/19/77		65.4		
D-2 LOW	1466	F	5	0.03	0.37	14.0	21.0	05/20/76		76.4		
D-2 LOW	1606	F	5	0.04	0.42	12.5	19.0	04/19/77		65.4		
D-2 LOW	1579	M	8	0.05	0.59	14.0	19.3	04/19/77		65.4		
D-2 LOW	1590	F	6	0.05	0.51	12.0	19.2	04/19/77		65.4		
D-2 LOW	1585	F	8	0.06	0.68	12.0	19.2	04/19/77		65.4		
D-2 LOW	1580	F	9	0.07	0.82	11.0	19.3	04/19/77		65.4		
D-2 LOW	1591	M	11	0.07	0.76	15.0	19.2	04/19/77		65.4		
D-2 LOW	1417	M	11	0.08	0.89	12.0	22.1	05/20/76		76.4		
D-2 LOW	1423	M	10	0.08	0.87	11.0	22.1	05/20/76		76.4		
D-2 LOW	1567	M	10	0.08	0.83	12.0	19.4	04/19/77		65.4		
D-2 LOW	1472	F	10	0.09	1.01	10.0	21.0	05/20/76		76.4		
D-2 LOW	1503	F	9	0.09	0.97	9.0	19.8	05/20/76		76.4		
D-2 LOW	1602	M	15	0.09	1.03	14.5	19.2	04/19/77		65.4		

* Indicates age in months since birth, all other ages are in months since exposure.

INHALED PLUTONIUM NITRATE IN DOGS

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION NCI	NCI/G LUNG	NCI/ KG	INHALATION EXPOSURE WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	MONTHS SINCE INHALATION 9/30/82	DEATH	COMMENTS ON DEAD DOGS
D-2 LOW	1484	F	11	0.10	1.08	10.0	20.5	05/20/76		76.4		
D-2 LOW	1599	F	10	0.10	1.14	9.0	19.2	04/19/77		65.4		
D-2 LOW	1490	F	16	0.15	1.65	9.5	20.5	05/20/76		76.4		
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	03/24/82		60.3	Sacrificed
D-3 MED-LOW	1386	M	34	0.21	2.36	14.5	22.0	04/20/76		77.3		
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	01/20/76		80.3		
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		66.5		
D-3 MED-LOW	1595	M	50	0.29	3.23	15.5	18.0	03/15/77		66.5		
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		77.3		
D-3 MED-LOW	1587	M	53	0.31	3.40	15.5	18.1	03/15/77		66.5		
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		74.3		
D-3 MED-LOW	1344	F	41	0.33	3.60	11.5	17.2	10/16/75	11/14/75		1.0	Sacrificed
D-3 MED-LOW	1589	F	41	0.34	3.75	11.0	18.0	03/15/77	06/08/82		62.8	Sacrificed, Lung Tumor
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	4.08	10.5	20.8	07/22/76	10/19/76		2.9	Sacrificed
D-3 MED-LOW	1574	M	46	0.38	4.21	11.0	18.2	03/15/77		66.5		
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		77.3		
D-3 MED-LOW	1439	F	53	0.42	4.61	11.5	21.0	04/20/76		77.3		
D-3 MED-LOW	1523	F	55	0.42	4.60	12.0	21.3	07/22/76		74.3		
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		80.3		
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		66.5		
D-3 MED-LOW	1576	M	70	0.53	5.86	12.0	18.2	03/15/77	03/17/82		60.1	Sacrificed
D-3 MED-LOW	1582	F	57	0.54	5.96	9.5	18.1	03/15/77		66.5		
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		77.3		
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		80.3		
D-3 MED-LOW	1604	M	85	0.74	8.10	10.5	18.0	03/15/77		66.5		
D-3 MED-LOW	1530	F	72	0.76	8.41	8.5	20.8	07/22/76		74.3		
D-3 MED-LOW	1456	F	61	0.79	8.68	7.0	20.5	04/20/76		77.3		
D-3 MED-LOW	1598	F	93	1.06	11.65	8.0	18.0	03/15/77	03/10/82		59.8	Sacrificed
D-3 MED-LOW	1422	F	99	1.12	12.35	8.0	18.1	01/20/76		80.3		
D-4 MEDIUM	1637	M	192	1.45	15.99	12.0	18.9	11/07/77		58.7		

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INHALED PLUTONIUM NITRATE IN DOGS

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION NCI	NCI/G LUNG	NCI/ KG	INHALATION EXPOSURE WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	MONTHS SINCE INHALATION 9/30/82	DEATH	COMMENTS ON DEAD DOGS
D-4 MEDIUM	1404	M	260	1.48	16.25	16.0	21.5	04/20/76		77.3		
D-4 MEDIUM	1521	F	205	1.49	16.37	12.5	21.3	07/22/76		74.3		
D-4 MEDIUM	1656	M	211	1.54	16.90	12.5	18.4	11/07/77		58.7		
D-4 MEDIUM	1379	M	278	1.74	19.16	14.5	19.1	01/20/76		80.3		
D-4 MEDIUM	1362	M	267	1.87	20.54	13.0	20.2	01/20/76		80.3		
D-4 MEDIUM	1639	F	248	2.05	22.57	11.0	18.5	11/07/77		58.7		
D-4 MEDIUM	1647	M	294	2.05	22.58	13.0	18.5	11/07/77		58.7		
D-4 MEDIUM	1640	M	307	2.06	22.71	13.5	18.5	11/07/77		58.7		
D-4 MEDIUM	1645	F	257	2.13	23.39	11.0	18.5	11/07/77		58.7		
D-4 MEDIUM	1534	M	295	2.14	23.57	12.5	20.8	07/22/76		74.3		
D-4 MEDIUM	1414	F	233	2.35	25.86	9.0	18.2	01/20/76		80.3		
D-4 MEDIUM	1618	F	277	2.40	26.36	10.5	20.3	11/07/77		58.7		
D-4 MEDIUM	1385	M	373	2.42	26.63	14.0	19.0	01/20/76		80.3		
D-4 MEDIUM	1408	F	331	2.62	28.77	11.5	18.5	01/20/76		80.3		
D-4 MEDIUM	1428	F	378	3.12	34.36	11.0	21.1	04/20/76		77.3		
D-4 MEDIUM	1535	F	345	3.13	34.48	10.0	20.7	07/22/76		74.3		
D-4 MEDIUM	1446	F	354	3.22	35.40	10.0	21.0	04/20/76		77.3		
D-4 MEDIUM	1364	M	463	3.24	35.65	13.0	20.2	01/20/76		80.3		
D-4 MEDIUM	1387	F	345	4.48	49.30	7.0	19.0	01/20/76	08/13/80		54.8	Bone Tumor
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		58.7		
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	1659	F	990	7.20	79.22	12.5	18.3	11/07/77		58.7		
D-5 MED-HIGH	1636	M	1212	8.48	93.25	13.0	18.9	11/07/77		58.7		
D-5 MED-HIGH	1621	M	1334	8.66	95.26	14.0	20.3	11/07/77		58.7		
D-5 MED-HIGH	1646	F	1061	8.77	96.45	11.0	18.5	11/07/77		58.7		
D-5 MED-HIGH	1429	M	1376	9.62	105.85	13.0	23.2	06/23/76	05/29/81		59.2	Bone Tumor, Lung Tumor
D-5 MED-HIGH	1641	M	1275	9.66	106.24	12.0	18.5	11/07/77		58.7		
D-5 MED-HIGH	1660	M	1518	10.22	112.41	13.5	18.3	11/07/77		58.7		
D-5 MED-HIGH	1508	M	1716	10.76	118.37	14.5	20.9	06/23/76	01/24/80		43.0	Bone Tumor
D-5 MED-HIGH	1655	M	1094	11.05	121.56	9.0	18.4	11/07/77		58.7		
D-5 MED-HIGH	1652	F	1320	12.00	131.95	10.0	18.4	11/07/77		58.7		
D-5 MED-HIGH	1619	F	1490	12.32	135.50	11.0	20.3	11/07/77		58.7		
D-5 MED-HIGH	1512	M	2411	14.61	160.71	15.0	20.9	06/23/76	12/23/79		42.0	Bone Tumor
D-5 MED-HIGH	1419	M	1559	14.92	164.11	9.5	23.3	06/23/76		75.2		
D-5 MED-HIGH	1498	F	2018	16.68	183.45	11.0	21.5	06/23/76	04/09/82		69.5	Bone Tumor, Lung Tumor
D-5 MED-HIGH	1502	F	3008	20.25	222.80	13.5	20.9	06/23/76	01/21/81		55.0	Bone Tumor, Lung Tumor
D-5 MED-HIGH	1485	F	2330	21.18	233.00	10.0	21.7	06/23/76	12/30/80		54.2	Bone Tumor
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	10/16/80		51.8	Bone Tumor
D-5 MED-HIGH	1459	F	2645	26.72	293.89	9.0	22.6	06/23/76	09/25/80		51.1	Rad. Pneumonitis, Lng Tmr
D-6 HIGH	1518	M	3565	29.46	324.09	11.0	20.6	06/23/76	12/18/79		41.8	Rad. Pneumonitis, Lng Tmr

* Indicates age in months since birth, all other ages are in months since exposure.

INHALED PLUTONIUM NITRATE IN DOGS

DOSE GROUP	DOG NUMBER	SEX	INITIAL ALVEOLAR DEPOSITION NCI	NCI/G LUNG	NCI/ KG	INHALATION EXPOSURE WEIGHT (KG)	AGE* (MO)	DATE	DATE OF DEATH	MONTHS SINCE INHALATION 9/30/82	MONTHS SINCE INHALATION DEATH	COMMENTS ON DEAD DOGS
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

* Indicates age in months since birth, all other ages are in months since exposure.

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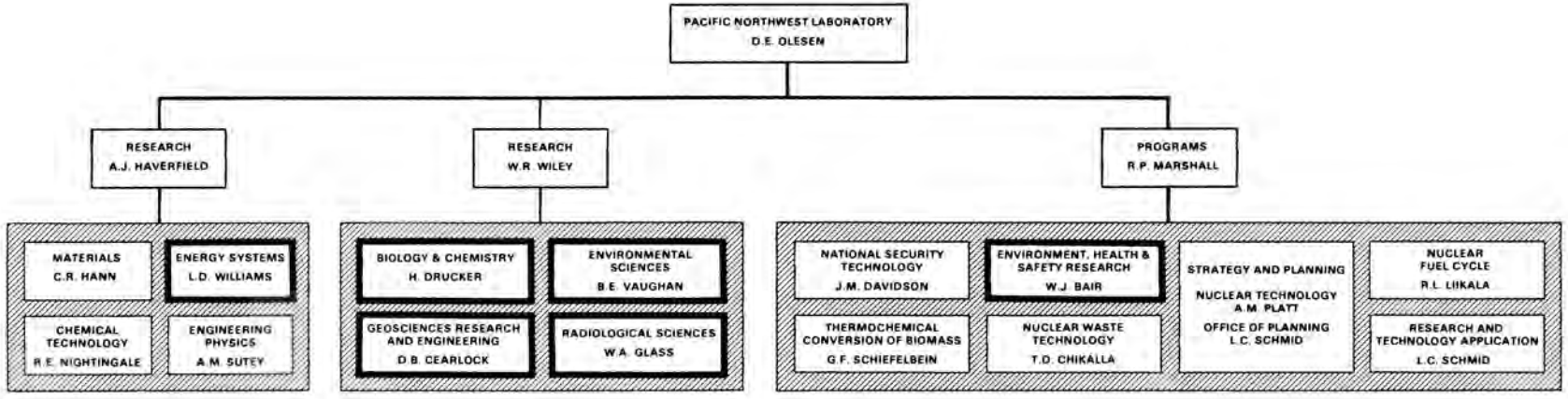

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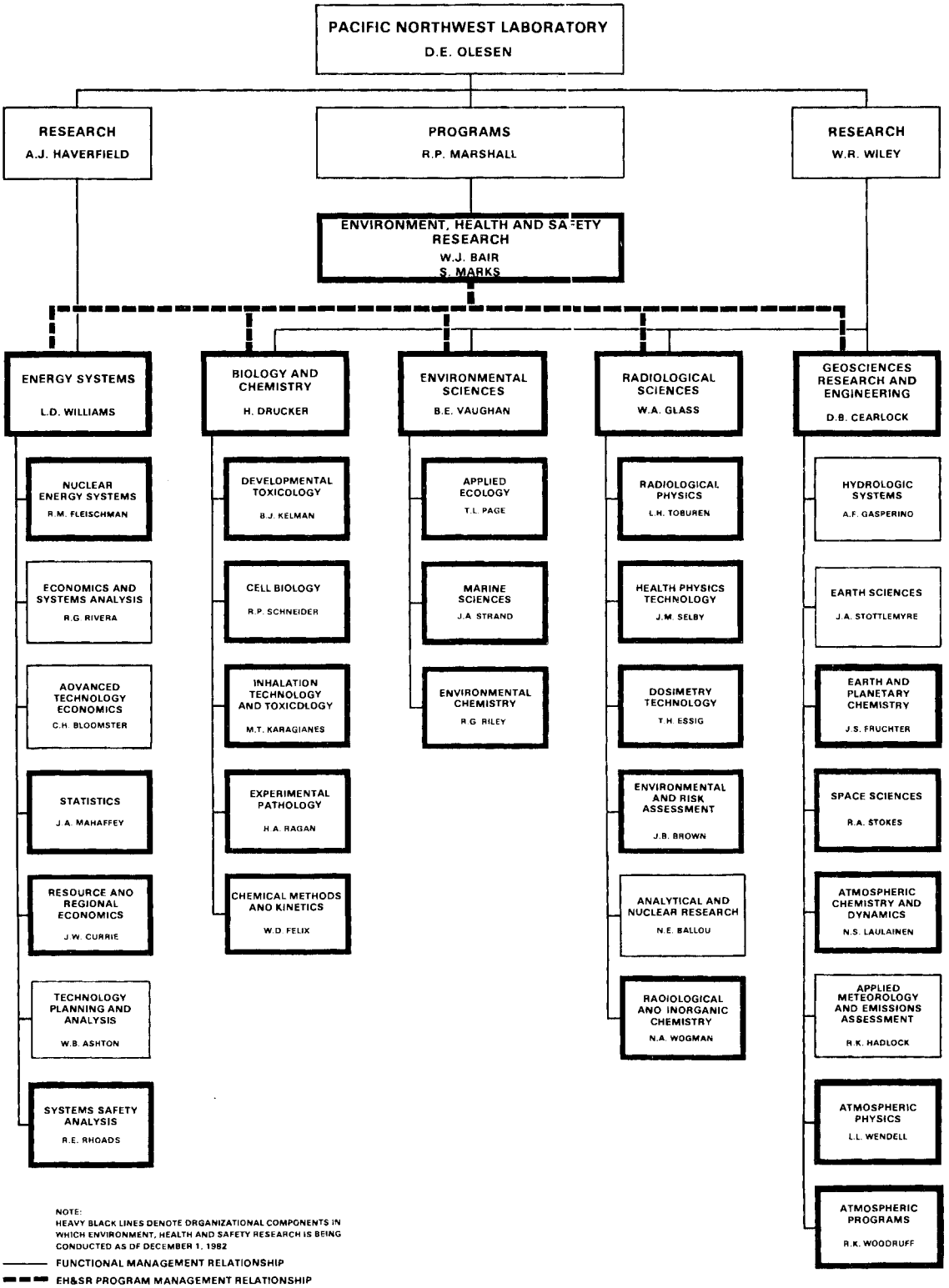
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