Pacific Northwest Laboratory Annual Report for 1986 to the DOE Office of Energy Research

**Part 1 Biomedical Sciences
February 1987**

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

**Pacific Northwest Laboratory
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J.F. Park and Staff

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Pacific Northwest Laboratory
Richland, Washington 99352

PREFACE

This 1986 annual report from Pacific Northwest Laboratory (PNL) to the Department of Energy (DOE) describes research in environment, health, and safety conducted during fiscal year 1986. 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 Assistant Secretary for Environment, Safety and Health. In some instances, the volumes report on research funded by other DOE components or by other governmental entities under interagency agreements. Each part consists of project reports authored by scientists from several PNL research departments, reflecting the multidisciplinary nature of the research effort.

The parts of the 1986 Annual Report are:

Part 1: Biomedical Sciences Program Manager - J. F. Park	D. L. Felton, Report Coordinator and Editor
Part 2: Environmental Sciences Program Manager - R. E. Wildung	R. E. Wildung, Report Coordinator K. A. Borgeson and S. G. Weiss, Editors
Part 3: Atmospheric Sciences Program Manager - C. E. Elderkin	C. E. Elderkin, Report Coordinator E. L. Owczarski, Editor
Part 4: Physical Sciences Program Manager - L. H. Toburen	L. H. Toburen, Report Coordinator P. L. Gurwell, Editor
Part 5: Nuclear and Operational Safety Program Managers - L. G. Faust W. E. Kennedy, Jr. J. M. Selby B. L. Steelman	L. G. Faust, Report Coordinator S. K. Ennor, 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.

A major effort was in response to OHER's request for assistance in collecting information on the release of radioactivity from the nuclear power plant accident at Chernobyl. Environmental and chemical scientists participated in collecting and analyzing atmospheric and environmental samples in Sweden, over the Pacific Ocean, along the northwest coast of North America and along the path of the radioactive plume as it passed across the United States.

Credit for this annual report goes to the 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.

Members of the Scientific Advisory Committee established last year are:

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1983	PNL-5000, Pt. 1-5
1984	PNL-5500, Pt. 1-5
1985	PNL-5750, Pt. 1-5

FOREWORD

This report summarizes progress on OHER biomedical and health-effects research conducted at PNL in FY 1986. The research develops the knowledge and scientific principles necessary to identify, understand and anticipate the long-term health consequences of energy-related radiation and chemicals. Our continuing emphasis is to decrease the uncertainty of health-effects risk estimates from existing and/or developing energy-related technologies through an increased understanding of how radiation and chemicals cause health effects.

The report is arranged to reflect the PNL research relative to OHER programmatic structure. The first section, on human health effects, concerns statistical and epidemiological methods for assessing health risks among nuclear workers. The next two sections, which contain reports of health-effects research in biological systems, include effects of radiation and of energy-related chemicals. The last section is related to medical applications of nuclear technology.

The section on human health effects reports analysis of data from an ongoing study of the mortality of Hanford workers and progress on development of statistical and epidemiological methods for assessing health risk from low-level radiation exposure. Death rates of Hanford workers from nearly all causes continue to be substantially lower than those of the general U.S. population, with no evidence of a positive correlation between radiation exposure and overall cancer mortality. Nor is there evidence of a positive correlation between radiation exposure and deaths from leukemia, the disease that has been most strongly linked with radiation in populations exposed to high levels of radiation. Cancer deaths among workers with confirmed plutonium deposition did not exceed that of workers without confirmed depositions. There is no evidence of increased lung-cancer risk with increasing radiation exposure, whether or not the analysis is controlled for smoking habits.

The section on health effects of radiation in biological systems contains results from inhalation dose-effect relationship studies with inhaled radionuclides. Lifespan studies in dogs with inhaled $^{239}\text{PuO}_2$, $^{238}\text{PuO}_2$, and $^{239}\text{Pu}(\text{NO}_3)_4$ are summarized to 15, 12, and 9 years after exposure, respectively. Lung cancer with inhaled $^{239}\text{PuO}_2$ and lung and bone cancer with inhaled $^{238}\text{PuO}_2$ and $^{239}\text{Pu}(\text{NO}_3)_4$ are the primary plutonium-exposure-related causes of death. Tissues from these dogs are being used to examine the role of oncogenes in plutonium-induced lung cancer and to determine whether there are tumor-specific changes in the DNA sequences of the genes associated with cancer causation. There is evidence that a specific oncogene, related to the *ras* gene, is activated in these tumors. Dose-effect relationship studies with inhaled $^{239}\text{PuO}_2$ in rats are in progress to obtain lung-tumor-incidence data at lifetime doses of 5 to 1500 rad. Thus far, the data suggest a threshold dose of about 100 to 200 rad for lung-tumor induction. Studies are also in progress in rats with inhaled radon daughters to determine the influence of dose and dose rate on lung-tumor incidence. Increased lung-tumor incidence has been observed in groups exposed to >320 working level month (WLM; see text) exposure. Lung-tumor incidence in the 100-WL dose rate groups was two to three times the incidence in the 1000-WL groups. In rats administered ^{232}U -nitrate, ^{228}Th decay progeny were retained more tenaciously than ^{232}U , suggesting

that ICRP-30 guidelines, which assume parent/daughter equilibrium, may seriously underestimate dose from decay progeny. Prenatal exposure of rats to citrated ^{233}U produced dose-dependent effects on the fetus which were attributable to chemical toxicity and maternal effects rather than to direct effects on the fetus. Studies on the mechanisms of gastrointestinal absorption in iron-deficient weanling rats suggest that pentavalent ^{237}Np and hexavalent ^{233}U utilize the iron transport system more efficiently than trivalent ^{241}Am and ^{244}Cm . This section also reports improved methods for aerosol generation, characterization and inhalation exposure of animals.

The section on health effects of energy-related chemicals reports mutational synergism between nonmutagenic aromatic chemical mixtures and several nonpolycyclic aromatic hydrocarbon (PAH) chemical mutagens. The synergism was maintained when the aromatic mixtures were replaced by individual nonmutagenic PAH. Studies to identify the influence of chemical class fractions from complex organic mixtures (COM) on the mouse-skin tumor-initiating activity of benzo[a]pyrene (BaP) demonstrated that the PAH and nitrogen-containing polycyclic aromatic compound fractions that contain structures similar to BaP were the most effective inhibitors; aliphatic and hydroxylated PAH were the least effective. Binding of BaP to mouse-skin DNA decreased in the presence of COM to a greater extent than did tumor-initiating activity. The biological half-life for BaP in mouse skin increased by a factor of 2 when it was administered with COM, and BaP was representative of other PAH fractions. Developmental studies demonstrated that both pregnant rats and mice treated dermally with high-boiling COM developed fetuses with major malformations at doses not unlike those anticipated from occupational exposures. The PAH fraction was responsible for all of the teratogenic activity. Adverse cardiovascular effects were observed following dermal exposure to a high-boiling COM but not after exposure to a middle-boiling-range material. Mice exposed by inhalation to a high-boiling COM developed lung and skin tumors after 4 and 12 weeks exposure. Initiation/promotion studies using the neonatal rat liver model indicated that animals responded in a dose-dependent manner to initiation by diethylnitrosamine and that females were more sensitive than males to promotion by COM. In vivo metabolism investigations have shown that the rates of removal of individual PAH from the blood are reduced when the compounds are co-administered with a COM. This co-administration also reduced the rates of exhalation of these compounds and the breath/blood partition coefficients.

Research on medical applications of a portable blood irradiator suggests that the irradiator may positively affect the outcome of kidney transplants.

This health-effects research is an interdisciplinary effort requiring scientific contributions from many research departments at PNL. The personnel in the Biology and Chemistry Department are the principal contributors to this report. Requests for reprints from the list of publications for 1986 will be honored while supplies last.

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- **Statistical Health Effects Studies**

Principal Investigator: E. S. Gilbert

Other Investigators: J. A. Buchanan, S. Marks, and R. G. Stevens

The objectives of this program are to provide statistical and epidemiological methods for assessing health risks from low-level exposures and to apply these methods to data on relevant human populations. A major component of this study is the analysis of data from an ongoing study of the mortality of Hanford workers. In the past year, updated analyses of deaths occurring through 1981 have been completed, data from a lung-cancer case-control study have been analyzed, and a statistical investigation of the effects of dose measurement errors on risk assessment has been conducted. In addition, a biological rationale for an epidemiological study of breast cancer and residential exposure to electric and magnetic fields has been developed.

The purpose of this project is to provide the data and methodology required to evaluate the impact of energy-related activities on health. This has been accomplished primarily by analyzing mortality data on Hanford workers, particularly in relation to the radiation exposure they have received. Methodological issues that must be dealt with in this and similar studies include analyzing exposures that change with time, quantifying risks and their uncertainty, and assessing the possible impact of exposure measurement errors.

Hanford worker mortality data, including deaths up to January 1, 1982, have been analyzed. As in earlier analyses, the relationship of cumulative radiation exposure to death rates from several diseases has been examined; in addition, death rates have been compared with those of the general U.S. population. Several aspects of these data have been analyzed in greater detail than previously. These include consideration of data on female workers, the limited internal depositions of radionuclides, and deaths occurring in the states of Washington and California in the years 1982-1984. Estimates and confidence limits for risks have also been calculated, based on methods developed previously.

As in earlier analyses based on deaths occurring up to January 1, 1979, Hanford workers continue to show a strong healthy-worker effect; death rates from nearly all causes are substantially lower than those of the general U.S. population. Based on internal comparisons, which provide the most meaningful analyses of the effects of occupational radiation exposure, there is no evidence of a positive correlation between radiation exposure and overall cancer mortality. The data were analyzed with several different choices of controlling factors, and in no case was the corre-

lation in the positive direction. The estimate of risk is negative, but the upper confidence limit for risk is positive, indicating that risks on the order of 5 to 10 times estimates given by the International Commission on Radiation Protection (ICRP) or by the BEIR III committee are consistent with the Hanford data.

Nor is there evidence of a positive correlation between radiation exposure and death from leukemia, the disease that has been most strongly linked with radiation in studies of populations exposed to high levels of radiation. For leukemia, the risk estimate is also negative, and the upper confidence limit is only slightly larger than risk estimates provided by ICRP and BEIR III. Difficulties related to comparability of dosimetry for Hanford workers and the Japanese atomic bomb survivors, which form the basis for estimates provided by ICRP and BEIR III, prevent a precise comparison of estimates and confidence limits.

The summary of results given above is based on analyses that included both male and female workers. However, since most of the radiation exposure has been received by males, results for males dominate the findings. Results of analyses of females alone are different from those of males; they show tentative evidence ($0.05 < P < 0.10$) of a correlation between cancer mortality and radiation exposure. However, if one takes into account that at least two tests (males and females) must be conducted to obtain this correlation, the revised p-value exceeds 0.10. Although it might be argued that females are more sensitive to radiation than males, the assumption of reasonable values for this differential sensitivity does not lead to an overall correlation, based on both males and females, that even approaches statistical significance. It is, perhaps, worth noting that breast

cancer shows no evidence of a correlation with radiation exposure in Hanford female workers. For the reasons noted, the correlation of all cancers in female workers with radiation exposure appears to be spurious, or it may result from some other factor associated with cumulative radiation exposure in females.

Data on internal depositions are limited in that there are only 457 workers with confirmed plutonium depositions. The number of cancer deaths in these workers does not exceed that expected, based on Hanford workers without confirmed depositions. Nor does the number of cancer deaths in workers with depositions greater than 5% of a maximum permissible body burden exceed expectation.

Because the workers with depositions tend to have larger external exposures than other workers, these exposures could confound analyses of external exposure. For this reason, an analysis of external exposure was conducted, excluding workers with internal depositions. Results of this analysis did not differ in any important way from analyses in which these workers were included.

Recent linkage with death files in the states of Washington and California have provided an opportunity to evaluate the completeness of Social Security Administration's death ascertainment. Overall, the Social Security Administration has identified over 97% of the deaths occurring in Washington. There is no strong trend in ascertainment associated with radiation exposure or other variables examined. About half of all Hanford worker deaths occur in the state of Washington, with a much higher proportion for those in higher exposure categories. Ascertainment for Washington is probably very close to 100% complete.

Because of the linkage noted above, information on deaths occurring in the states of Washington and California is complete 3 or 4 years sooner than information on deaths occurring in other states. These deaths cannot be analyzed with cohort-based methods, since it is not known which workers are at risk of dying in Washington and California. However, by using proportional mortality analyses for the 1982-1984 deaths, they can be included in the overall results. This has not modified results greatly except for an additional death, with a relatively high exposure, due to multiple myeloma.

Multiple myeloma continues to exhibit a statistically significant correlation with radiation exposure, based on three

deaths with relatively high exposures that occurred before January 1, 1982. The additional high-exposure death noted above, which occurred in 1984 in Washington, as well as a reported association between radiation exposure and multiple myeloma among workers at the Sellafield plant of British Nuclear Fuels (Smith and Douglas 1986), strengthens the case for interpreting the multiple myeloma correlation in Hanford workers as a cause-and-effect relationship.

Analyses of the lung-cancer case-cohort study have been completed. In this study, smoking histories were extracted from Hanford Environmental Health Foundation (HEHF) files for lung-cancer deaths that met certain criteria and from a stratified random control sample of workers who met the same criteria. The objectives of this study are to examine the association between lung-cancer mortality and radiation exposure with allowance for smoking and to examine the association of smoking with variables such as radiation exposure and job category.

Data on smoking have been routinely collected as a part of employee medical examinations at HEHF since 1964. Because many workers had several years of smoking data, based on responses to different questionnaires used in various years, it was necessary to develop a procedure for summarizing this information appropriately. The validity of the smoking data was checked by calculating relative risk estimates for several smoking status categories and comparing them with results obtained from other studies of smoking and lung cancer. The effect of smoking on lung-cancer risk in Hanford workers was found to be quite comparable to that reported in the U.S. Surgeon General's Report. The prevalence of smoking and the amount smoked are also comparable to values reported in other studies.

Controlling for smoking habits in analyses of the association of lung cancer and radiation exposure did not greatly modify the results obtained when no such control was included. There is no evidence of increased lung cancer risk with increasing radiation exposure, whether or not the analysis is controlled for smoking habits. However, workers in the "middle" exposure categories of about 2 to 15 rem do have higher lung-cancer risks than those with either less than 2 rem or greater than 15 rem. This result, obtained earlier, was interpreted as possibly reflecting differences in smoking habits. This explanation was not confirmed by the lung-cancer case-cohort study, and there is no indication in this

study that smoking habits vary greatly by radiation exposure.

An advantage of occupational studies of external radiation exposure for assessing radiation risks is that doses are probably more accurately measured than in many of the high-level studies. Thus, in order to fully assess the appropriate roles of high- and low-level studies for the purpose of risk estimation, it is important to understand the possible uncertainties introduced by errors in measuring doses. The effects of measurement error, when such errors are not taken into account in analyzing the data, have been statistically evaluated. For a wide range of assumptions, the most serious consequence of ignoring error is downward bias in estimating regression coefficients. In addition, the shape of the dose-response function may be distorted, and variances of estimated parameters may be underestimated. Except for the case of very large errors combined with highly skewed exposure distributions, tests of the null hypothesis of no effect that ignore error are found to be nearly as powerful as an optimal test that is available if the error structure is known. Results of this investigation have been submitted for publication.

In order to assess the specific effect of measurement error in doses of Hanford workers, the doses of workers who died of leukemia and those of an appropriate control group are being specially investigated. The objectives of this investigation are to gain a better understanding of errors in dosimetry and provide a more precise quantification of risks due to leukemia. Although there is no indication of a correlation of radiation exposure with death from leukemia in Hanford workers, we wish to use these data to ob-

tain upper limits on risks and to compare these limits with estimates and limits based on the Japanese atomic-bomb survivors and other populations exposed at high levels. A more precise comparison can be made if the best available dose estimates are used and possible dose measurement errors are taken into account.

A study of congenital malformations that occurred during the period 1957-1980 in the three hospitals in Richland, Kennewick and Pasco has been completed. Two papers describing the results of this study have been submitted for journal publication.

The biological rationale was developed for an epidemiological study of breast cancer and residential exposure to extremely low-frequency (ELF) electric and/or magnetic fields and exposure to light at night. A paper presenting this work will appear in the April, 1987, issue of the American Journal of Epidemiology. In addition, the design of the epidemiological study has been refined and updated as further work on the biology of the pineal gland has revealed important new biological considerations that should be incorporated in the human study. A study of about 700 incident cases of breast cancer identified in a population-based cancer registry and an equal number of controls has been proposed. Data would be collected on all of the known risk factors for breast cancer as well as on exposure to ELF fields and light at night. Meters capable of measuring ELF fields and the light level in the subject's bedroom at night would be used to provide more exposure data. In addition, questionnaire data on past residences and exposures to such sources as electric blankets would be gathered.

• Inhaled Plutonium Oxide in Dogs

Principal Investigator: J. F. Park

Other Investigators: R. L. Buschbom, G. E. Dagle, D. R. Fisher, E. S. Gilbert, J. D. Kaschmitter, G. J. Powers, H. A. Ragan, C. O. Romsos, R. E. Weller, and E. L. Wierman

Technical Assistance: K. H. Debban, R. F. Flores, B. B. Kimsey, B. G. Moore, R. P. Schumacher, M. J. Steele, N. B. Valentine, and D. H. Willard

This project is concerned with long-term experiments to determine the lifespan dose-effect relationships of inhaled $^{239}\text{PuO}_2$ or $^{238}\text{PuO}_2$ in beagles. The data will be used to estimate the health effects of inhaled transuranics. Beagle dogs given a single exposure to $^{239}\text{PuO}_2$ or $^{238}\text{PuO}_2$ aerosols to obtain graded levels of initial lung burdens (ILB) are being observed for lifespan dose-effect relationships. Mortality due to radiation pneumonitis and lung tumor increased in the four highest dose-level groups exposed to $^{239}\text{PuO}_2$ during the 15-year postexposure period. During the 12 1/2 years after exposure to $^{238}\text{PuO}_2$, mortality due to lung and/or bone tumors increased in the three highest dose-level groups. Chronic lymphopenia, occurring 0.5 to 2 years after exposure, was the earliest observed effect after inhalation of either $^{239}\text{PuO}_2$ or $^{238}\text{PuO}_2$ in the four highest dose-level groups that had ILB of ≥ 80 nCi. Other plutonium-exposure-related effects include sclerosis of the tracheobronchial lymph nodes, focal radiation pneumonitis, adenomatous hyperplasia of the liver, and dystrophic osteolytic lesions in the skeleton.

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

One hundred thirty dogs exposed to $^{239}\text{PuO}_2$ in 1970 and 1971 were selected for long-term studies; 14 were sacrificed to obtain plutonium distribution and pathology data;

116 were assigned to lifespan dose-effect studies (Table 1). One hundred sixteen dogs exposed to $^{238}\text{PuO}_2$ in 1973 and 1974 were selected for lifespan dose-effect studies (Table 2). Twenty-one 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 15 years after exposure to $^{239}\text{PuO}_2$. During this period, all of the dogs in the highest-level dose group and in Dose-Level Groups 5 and 4, 15 in Group 3, 18 in Group 2, 22 in Dose-Level Group 1 and 16 in the control group were euthanized when death was imminent.

TABLE 1. Lifespan Dose-Effect Studies with Inhaled $^{239}\text{PuO}_2$ in Beagles.^(a)

Dose Level Group	Number of Dogs		Initial Lung Deposition ^(b)	
	Male	Female	nCi ^(c)	nCi/g Lung ^(c)
Control	10	10	0	0
1	12	12	3.5 ± 1.3	0.029 ± 0.011
2	10	11	22 ± 4	0.18 ± 0.04
3	10	10	79 ± 14	0.66 ± 0.13
4	11	11	300 ± 62	2.4 ± 0.4
5	10	11	1100 ± 170	9.3 ± 1.4
6	3	5	5800 ± 3300	50 ± 22
	66	70		

^(a) Exposed in 1970 and 1971

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

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

TABLE 2. Lifespan Dose-Effect Studies with Inhaled $^{238}\text{PuO}_2$ in Beagles. (a)

Dose Level Group	Number of Dogs		Initial Lung 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	11	10	18 ± 3	0.15 ± 0.03
3	12	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
	70	66		

(a) Exposed in 1973 and 1974

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

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

TABLE 3. Summary of Lesions in Dogs Euthanized During the 15-yr Period After Inhalation of $^{239}\text{PuO}_2$.

	Dose Group						
	6	5	4	3	2	1	Control
Number of Dogs/Group	8	21	22	20	21	24	20
Number of Dead Dogs/Group	8	21	22	15	18	22	16
Mean Survival Postexposure, yr	2	6	10	12	13	12	12
Condition ^(a)							
Radiation Pneumonitis	7	1					
Radiation Pneumonitis and Lung Tumor	1						
Lung Tumor		20	14	5	2		4
Lung Tumor and Bile Duct Carcinoma			1				
Malignant Lymphoma and Lung Tumor				2			
Nephropathy and Lung Tumor			1	1	1		
Bone Tumor					1	2	
Malignant Lymphoma				1		4	2
Malignant Lymphoma and Bile Duct Carcinoma							1
Hemangiosarcoma (Heart, Spleen, Liver)						3	2
Pituitary Tumor, Cushing's			1			1	
Reticulum Cell Sarcoma			1				
Ovarian Tumor					1		
Oral Tumor							1
Round Cell Sarcoma and Bile Duct Adenoma						1	
Hemangioma (Spleen)					1		
Malignant Melanoma					1		1
Pheochromocytoma					1		
Urinary Bladder Tumor				1	2		
Neurofibrosarcoma				1			
Meningioma						1	
Pneumonia			2	1	3	4	
Epilepsy					1	1	1
Thromboembolism				1			1
Pyometra			1	1			
Unknown					1	1	
Liver Cirrhosis			1				
Septicemia						1	
Cardiac Insufficiency				1	1		
Peritonitis					1		
Adrenitis							1
Kidney Failure						1	
Nephrosclerosis							1
Chronic Nephropathy					1	1	1
Glomerulosclerosis						1	

(a) Number of dogs with lesion associated with death

Mean survival time was decreased in the three highest dose-level groups compared to that in the other groups. Fourteen

dogs were sacrificed for comparison of plutonium tissue distribution. Table 4 shows the primary cause of death and the

distribution of ^{239}Pu in the tissues of these animals as percent of final body burden. Figure 1 shows the plutonium tissue distribution as percent of initial lung burden (ILB).

Table 4 indicates that, as survival time increased, the fraction of plutonium in the lung decreased to 16% of the final body burden by 14 to 15 years after exposure. During the first year after exposure, plutonium was translocated primarily to the thoracic lymph nodes; little plutonium was translocated to other tissues. Plutonium content of the thoracic lymph nodes increased to 64% of the final body burden at 14 to 15 years after exposure; the abdominal lymph nodes, principally the hepatic nodes, contained ~4%. The fraction of plutonium in liver increased, accounting for 25% of the final body burden at 14 to 15 years after exposure in the higher (≥ 75 nCi final body burden)-dose-level groups. The organ distribution of plutonium in the periodically sacrificed dogs was generally similar to that of the higher-dose-level dogs euthanized when death was imminent during the first 2 years after exposure. The lower-dose-level (≤ 75 nCi final body burden) dogs sacrificed or euthanized during the 4th to 15th postexposure years generally 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 1% of the final body burden was in the skeleton at 14 to 15 years after exposure. Plutonium analyses are still in progress on one of the dogs.

Figure 1 shows the ^{239}Pu tissue distribution as percent of the ILB for all dogs for which tissue radiochemical analyses are complete. The ILB for those dogs for which radiochemical analysis of excreta were not complete were estimated from external thorax counts at 14 and 30 days after exposure. For dogs whose analyses were complete, ILB were estimated from the summation of the tissue burdens of plutonium, plus the plutonium excreted, minus plutonium excreted in the feces during the first 3 days after exposure. The latter was assumed to be deposited in the upper respiratory tract. Uptake and retention functions were fitted to the organ burden data. Based on the premise that the organ burdens were interrelated, the uptake and retention function for all organs was fitted simultaneously instead of fitting isolated functions for each organ. The organs were treated as compartments of a single system, with transfer rates specifying the total amount, leaving a compartment per unit time and the fractional distribution of that amount among the other compartments. The transfer rates assumed that plutonium moved through the body in a single pass. The material initially depos-

ited in the lung was either excreted or moved to some other organ, from which it was excreted. It was assumed that there were no feedback loops in the system. Organ systems included lung, thoracic lymph nodes, liver, skeleton, and all other tissues. The functions were estimated using weighted, nonlinear least squares. The weights were estimated by biweighting procedures that give the more extreme data values very little weight. The curves for liver were based on dogs with final body burdens ≥ 75 nCi because dogs with < 75 nCi had less plutonium translocated to the liver.

The nine dogs euthanized because of radiation pneumonitis during the 3-year post-exposure 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.

Forty-eight of the 98 exposed dogs euthanized 3 to 15 years after exposure had lung tumors. Radiographic evidence of pulmonary neoplasia frequently preceded development of respiratory insufficiency. In dogs with neoplasia in the lung, respiratory insufficiency, when it was observed, was usually a late clinical finding that occurred shortly before euthanasia. All of the exposed dogs with lung tumors were in Dose-Level Groups 2, 3, 4, 5, and 6. Two dogs in Dose Level 1 were euthanized 11.7 and 12.1 years, respectively, after exposure: one had an osteosarcoma involving the nasal cavity and maxilla; the other had a chondrosarcoma involving the nasal cavity. One dog in Dose Level 2, euthanized 12.8 years after exposure, had a multilobular sarcoma of the skull. Four control dogs were euthanized because of lung tumors. Dogs 794M, 803M, 809M, 824F, 833F, and 835F (Dose Level 4), 697M, 778M, 782M, 823M, 827F, 834F and 905F (Dose Level 3), 748F, 754M, 769F, 767M, 776M, 780F, 802M, 806F, 826F, 831F, 845F, 859M, 862M and 874M (Dose Level 2), and 756M, 770F, 788M, 807F, 825F, 832F, 841F, 847M, 853M, 867M, 870M, 875M, 879M, 886F, 891M, 893M, 899F, 900M, 907F, and 908M (Dose Level 1) died during the 7- to 15-year postexposure period of causes presently thought to be unrelated to plutonium exposure.

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

Dog Number	Time After Exposure, mo	Final Body Burden, μCi	Percent of Final Body Burden					Cause of Death
			Lungs	Thoracic Lymph Nodes ^(a)	Abdominal Lymph Nodes ^(b)	Liver	Skeleton	
478M	0.25	0.293	98	0.15	0.02	0.24	6.18	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	30	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	Ovary 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

^(a) Includes tracheobronchial, mediastinal and sternal lymph nodes

^(b) Includes hepatic, splenic and mesenteric lymph nodes

TABLE 4. Continued.

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	
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, Addison's
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 and Bile Duct Adenoma
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
805F	134	0.169	5.8	55	8.9	21	2.8	Esophageal Leiomyoma, Lung Tumor
780F	135	0.0074	28	69	0.37	0.02	0.79	Pheochromocytoma
905F	135	0.080	13	50	10	19	1.7	Malignant Lymphoma
825F	137	0.0020	9.5	85	0.74	0.54	2.7	Hemangiosarcoma, Spleen
764F	139	0.081	15	75	3.9	4.9	0.73	Lung Tumor
808F	139	0.206	11	30	1.8	53	3.0	Lung Tumor
806F	140	0.010	11	78	1.8	5.1	2.3	Malignant Melanoma, Palate
850F	140	0.00062	12	82	0.61	0.11	2.0	Bone Tumor
833F	143	0.157	3.1	40	22	31	1.1	Metritis, Adrenal and Thyroid Carcinoma
862M	145	0.0026	21	56	0.85	4.4	6.9	Peritonitis
904F	145	0.0013	8.9	87	0.30	0.88	1.0	Chondrosarcoma
756M	147	0.0016	15	75	1.0	1.6	4.1	Epilepsy
782M	148	0.043	12	72	4.9	9.0	0.86	Neurofibrosarcoma
886F	149	0.00085	13	51	15	3.6	13	Meningioma
795F	152	0.030	24	26	8.3	38	1.5	Lung Tumor
771F	153	0.019	20	71	1.0	5.8	1.1	Lung Tumor
813F	153	0.036	22	44	4.7	27	1.1	Multilobar Sarcoma, Skull
826F	153	0.0033	8.1	88	0.37	0.94	1.2	Hemangioma, Spleen
859M	154	0.048	19	31	29	7.3	0.79	Urinary Bladder Tumor
870F	154	0.00062	8.2	70	4.9	9.6	4.8	Pneumonia
879M	154	0.00093	19	75	0.52	0.81	1.6	Hemangiosarcoma
884M	155	0.077	13	45	9.4	30	1.6	Lung Tumor
831F	155	0.0087	24	71	0.65	3.3	1.0	Pneumonia
866M	156	0.145	15	41	9.3	34	0.20	Lung Tumor
823M	157	0.072	7.3	83	1.8	6.0	1.5	Urinary Bladder Tumor
838M	157	0.044	18.0	73	0.77	5.4	1.4	Malignant Lymphoma, Lung Tumor
788M	158	0.0022	22	70	2.0	1.8	0.11	Chronic Nephropathy
845F	158	0.012	28	69	0.25	1.5	0.63	Urinary Bladder Tumor
853M	158	0.0081	13	77	2.2	5.4	0.54	Bronchopneumonia
750M	161	0.071	20	51	13.0	9.5	2.4	Lung Tumor, Malignant Lymphoma
847M	163	0.00061	22	75	0.15	0.60	1.2	Kidney Failure
776M	163	0.0020	29	67	0.11	1.2	1.1	Bronchopneumonia
802M	164	0.019	13	45	33	6.7	1.3	Pneumonia
827F	164	0.075	4.5	49	17	27	1.5	Acute Pneumonia
874M	165	0.0048	5.6	90	0.54	1.4	0.56	Chronic Nephropathy
842M	166	0.0054	4.7	90	0.76	3.2	0.75	Lung Tumor, Chronic Nephropathy
770F	166	0.0023	17	80	0.15	0.69	0.52	Glomerulosclerosis
844F	170	0.097	19	50	8.9	19	1.3	Nephropathy, Lung Tumor
819F	170	0.085	18	42	4.2	30	3.0	Nephropathy, Lung Tumor
907F	174	0.00097	7.4	89	1.2	0.78	0.61	Pneumonia
876F	175	0.0080	10	80	1.8	6.1	0.88	Nephropathy, Lung Tumor
877F	175	0.011	13	79	2.2	3.8	0.70	Lung Tumor
867M	175	0.0027	23	52	5.8	16	1.6	Malignant Lymphoma
893M	177			-----Processing-----				Pneumonia
839F	177	0.105	6.4	53	11	27	2.0	Lung Tumor, Bile Duct Carcinoma
841F	178	0.0028	6.8	89	0.13	2.1	0.84	Malignant Lymphoma
832F	178	0.0020	8.3	87	0.17	1.5	0.88	Malignant Lymphoma
767M	180	0.0088	33	64	0.22	1.1	0.96	Valvular Endocardopathy

(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 $^{239}\text{PuO}_2$. Points represent data from individual dogs (● = ≥ 75 nCi, ○ = ≤ 75 nCi final body burden). The uptake and retention curves and function were based on dogs in which initial lung burdens were estimated from external thorax counts at 14 and 30 days after exposure. The curves for liver were based on dogs with final body burdens ≥ 75 nCi.

In 21 of the dogs, the lung tumors were classified as bronchiolar-alveolar carcinoma; in six dogs as adenosquamous carcinoma; in eight dogs, adenocarcinoma; in four dogs, epidermoid and adenocarcinoma; in three dogs, epidermoid carcinoma; in one dog, epidermoid and bronchiolar-alveolar carcinoma; in three dogs, adenocarcinoma and bronchiolar-alveolar carcinoma; in one dog, epidermoid carcinoma, adenocarcinoma, and bronchiolar-alveolar carcinoma; and in another dog, adenocarcinoma, adenosquamous carcinoma and bronchiolar-alveolar adenocarcinoma. The epidermoid carcinomas metastasized to the lungs, skeletons, brains, intestines and thoracic lymph nodes; the bronchiolar-alveolar carcinomas metastasized only to the thoracic lymph nodes in eight dogs, and to several organs (including mediastinum; kidney; thyroid; skeleton; heart; adrenal gland; aorta; and axillary, pre-scapular, cervical, splenic, thoracic, 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, hepatic, splenic, sternal and axillary lymph nodes; heart, kidney, and esophagus in five dogs.

The lung tumors in the control dogs were classified as bronchiolar-alveolar adenocarcinomas in two dogs with metastases to thoracic and abdominal lymph nodes, trachea, esophagus and mediastinum; adenocarcinoma with metastases to the diaphragm and abdominal lymph nodes in one dog; and combined epidermoid and adenocarcinoma with metastases to the thoracic lymph nodes, diaphragm, liver and kidney in another.

Three of the exposed 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 2, 3, 4, 5 and 6. There was also a generalized lymphoid atrophy that may be related, in the dogs with respiratory insufficiency, to debilitation or to lymphopenia. Livers of the dogs in Dose-Level Groups 4 and 5, which were euthanized during the 4- to 13-year 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.

Bile-duct tumors have been reported by other laboratories in beagles exposed to

plutonium. In our $^{239}\text{PuO}_2$ study, one Dose-Level 4 dog and one control dog had a bile-duct carcinoma as an incidental lesion not related to the death of the dog. One Dose-Level 1 dog had an incidental bile-duct adenoma. In the $^{238}\text{PuO}_2$ study, one Dose-Level 4 dog also had a bile-duct carcinoma as an incidental observation. These four are the total number of bile-duct tumors observed thus far in these studies.

Lymphopenia developed after inhalation of $^{239}\text{PuO}_2$ in dose-level groups with mean initial lung depositions of 79 nCi or more (Figure 2). Through 123 months 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 months 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 lung 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 $^{239}\text{PuO}_2$ 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 $^{239}\text{PuO}_2$.

Table 5 summarizes, by dose-level group, mortality and lesions associated with death through 12½ years after exposure to $^{238}\text{PuO}_2$. During this period, all of the dogs in the highest-level dose group and in Dose-Level Group 5, fourteen dogs in Group 4, eight dogs in Group 3, eight dogs in Group 2, and nine dogs in Dose-Level Group 1 were euthanized when death was imminent. Seven control dogs were euthanized during the 12½-year postexposure period. Mean survival time was decreased in the three highest dose-level groups compared to the other groups. Twenty-one dogs were sacrificed for comparison of plutonium tissue distribution. Table 6 shows the primary causes of death and the

distribution of ^{238}Pu in the tissues of these animals as percent of final body

burden. Figure 3 shows the plutonium tissue distribution as percent ILB.

FIGURE 2. Mean Leukocyte, Neutrophil and Lymphocyte Values in Dogs After Inhalation of $^{239}\text{PuO}_2$.

TABLE 5. Summary of Lesions in Dogs Euthanized During the 12½-yr Period After Inhalation of $^{238}\text{PuO}_2$.

	Dose Group						Control
	6	5	4	3	2	1	
Number of Dogs/Group	13	20	20	22	21	20	20
Number of Dead Dogs/Group	13	20	14	8	8	9	7
Mean Survival Postexposure, yr	5	7	11	12	12	12	12
Condition(a)							
Bone Tumor	2	11	4			1	
Lung Tumor	3			1			
Bone and Lung Tumor	6	4	2				
Bone Tumor and Bile Duct Carcinoma			1				
Bone Tumor and Addison's Disease	1						
Bone and Lung Tumor and Addison's Disease		1					
Addison's Disease	1	2					
Malignant Mesothelioma					1		
Malignant Lymphoma			1	3		2	3
Hemangioma; Spleen			1				
Hemangiosarcoma; Heart and Spleen			1		1		
Pituitary Tumor, Cushing's			1	1			1
Urinary Bladder Tumor			1				2
Brain and Heart Tumor						1	
Brain Tumor					1		
Parathyroid Adenoma				1			
Adrenal Carcinoma						1	
Round Cell Sarcoma; Kidney					1		
Adrenal and Pituitary Tumor			1				
Lung Tumor, Metastatic					1		
Pneumonia		1	1	1	2		
Radiation Pneumonitis				1			
Immune Hemolytic Anemia						1	
Spinal Cord Degeneration					1		
Pyometra							1
Herniated Vertebral Disk		1					
Anesthesia						1	
Liver Abscess						1	

(a) Number of dogs with lesion associated with death

At 11½ to 12½ years after exposure, the fraction of the final body burden in the lungs of the ^{238}Pu -exposed dogs was about 2%, compared to 12% in the ^{239}Pu -exposed dogs (Table 6). At that time, ~7% of the ^{238}Pu was in the thoracic lymph nodes, compared to 64% of the ^{239}Pu . Livers of the ^{238}Pu -exposed dogs contained 38% of the plutonium burden, compared to 14% in the livers of the ^{239}Pu -exposed dogs. About 46% of the final body burden was in the skeletons of the ^{238}Pu -exposed dogs, at that time, compared to ~2% 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. Plutonium analyses are still in progress on one dog.

Figure 3 shows the ^{238}Pu tissue distribution as percent of ILB for all dogs for which tissue radiochemical analyses are complete. The ILB and uptake and retention curves were estimated as described previously for ^{239}Pu . The uptake and retention curves were based on dogs in which ILB were estimated from the final plutonium body burden, plus the plutonium excreted, minus that excreted in the feces during the first 3 days after exposure.

Of the 72 exposed dogs euthanized, 33 were killed because of bone tumors, 4 because of lung tumors, and 1 because of radiation pneumonitis. Thirteen of the dogs that had bone tumors also had lung tumors.

TABLE 6. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{238}\text{PuO}_2$.

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

^(a)Includes tracheobronchial, mediastinal and sternal lymph nodes

^(b)Includes hepatic, splenic and mesenteric lymph nodes

TABLE 6. Continued.

Dog Number	Time After Exposure, mo	Final Body Burden, μ Ci	Percent of Final Body Burden					Cause of Death
			Lungs	Thoracic Lymph Nodes ^(a)	Abdominal Lymph Nodes ^(b)	Liver	Skeleton	
1063M	94	0.00060	3.4	15	1.3	22	43	Brain 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	Empyema, Pituitary Tumor, Cushing's
1192F	109	0.345	2.4	7.3	4.6	36	46	Bone Tumor
1178M	110	0.594	0.86	17	2.0	33	42	Bone Tumor, Lung Tumor
1047M	115	0.241	1.4	7.8	11	28	48	Herniated Vertebral Disc
1106F	117	0.0029	1.3	16	1.8	9.9	57	Adrenal Carcinoma
1103F	118	0.232	0.76	18	3.1	45	32	Bone Tumor, Lung Tumor
1188M	119	0.0089	0.71	2.5	0.94	68	24	Metastatic Lung Tumor
1066M	121	0.035	1.1	4.4	0.52	57	32	Malignant Lymphoma
1069F	121	0.0022	9.1	2.1	1.6	51	34	Malignant Lymphoma
1030F	122	0.160	1.5	15	1.1	22	56	Pneumonia
951M	122	0.0023	3.3	8.9	0.77	47	35	Anesthesia
1229M	123	0.0060	0.94	11	0.73	35	49	Pneumonia
1072M	124	0.079	0.65	4.1	1.6	57	34	Radiation Pneumonitis
1157M	124	0.294	0.55	3.5	3.7	41	44	Bone Tumor
971F	125	0.0095	1.7	5.5	0.44	49	41	Hemangiosarcoma, Spleen
1078F	125	0.025	0.98	9.6	0.60	46	41	Meningioma
952F	125	0.106	1.0	4.4	2.1	39	48	Bone Tumor
1059F	126	0.050	4.2	7.4	0.99	45	39	Malignant Lymphoma
991F	126	0.058	1.8	14	0.81	36	41	Urinary Bladder Tumor
1070M	126	0.011	1.9	9.5	0.70	51	34	Round Cell Sarcoma, Kidney
1166M	128	0.354	1.8	11	1.6	47	35	Malignant Lymphoma
983M	132	0.274	1.5	5.9	2.9	47	37	Adrenal Tumor, Pituitary Tumor
1035F	132	0.172	2.8	10	1.9	19	53	Bone Tumor, Cushing's
1031F	134	0.025	1.9	13	0.97	17	65	Pneumonia
1190F	134	0.033	0.84	4.4	1.2	49	41	Lung Tumor
1062M	135	0.270	0.63	2.6	3.9	46	44	Bone Tumor, Lung Tumor
1177M	136	0.142	0.77	5.0	0.89	36	53	Bone Tumor
959M	138	0.0025	3.4	14	0.62	33	48	Liver Abscess
992F	139	0.264	0.73	8.0	2.7	42	42	Bone Tumor
1194F	140	0.0014	0.67	10	9.0	20	56	Malignant Lymphoma
1105F	140	0.00074	0.62	5.6	0.70	44	45	Malignant Lymphoma
1193F	141	0.0037	0.58	9.2	1.0	37	48	Immune Hemolytic Anemia
973F	142	0.127	3.7	7.0	1.8	44	39	Bone Tumor
1060F	142	0.011	0.61	10	0.68	39	47	Pneumonia
1114M	143	0.272	0.51	7.4	2.9	39	47	Bone Tumor, Bile Duct Carcinoma
1222M	143	0.0051	8.5	4.0	0.82	36	47	Malignant Mesothelioma (mediastinal)
1053F	143	0.061	1.9	5.0	0.83	45	41	Cushing's Disease
1176M	145				-----Processing-----			Hemangioma, Spleen

^(a)Includes tracheobronchial, mediastinal and sternal lymph nodes^(b)Includes hepatic, splenic and mesenteric lymph nodes

FIGURE 3. Plutonium in Tissues of Dogs After Inhalation of $^{238}\text{PuO}_2$. Points represent data from individual dogs. The uptake and retention curves and functions were based on dogs in which initial lung burdens were estimated from the final plutonium body burden, plus the plutonium excreted, minus that excreted in the feces during the first 3 days after exposure.

Thirty-one of the 33 dogs with bone tumors had osteosarcomas, one Dose-Level Group 1 dog (989F) had a fibrosarcoma in the ilium, and one Dose-Level Group 4 dog (1103F) had a fibrosarcoma in a vertebra. All of the exposed dogs with osteosarcomas and/or lung tumors were in Dose-Level Groups 3, 4, 5, and 6. Thirteen of the 31 osteosarcomas were in vertebrae; two in femora; four in ribs; three in the scapulae; five in the pelvis; one in the tibia; one in the sternum; one in the sacrum; and one in the humerus. Dog 994F (Dose Level 6); dogs 1047M, 1079M, 1096M, and 1191F (Dose Level 5); 983M, 991F, 1030F, 1053F, 1081M, 1166M and 1176M (Dose-Level 4); 960M, 1031F, 1040M, 1043M, 1059F, and 1066M (Dose Level 3); 971F, 1060F, 1070M, 1078F, 1082M, 1188M, 1222M and 1229M (Dose Level 2); and 951M, 959M, 1063M, 1069F, 1105F, 1106F, 1193F and 1194F (Dose Level 1) died during the 12½-year postexposure period of causes presently thought to be unrelated to plutonium exposure.

The lung tumors were classified as bronchiolar-alveolar carcinomas in 12 dogs, bronchiolar-alveolar adenoma in one dog, adenocarcinoma in one dog, and adenosquamous carcinoma in two dogs. In one dog, three lung-tumor types were observed: bronchiolar-alveolar, adenocarcinoma and fibrosarcoma. Metastases were observed in the lungs, thoracic lymph nodes, trachea, mediastinum, and heart of the dog with pulmonary adenocarcinoma. Bone-tumor metastases were found in the lungs of six dogs; in three dogs, the bone tumor metastasized to lungs, thoracic lymph nodes, liver, spleen and heart; in one dog, the bone tumor metastasized to the iliac lymph nodes; and in one dog, the bone tumor metastasized to the lungs, pleura, diaphragm 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 4, 5, and 6 dogs included focal alveolar histiocytosis, alveolitis, alveolar epithelial cell hyperplasia, alveolar emphysema, pleural fibrosis, and interstitial fibrosis. Numerous alpha stars were observed, mainly in foci of fibrosis, and single alpha tracks were scattered throughout sections in foci of alveolar histiocytosis and in alveolar septa. Sclerosing lymphadenopathy in the tracheobronchial and mediastinal lymph nodes was associated with high concentrations of plutonium observed as alpha stars in Dose-Level Groups 3, 4, 5 and 6. Similar but less severe lesions were seen in the hepatic lymph nodes. In Dose-Level Groups 5 and 6, there were extensive alterations in bone, including multiple areas of focal atrophy of bone; endosteal, trabecular and peritrabecular bone fibro-

sis; and osteolysis of cortical, endosteal and trabecular bone. One dog had lesions of secondary hypertrophic osteoarthropathy. Radioactivity in the bone was present as single tracks, generally scattered throughout the bone, cartilage, and bone marrow. The liver contained foci of hepatocellular fatty change, where small clusters of single tracks were seen. There was also mild, focal, nodular hyperplasia of hepatocytes in Dose-Level Groups 3, 4, 5 and 6. Elevated serum GPT levels, suggestive of liver damage, were observed in Dose-Level Groups 3, 4, 5 and 6 dogs.

Dose-related lymphopenia was observed in groups with mean lung $^{238}\text{PuO}_2$ deposition of 77 nCi or more (Figure 4). The lymphocyte depression was more pronounced in magnitude and appeared earlier than in dogs exposed to similar doses of $^{239}\text{PuO}_2$. Through 126 months after exposure, mean lymphocyte values were significantly lower ($P < 0.05$) for Dose-Level Groups 4 and 5 than for the control group. However, lymphocyte values in the $^{238}\text{PuO}_2$ -exposed dogs tended to increase sooner after reaching a minimum than in $^{239}\text{PuO}_2$ -exposed dogs, and mean lymphocyte concentrations in Group 3 dogs were not significantly different from values of control dogs 86 to 94 months 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 because of lymphopenia, except in Groups 5 and 6, in which neutropenia was also observed. Through 118 months 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 $^{238}\text{PuO}_2$ inhalation. No chronic effects have been observed in red-cell parameters.

Lymphopenia, the earliest observed effect after inhalation of either $^{239}\text{PuO}_2$ or $^{238}\text{PuO}_2$, occurred 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/week to the lung.

In serum chemistry assays of $^{238}\text{PuO}_2$ dogs, performed more than 118 months following exposure, ALP and GPT values were higher than those of the control group only in Dose-Level Groups 3, 4, and 5 dogs. Elevations in GPT are consistent with liver histopathologic findings and radiochemical analyses indicating ^{238}Pu translocation to the liver. Alkaline phosphatase elevations occurred in some of the dogs with

primary bone tumors and in others in which the increase was attributable to the liver (by heat inactivation of ALP) as the source of the largest portion of the ALP.

Using the uptake and retention curves shown in Figures 1 and 3, cumulative radi-

ation doses to death were estimated for the lungs of the ^{239}Pu dogs and the lungs and skeletons of the ^{238}Pu dogs (Table 7). For the dose calculations, mean plutonium concentration in the entire lung and skeleton was used.

FIGURE 4. Mean Leukocyte, Neutrophil and Lymphocyte Values in Dogs After Inhalation of $^{238}\text{PuO}_2$.

TABLE 7. Estimates of Cumulative Radiation Doses to Lungs (^{239}Pu -Exposed) or Lungs and Skeletons (^{238}Pu -Exposed) of Dogs with Lung and/or Bone Tumors After Inhalation Exposure.

	Dose Level Group	Number of Dogs with Tumors	Survival Time Postexposure, mo	Cumulative Dose to Organ, rad
$^{239}\text{PuO}_2$ - Lung Tumors	6	1	69	7400 ^(a)
	5	20	37 - 115	1700 - 4000
	4	16	93 - 177	550 - 1500
	3	8	98 - 170	150 - 550
	2	3	166 - 175	30 - 120
	1	0	---	---
$^{238}\text{PuO}_2$ - Lung Tumors	6	9	49 - 84	2300 - 9800 ^(a)
	5	5	70 - 110	1350 - 2900
	4	2	118 - 135	400 - 450
	3	1	134	100
	2	0	---	---
	1	0	---	---
$^{238}\text{PuO}_2$ - Bone Tumors	6	9	49 - 84	180 - 480 ^(b)
	5	16	61 - 132	80 - 230
	4	7	118 - 143	50 - 100
	3	0	0	---
	2	0	0	---
	1	1	99	<1

^(a)Dose to lungs

^(b)Dose to skeleton

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• Inhaled Plutonium Nitrate in Dogs

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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 $^{239}\text{Pu}(\text{NO}_3)_4$ in 1976 and 1977. The earliest biological effect was on the hematopoietic system; lymphopenia and neutropenia occurred at the two highest dose levels. We have also observed radiation pneumonitis, lung cancer, and bone cancer at the three 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 being studied with intravenously injected radionuclides at the University of Utah; inhalation studies with beta-, gamma- and alpha-emitting radionuclides at the Inhalation Toxicology Research Institute (Lovelace); and external irradiation at the University of California (Davis) and Argonne National Laboratory.

Six dose groups (105 dogs) were exposed, in 1976 and 1977, to aerosols of $^{239}\text{Pu}(\text{NO}_3)_4$ 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 $^{239}\text{Pu}(\text{NO}_3)_4$ for periodic sacrifice to study plutonium metabolism and the pathogenesis of developing lesions; 7 dogs were selected as controls for periodic sacrifice; and 20 dogs were selected as untreated controls for lifespan observations. The Appendix (following the entire Annual Report) shows the current status of each dog on these experiments.

The average amount of plutonium in the lung decreased to approximately 1% of the final body burden in dogs surviving 5 years or more (Table 2). There was early translocation to the liver and skeleton, with only minimal amounts translocated to thoracic or abdominal lymph nodes. This

was in contrast to dogs that inhaled $^{239}\text{PuO}_2$, where a considerable amount translocated to the thoracic lymph nodes, but only small amounts translocated to skeleton at these time periods.

TABLE 1. Lifespan Dose-Effect Studies with Inhaled $^{239}\text{Pu}(\text{NO}_3)_4$ in Beagles.

Dose Level Group	Number of Dogs		Initial Lung Deposition ^(b)	
	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	56 ± 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 weeks after exposure to $^{239}\text{Pu}(\text{NO}_3)_4$. The results of these continuing evaluations are shown in Figure 1.

TABLE 2. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$.

Dog Number	Time After Exposure, mo	Final Body Burden, μCi	Percent of Final Body Burden					Cause of Death
			Lungs	Thoracic Lymph Nodes ^(a)	Abdominal Lymph Nodes ^(b)	Liver	Skeleton	
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
1485F	54	1.052	0.82	0.35	0.07	31.13	63.94	Bone Tumor
1502F	55	3.113	0.80	0.39	0.09	33.33	62.51	Bone Tumor, Lung Tumor
1387F	55	0.167	1.41	0.22	0.12	45.48	49.10	Bone Tumor
1429M	59	1.159	4.14	0.35	0.10	37.06	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
1646F	60	0.806	0.72	0.20	0.40	46.92	48.42	Bone Tumor
1619F	62	1.361	0.55	0.59	0.13	37.87	58.63	Bone Tumor
1589F	63	0.029	0.68	0.04	0.13	46.43	50.32	Sacrifice
1636M	66	0.634	1.21	0.27	0.52	53.97	39.09	Bone Tumor
1652F	68	0.658	1.46	0.23	0.29	50.47	44.32	Bone Tumor, Lung Tumor
1498F	69	0.845	0.59	0.32	0.13	26.63	53.37	Bone Tumor, Lung Tumor
1659F	69	0.736	1.14	0.34	0.40	38.90	55.89	Bone Tumor
1640M	76	0.177	4.01	0.64	0.63	54.41	36.59	Lung Tumor
1419M	76	0.873	0.69	0.28	0.39	44.06	50.70	Bone Tumor, Lung Tumor
1660M	82	0.854	0.76	0.53	0.53	37.51	56.17	Bone Tumor, Lung Tumor
1621M	84	0.840	0.94	0.56	0.29	40.87	54.55	Bone Tumor, Lung Tumor
1655M	88	0.505	1.05	0.22	0.93	41.83	52.14	Lung Tumor, Bone Tumor
1501M	92	0.002	1.62	0.50	0.79	38.05	48.41	Thyroid Tumor
1648M	92	0.639	1.12	0.25	0.73	42.83	50.61	Bone Tumor, Lung Tumor
1641M	92	0.869	0.78	0.	0.48	45.72	48.89	Lung Tumor
1408F	93	0.181	0.60	0.19	0.37	49.47	45.52	Bone Tumor
1404M	93	0.217	0.82	0.28	0.72	46.24	48.62	Pleuritis
1470F	95	0.001	1.11	0.48	0.34	43.21	50.23	Meningioma
1489F	98				-----Processing-----			Esophageal Tumor
1565F	101				-----Processing-----			Hemangiosarcoma
1503F	103				-----Processing-----			Thyroid Tumor
1645F	105				-----Processing-----			Lung Tumor
1587M	106				-----Processing-----			Hemangiosarcoma, Lung Tumor
1534M	106				-----Processing-----			Congestive Heart Failure
1521F	106				-----Processing-----			Bone Tumor, Lung Tumor
1599F	107				-----Processing-----			Adrenal Tumor

^(a)Includes tracheobronchial, mediastinal and sternal lymph nodes^(b)Includes hepatic, splenic and mesenteric lymph nodes

FIGURE 1. Mean Leukocyte, Neutrophil and Lymphocyte Values in Dogs After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$.

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 (neutrophils, lymphocytes, monocytes and eosinophils). This is in contrast to the effects of both $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$, which significantly depressed lymphocyte concentrations by 21 months after exposure to initial lung burdens of ~80 nCi or more. The lymphopenia at lower dose levels of plutonium oxides may be related to the more-extensive translocation of plutonium oxide to the tracheobronchial lymph nodes and subsequent higher dosage levels to lymphocytes circulating through those lymph nodes.

Serum enzyme assays have been performed throughout the postexposure period in an attempt to diagnose specific damage to liver and/or bone by plutonium translocated from the lung. Prior evaluations revealed periodic elevations in mean values for glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase, and alkaline phosphatase (ALP); however, there were no consistent dose-related elevations in these values. Currently (more than 9 years following exposure), GPT and ALP values in Dose-Level Groups 3, 4 and 5 are significantly ($P < 0.05$) higher than those for the control group.

Table 3 summarizes, by dose-level group, the mortality and lesions associated with deaths through 9 years after exposure to $^{239}\text{Pu}(\text{NO}_3)_4$. All five dogs at the highest dose level (Group 6) died from radiation pneumonitis 14 to 41 months 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. One dog at the highest dose level had a small bronchiolo-alveolar carcinoma as well as radiation pneumonitis.

All the dogs in Dose-Level Group 5 died or were euthanized 34 to 92 months after plutonium exposures. The principal cause of death at this exposure level was osteosarcoma, which occurred in 17 of 20 dogs; several had more than one site. The sites of the osteosarcomas were lumbar vertebrae (four dogs), cervical vertebrae (three dogs), thoracic vertebrae (two dogs), humerus (five dogs), pelvis (two dogs), facial bones (two dogs), ribs (two dogs), and nasal turbinates (one dog). Metastases to distal sites occurred in six dogs; these dogs also had radiation osteosis, generally characterized by peritrabecular fibrosis.

TABLE 3. Lesions in Beagle Dogs 9 Years After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$.

	Dose Group						Vehicle	Control
	6	5	4	3	2	1		
Number of Dogs/Group	5	20	20	20	20	20	20	20
Number of Dead Dogs/Group	5	20	9	1	2	4	1	2
Condition ^(a)								
Radiation Pneumonitis	4	1						
Radiation Pneumonitis and Lung Tumor	1	1						
Bone Tumor		8	2					
Bone and Lung Tumor		9	2					
Lung Tumor		1	3					
Hemangiosarcoma and Lung Tumor				1				
Pneumonia or Pleuritis			1					1
Lymphoma							1	
Thyroid Tumor					1	1		
Meningeal Tumor						1		
Status Epilepticus								1
Congestive Heart Failure			1					
Hemangiosarcoma						1		
Adrenal Tumor					1			
Esophageal Tumor						1		

^(a)Number of dogs with lesions associated with death

Other deaths in Dose-Level Group 5 were caused by radiation pneumonitis (two dogs) and multiple lung tumors (one dog). The multiple lung tumors, in different lobes, were papillary adenocarcinomas, combined epidermoid and adenocarcinoma, and bronchioloalveolar carcinoma; metastases were present in the tracheobronchial lymph nodes.

Malignant but nonfatal lung tumors were also present in nine dogs from Dose-Level Group 5 that died from osteosarcomas and in one dog that died from radiation pneumonitis. Typically, these arose subpleurally, proximal to areas of interstitial fibrosis or small cavities communicating with bronchioles. They consisted of bronchioloalveolar carcinomas in four dogs; papillary adenocarcinomas in two dogs; both bronchioloalveolar carcinoma and papillary adenocarcinoma in one dog; both papillary and tubular adenocarcinomas in one dog; a combined epidermoid and adeno-

carcinoma in one dog; and a bronchioloalveolar carcinoma, papillary adenocarcinoma, and a mixed lung tumor in one dog. No metastases of these lung tumors were observed.

In Dose-Level Group 4, nine dogs have died 54 to 106 months after plutonium exposure. The principal causes of death were bone tumors (four dogs), lung tumors (three dogs), suppurative pleuritis (one dog), and congestive heart failure (one dog). Two dogs that died because of bone tumors also had nonfatal lung tumors.

In Dose-Level Group 3, one dog died 106 months after plutonium exposure; the dog had a large subcutaneous hemangiosarcoma, with pulmonary metastases. Although the hemangiosarcoma was probably unrelated to plutonium exposure, the dog also had a small lung tumor, still under study, that is tentatively considered a giant cell carcinoma.

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• Oncogenes in Radiation Carcinogenesis

Principal Investigator: M. E. Frazier

Other Investigators: R. F. Jostes, L. L. Scott, and G. L. Stiegler

Technical Assistance: S. R. Peterson and R. J. Rausch

Lung tumors obtained from studies of lifespan, dose-effect relationships in beagle dogs exposed to plutonium by inhalation are being used for studying mechanisms of radiation-induced carcinogenesis. Specifically, we are examining the role of known oncogenes in lung tumors induced by the internally deposited radionuclides plutonium-238 and plutonium-239. The initial phase of this research is attempting to determine whether oncogenes are involved in plutonium-induced lung cancers and, if so, their role in the carcinogenic process.

In this study we are examining DNA from plutonium-induced lung tumors to determine whether dominant-acting transforming genes are present in the tumors. In addition, we are analyzing DNA (Southern blot hybridization) from tumor cells and radiation-exposed nontumorous cells, from the same animals, to determine whether there are tumor-specific changes in the DNA sequences of the genes associated with cancer causation.

Some carcinogens cause unique or unusual types of cancer; for example, vinyl chloride causes hemangiosarcomas of the liver, and asbestos induces mesotheliomas in lungs of exposed individuals. Unfortunately, the causative agents responsible for most types of cancers are not identifiable in this manner. A long-term goal of our research is to devise methods for detecting molecular differences in the DNA of tumor cells that will identify the causative agent.

Not all DNA damage results in cancer; existing evidence implicates a subset of genes in carcinogenesis. Although these genes (proto-oncogenes) are important for normal physical growth and development, their exposure to either physical or chemical mutagens can cause alterations which result in cancer. When proto-oncogenes (Figure 1) are activated, they may contribute to the neoplastic transformation of cells through point mutations, frame shifts, deletions, translocations, gene amplification or other genetic mechanisms. This activation results in either an altered protein with a modified function, increased amounts of gene product, or some combination of these events, each of which can contribute to the carcinogenic pro-

cess. Evidence is accumulating that a given carcinogen produces characteristic changes in DNA that can convert a proto-oncogene into an activated oncogene (cancer-causing gene). For example, certain chemical agents appear to cause characteristic point mutations in specific oncogenes.

Table 1 shows some specific relationships between certain insults and specific tumors. For example, activated Ha-ras oncogenes were detected in 48 of 58 N-nitroso-N-methylurea (NMU)-induced rat mammary carcinomas. In three different strains of rats, all 48 activated oncogenes had the same point mutation in the second nucleotide of the twelfth codon, a guanine-to-adenine transition. Activated Ha-ras genes were detected in only 21% of 7,12-dimethylbenz[a]anthracene (DMBA)-induced rat mammary carcinomas, and the activating lesion was in the 61st codon. Skin tumors that were DMBA-induced and promoted with tetradecanoylphorbol-13-acetate (TPA) in mice also caused activation of the Ha-ras oncogene as a result of a point mutation in the 60th codon. In 33 of the 37 skin tumors, the mutation detected was an adenine-to-thymidine transversion. Guerrero et al. have reported that thymomas were induced in the same strain of mice by two different agents, NMU (a chemical) and gamma rays, each of which activated a different oncogene. The gamma radiation caused a mutation in the 12th codon of the Ki-ras gene. The striking specificity suggested by these data implies that the activating mutations are the direct consequence of the agents' specific mutagenic activity. If this is true, it should be possible to use retrospective molecular analyses to determine cancer causation.

FIGURE 1. Types of DNA Mutation Caused by Oncogenes.

TABLE 1. Insult-Specific Oncogene Activation.

Insult(a)	Tumor	Activated Oncogene	Mutation
NMU(b,c)	Mammary Carcinoma	Ha-ras	G→A, 12th Codon
DMBA(d,e)	Mammary Carcinoma	Ha-ras	?, 60th Codon
DMBA/TPA(f,g)	Skin Carcinoma	Ha-ras	A→T, 60th Codon
NMU(h,i,j)	Thymoma	N-ras	?, ? Codon
γ-radiation(h,i,j)	Thymoma	Ki-ras	G→A, 12th Codon

(a) NMU = N-nitroso-N-methylurea
DMBA = 7, 12-dimethylbenz(a)anthracene
TPA = 12-O-tetradecanoylphorbol-13-acetate

(b) Zarbl et al., *Nature* 315: 382 (1985)

(c) Sukumar et al., *Nature* 306: 658 (1983)

(d) Yuasa et al., *Nature* 303: 775 (1983)

(e) Taparowsky et al., *Science* 217: 934 (1982)

(f) Balmain et al., *Nature* 307: 658 (1984)

(g) Balmain et al., *Nature* 303: 72 (1983)

(h) Guerrero et al., *Proc. Natl. Acad. Sci. USA* 81: 202 (1984)

(i) Guerrero et al., *Science* 225: 1041 (1984)

(j) Guerrero et al., *Science* 225: 1159 (1984)

We are using normal and cancer tissues from plutonium-exposed dogs to determine whether radiation causes such distinctive patterns of genetic change. Our approach to this problem attempts to answer three questions? First, do the canine lung-tumor cells contain dominant-acting oncogenes? Second, are there tumor-specific changes in the DNA adjacent to or within known oncogenes? And third, are levels of oncogene messenger-RNA expression within tumors altered (increased or decreased) relative to expression observed in normal cells? Initial studies suggest that a specific oncogene related to the *ras* gene is activated in these tumors. Last year we reported that DNA from plutonium-induced lung tumors caused the malignant transformation of indicator cells in the standard transformation assay, and a homolog of the *ras* sequence had been detected in some of these transformed cell. Furthermore, DNA from plutonium-induced lung tumors contained tumor-specific changes in the Ki-ras-related sequences. DNA from many of these same dogs has now been analyzed for alterations in the DNA sequences of two additional oncogenes, using the Southern blot hybridization technique. Bam HI restriction fragment length polymorphisms (RFLP) of sequences homologous to the Ha-ras locus are shown in Figure 2. DNA from lung tumor, normal lung, or normal spleen was digested with Bam HI and hybridized to

the Ha-ras fragment. In this particular blot, the Ha-ras gave a band at 6.6 kbp in all of the tissues. Additional fragments were observed in lung tumors from dogs 880 and 889. Table 2 summarizes the data obtained from analyses conducted to date: three of seven dogs displayed tumor-specific RFLP in Ha-ras-sequences. Table 3 shows the results of analyzing the plutonium-induced tumors for the presence of RFLP in the myc oncogene; in this instance, only one of nine dogs had a tumor-specific RFLP.

Evidence from these studies suggests that the changes detected in oncogene sequences of tumor cell DNA are more extensive than the single-base changes which have been observed in chemically induced or spontaneous tumors in other studies. Data from other laboratories, using different endpoints (such as mutations) indicate that large deletions and chromosomal rearrangements may result from exposure to alpha and/or gamma radiation.

FIGURE 2. Tumor-Specific Restriction Fragment Length Polymorphisms Detected in Plutonium-Induced Lung Tumors. High-molecular-weight DNA from dogs 727, 880, 889 and 1655, all with plutonium-induced lung tumors, were digested with Bam H1 and transferred to a nitrocellulose membrane. The nitrocellulose filter was hybridized with ³²P-labeled Ha-ras. NL = normal lung, LT = lung tumor and SP = spleen.

TABLE 2. Southern Blot Analysis of Plutonium-Induced Lung Tumors Using Ha-ras as the Probe.

Dog No. (a)	Restriction Endonuclease Fragment Size	
	<u>Bam</u> H1	
880 NL	6.6	
880 LT(b)	6.6, 4.4	
889 NL	6.6	
889 LT(b)	8.3, 6.6, 6.1	
1391 NL	6.6	
1391 LT(b)	7.6, 6.6	
727 NL	6.6	
727 LT	6.6	
783 NL	7	
783 LT	7	
796 NL	6.6	
796 LT	6.6	
1655 NL	6.6	
1655 LT	6.6	

(a) NL = normal lung, LT = lung tumor

(b) Lung tumors displaying tumor-specific restriction fragment length polymorphisms

TABLE 3. Southern Blot Analysis of Plutonium-Induced Lung Tumors Using myc as the Probe.

Dog No. (a)	Restriction Endonuclease Fragment Size		
	<u>Bam</u> H1	<u>Eco</u> R1	<u>Hind</u> III
1391 NL	10.8, 9.8, 6.2, 5.6	10, 6.6	10, 6, 5.8
1391 LT(b)	10.8, 9.8, 6.2	6.6	6
727 NL	10.8, 6.2	6.6	10
727 LT	10.8, 6.2	6.6	10
777 NL	NT(c)	6.6	10
777 LT	NT	6.6	10
796 NL	6.2	6.6	6.5
796 LT	6.2	6.6	6.5
880 NL	NT	10.3, 8	NT
880 LT	NT	10.3, 8	NT
889 NL	NT	10.3, 8	NT
889 LT	NT	10.3, 8	NT
1342 NL	NT	6.6	NT
1342 LT	NT	6.6	NT
1640 NL	8	10.3, 8	NT
1640 LT	8	10.3, 8	NT
1655 NL	8	10.3, 8	NT
1655 LT	8	10.3, 8	NT

(a) NL = normal lung, LT = lung tumor

(b) Lung tumors displaying tumor-specific restriction fragment length polymorphisms

(c) NT = not tested

Although observation of these RFLP does not itself prove that a specific oncogene is involved in the carcinogenic process, it helps us set priorities regarding which genes may be the most important to study. We also believe that analyzing all our data may provide some evidence for a specific pattern of oncogene activation. To determine whether such a pattern exists we have tabulated the data (Table 4), but it is not yet possible to determine whether definite patterns are present. The only

consistent factor appears to be the high percentage of dominant-acting transforming oncogene activity associated with these tumors.

Since we have also observed increased expression of ras gene messenger-RNA in lung tumor cells, we are attempting to isolate and sequence the activated ras genes from cell lines which were transformed with DNA from a canine lung tumor. This will allow us to precisely characterize the molecular lesions.

TABLE 4. Summary of Oncogene Research with ²³⁹Plutonium.

Dog No.	Exposure	Tentative Diagnosis	Dominant-Acting Oncogene	Tumor-Specific Changes in Oncogene Restriction Patterns			
				<u>src</u>	<u>myc</u>	<u>Ha-ras</u>	<u>Ki-ras</u>
727	Oxide	Mesothelioma	+(a)	-(b)	-	-	O(c)
777	Oxide	Adenosquamous Carcinoma	O	+	-	O	O
777	Oxide	Bronchiolar Adenocarcinoma	O	O	O	O	O
783	Oxide	Adenosquamous Carcinoma	O	O	O	-	+
796	Oxide	Squamous Cell Carcinoma	+	-	O	-	-
880	Oxide	Adenosquamous Carcinoma	+	-	-	+	+
889	Oxide	Adenosquamous Carcinoma	+	O	-	+	+
1391	Nitrate	O	O	O	+	+	+
1640	Nitrate	Adenosquamous Carcinoma	+	O	-	O	+
1640	Nitrate	Bronchiolar Adenocarcinoma	+	O	O	O	O
1655	Nitrate	Adenosquamous Carcinoma	+	O	-	-	-

(a) + = positive

(b) - = negative

(c) O = data not yet available

• Low-Level $^{239}\text{PuO}_2$ Lifespan Studies

Principal Investigator: C. L. Sanders

Other Investigators: E. S. Gilbert, K. E. Lauhala, J. A. Mahaffey, and K. E. McDonald

A total of 3192 female Wistar, 198 male Wistar, 192 female Long-Evans and 200 female Fischer-344 young adult rats were either sham-exposed or given a single inhalation to ^{169}Yb , $^{239}\text{PuO}_2$ and examined for their lifespan for lung tumor formation. An additional 200 exposed, female Wistar rats are being serially studied for cellular changes leading to lung-tumor formation. Histopathological analyses have been completed on 1149 of the 3192 Wistar rats in the initial lifespan study. Dose-response relationships were similar for pulmonary fibrosis, metaplasia and lung tumor formation. Percent of animals with lung tumors were: 0.6% (0 rad); 0.5% (5.9 rad); 0% (11 rad); 0% (23 rad); 4.5% (46 rad); 0% (84 rad); 14% (190 rad), 19% (350 rad); 73% (740 rad); and 85% (1500 rad). The dose-response curve was best fit by a quadratic function. Most multistage models of cancer induction used in the study of chemical and radiation carcinogenesis have assumed that both initiating and promoting events are required for greatest expression of tumors, and that the shape of the dose-response curve is dependent on the sequence of cellular events. We propose that the low-dose portion of the curve represents initiation event(s), while the much steeper, high-dose portion of this curve represents mostly promotion event(s). We believe that promotion results from an increased $^{239}\text{PuO}_2$ clustering, which leads to a cellular evolution of focally intense inflammation, fibrosis, metaplasia and carcinoma formation.

Plutonium dioxide, an abundant by-product of uranium-fueled reactors, constitutes a lung cancer risk when inhaled. Methods of exposure to ^{169}Yb , $^{239}\text{PuO}_2$, dose calculation and histopathological examination have been described in Annual Reports for 1981-1985. A total of 3192 female Wistar rats were exposed or sham-exposed to ^{169}Yb , $^{239}\text{PuO}_2$ in the initial lifespan study. The lowest dose group (5.9 ± 1.3 rad), equivalent to the current maximum permissible lung dose for nuclear industry workers (1.5 rad/yr from inhaled trans-uranics), contained 919 rats. An additional 198 male Wistar rats, 192 female Long-Evans rats and 200 female, Fischer-344 rats were exposed for lifespan study, in order to examine lung tumor response in two other strains and in the males at ~100-rad and 1000-rad doses. Two hundred female Wistar rats were exposed to ^{169}Yb , $^{239}\text{PuO}_2$ (Table 1) for study of the cellular evolution of lung tumors (using quantitative autoradiographic, morphometric, ultrastructural and cell-kinetic ^3H -thymidine labeling techniques). As in the initial lifespan study, correlation was excellent between estimated initial alveolar deposition (IAD) in individual rats, using whole-body counting of ^{169}Yb activity at 14 days after inhalation, and actual measurements of ^{239}Pu in lungs, made with liquid scintillation in the same animals (Figure 1).

All 3192 of initial lifespan $^{239}\text{PuO}_2$ rats have died; histopathological evaluations have been completed on 1149 rats, including 366 sham-exposed controls (Figure 2).

TABLE 1. Status of Study Groups as of October 1986.

Strain	Sex	Mean IAD, nCi(a)	Number of Rats	
			Alive	Dead
Wistar	F	0	0	1058
Wistar	F	0.44 ± 0.09	0	919
Wistar	F	0.79 ± 0.20	0	610
Wistar	F	1.7 ± 0.33	0	163
Wistar	F	3.5 ± 0.90	0	112
Wistar	F	6.1 ± 1.2	0	105
Wistar	F	15 ± 3.1	0	36
Wistar	F	26 ± 5.7	0	68
Wistar	F	58 ± 20	0	46
Wistar	F	120 ± 37	0	75
Wistar	M	0	60	0
Wistar	M	6.8 ± 2.8	78	0
Wistar	M	94 ± 21	60	0
Long-Evans	F	0	60	0
Long-Evans	F	5.1 ± 1.3	72	0
Long-Evans	F	77 ± 16	60	0
Fischer-344	F	0	60	0
Fischer-344	F	5.6 ± 1.1	80	0
Fischer-344	F	75 ± 7.9	60	0
Wistar(b)	F	0	40	10
Wistar(b)	F	104 ± 32	125	25

(a) Initial alveolar deposition + standard deviation

(b) Serially killed at 1, 14, 30, 60, 90, 120, 151, 180, 210, 240, 270, 300, 350, 400, 450, 500, 550 and 600 days after inhalation

Survival time for exposed animals was significantly less than for sham-exposed controls only in the highest exposure group (1500 rad). This was because of both a high incidence of lung-tumor formation and several deaths from radiation pneumonitis (Figure 3). Other than lung tumors in the highest dose levels, the most prevalent tumors continue to be pituitary adenomas, mammary fibroadenomas and uterine carcinomas and sarcomas (Table 2). Except for lung tumors, no significant difference was noted between any exposed group and sham-exposed controls in tumor type or location.

FIGURE 1. Correlation of Estimated (Using ^{169}Yb Whole-Body Counts) and Actual (Using Liquid Scintillation Counts for ^{239}Pu in Lung in Same Animal) IAD Values.

FIGURE 2. Fraction of Lifespan Rats with Completed Histopathology.

FIGURE 3. Survival of Female Wistar Rats as a Function of Dose to Lung Following Inhalation of $^{239}\text{PuO}_2$.

TABLE 2. Distribution of Tumors, According to Location, in the Initial $^{239}\text{PuO}_2$ Lifespan Study using Female Wistar Rats.

Location	Number of Tumors	% of all Tumors
Pituitary Gland	502	32.6
Mammary Gland	365	23.7
Uterus	272	17.7
Lung	104	7.2
Skin	90	5.9
Thyroid	34	2.2
Adrenal	25	1.6
Lymph Node	19	1.2
Thymus	16	1.0
Brain	15	1.0
Kidney	14	0.9
Auditory	10	0.7
Spleen	9	0.6
Abdominal Cavity	8	0.5
Ovary	7	0.5
Bone	5	0.3
Heart	5	0.3
GI Tract	5	0.3
Liver	4	0.3
Salivary Gland	3	0.2
Pancreas	3	0.2
Muscle	2	0.1
Bone Marrow	2	0.1
Urinary Bladder	1	0.1
Eye	1	0.1
Harderian Gland	1	0.1
Nasal Cavity	1	0.1
Oral Cavity	1	0.1
Stomach	1	0.1
Others	6	0.3
Total	1531	100.0

Dose-response relationships were similar for severity of pulmonary fibrosis, incidence of pulmonary metaplasias and incidence of pulmonary tumors (Table 3; Figures 4, 5, and 6). The severity of pulmonary fibrosis was significantly

greater than that of controls only at the highest dose levels (740 and 1500 rad); increases were nonsignificant at 190 and 350 rad (Figure 4). The incidence of pulmonary metaplasias was significantly greater than in controls at dose levels of 190, 350, 740 and 1500 rad. Squamous cell metaplasia incidence was significantly greater than in controls only at dose levels of 740 and 1500 rad. Adenomatous metaplasia incidence was significantly greater at dose levels of 190 rad and all higher dose levels (Figure 5).

A large proportion of tumors (primarily those in lungs) exhibited a high degree of malignancy, indicated by multicentricity, invasion into the thoracic cavity, and metastasis to contralateral lobes and extrapulmonary sites (Table 4).

The percentages of all rats with lung tumors were 0.6% (0 rad); 0.5% (5.9 rad); 0% (11 rad); 0% (23 rad); 4.5% (46 rad); 0% (84 rad); 14% (190 rad); 19% (350 rad); 73% (740 rad); and 85% (1500 rad). Only two primary tumors (1 mesothelioma, 1 adenocarcinoma for 0.5% incidence) were seen in 366 sham-exposed rats. A total of 102 primary lung tumors were found in exposed rats (60 squamous carcinomas, 27 adenocarcinomas, 10 hemangiosarcomas, 3 fibrosarcomas, 1 mesothelioma, and 1 carcinosarcoma). Overall, only four primary lung tumors (1 squamous carcinoma, 1 adenocarcinoma, 1 fibrosarcoma, 1 mesothelioma) have been found in 594 rats (<1%) with lung doses <128 rad. However, 98 primary lung tumors (49 squamous) have been found in 189 rats (52%) with lung doses >128 rad. This indicates the presence of a possible "practical" or "quasi"-threshold dose, below which a tumor is much less likely, of 100-200 rad for lung-tumor formation from inhaled $^{239}\text{PuO}_2$ (Figure 6).

TABLE 3. Pulmonary Pathology in Female Wistar Rats as a Function of Dose to Lung.

Dose Group	Dose to Lung, rad	Number of Rats	Grade of Pulmonary Fibrosis(a)	Percent of Rats with Pulmonary Metaplasia		Percent of Rats with Pulmonary Tumors				
				Adenomatous	Squamous	Squamous Carcinoma	Adenocarcinoma	Hemangiosarcoma	Other	Total
0	0	366	0.13 ± 0.40	0.8	1.4	0	0.3	0	0.3	0.6
1	5.9 ± 1.3	205	0.16 ± 0.45	1.5	1.5	0	0.5	0	0	0.5
2	11 ± 2.1	147	0.10 ± 0.35	0.7	0	0	0	0	0	0
3	23 ± 4.2	106	0.18 ± 0.43	1.9	0	0	0	0	0	0
4	46 ± 9.4	68	0.21 ± 0.50	1.5	0	1.5	0	0	3.0	4.5
5	84 ± 15	68	0.31 ± 0.55	5.9	0	0	0	0	0	0
6	190 ± 39	29	0.52 ± 0.74	13.8	3.4	10.3	3.5	0	0	13.8
7	350 ± 70	54	1.00 ± 0.86	25.9	1.9	3.7	13.0	0	1.9	18.6
8	740 ± 140	40	2.03 ± 0.95	60.0	25.0	45.0	15.0	10.0	2.5	72.5
9	1500 ± 360	66	2.92 ± 0.83	84.9	66.7	54.6	18.2	9.1	3.0	84.9

(a) Pulmonary fibrosis graded on a scale of 0 to 5, with 0 being none and 5 extreme

FIGURE 4. Pulmonary Fibrosis in Female Wistar Rats as a Function of Dose to Lung Following Inhalation of $^{239}\text{PuO}_2$.

FIGURE 5. Percent of Female Wistar Rats with Pulmonary Metaplasias as a Function of Dose to Lung Following Inhalation of $^{239}\text{PuO}_2$.

FIGURE 6. Percent of Female Wistar Rats with Pulmonary Tumors as a Function of Dose to Lung Following Inhalation of $^{239}\text{PuO}_2$.

TABLE 4. Indicators of Primary Lung Tumor Malignancy in Female Wistar Rats.

Tumor Type	% Invasive(a)	% Multicentric(b)	% Metastatic at Extra-Pulmonary Sites
Squamous Carcinoma	45	80	28
Adenocarcinoma	57	58	14
Hemangiosarcoma	30	70	60
All Others	33	67	33

(a) Into thoracic cavity tissues

(b) Tumor found in more than one lung lobe

The relative risk of lung-tumor occurrence and associated 90% confidence limits for 12 dose groups are presented in Figure 7. The relative risk is defined as the risk of lung tumor for the exposed group compared to the risk for sham-exposed controls. The calculations take time into account. Statistical significance was calculated with one-sided P-values, comparing each exposed group with controls. A clear-cut statistical significance from controls is not achieved until the 128- to 250-rad dose range (mean, 188 rad).

FIGURE 7. Radiation Dose and Relative Risk of Lung Tumor Formation in Female Wistar Rats.

A linear dose-response function, fitted over the entire dose range, can be clearly rejected; a linear-quadratic function provides no improvement in fit over a quadratic function. However, the dose-response relationship is well fit by a pure quadratic function; on that basis, the relative risk at a dose X is given by $1 + 0.00074 X^2$. The 90% confidence limits for the regression coefficient are 0.00033 and 0.0022. The mean dose for adenocarcinoma formation is close to being significantly lower than the mean dose for squamous carcinoma formation. A quadratic function provided the best fit for both lung-tumor types. We propose that the lower dose-

range of the quadratic curve (between 100 and 200 rad) represents primarily initiation (mutation) events. We believe that the much steeper, high-dose portion of the curve represents mostly promotion (progressive expression) of carcinogenesis. We believe this is caused by greatly increased clustering of $^{239}\text{PuO}_2$ particles, leading to cellular evolution of focally intense inflammation, fibrosis, epithelial metaplasia and carcinoma formation in or near areas of particle clustering. The serial sacrifice study now in progress will quantify the cellular events involved in the evaluation of normal lung to tumor formation.

• Inhalation Hazards to Uranium Miners

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Using both large and small experimental animals, this project is investigating levels of uranium-mine air contaminants that produce respiratory system disease in miners. Lung cancer incidence and deaths from degenerative lung disease are significantly elevated 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. Histopathologic data for 100-working-level (WL) exposure rates show a significant increase in lung tumor risk over 1000-WL exposure rates for comparable cumulative radon-daughter exposures. Exposure of rats to radon daughters and other contaminants continues; the exposure of beagle dogs to uranium ore dust alone was terminated. Renal function and hematology data on ore-dust-exposed dogs are reported.

Small-Animal Studies

The 6000 (1000-working level; WL) and 7000 (100-WL) Series experiments (Table 1) 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 (100-WL) Series experiments (Table 2) are designed to extend the exposure-response relationships to levels appropriate to current conditions in the mines and to lifetime environmental exposures. The 9000 Series experiments (Table 3) continue the "low-dose" studies at exposure rates comparable to former occupational working levels (10 WL). They will help to further evaluate the hypothesis that the tumor probability per working-level-month (WLM) exposure increases with decrease in exposure and exposure rate. In addition, concurrent exposure to varying levels of uranium ore dust tests the hypothesis that irritants (both specific and nonspecific) act synergistically with radiation exposures, the synergism increasing with decrease in exposure level. The exposures of 6000, 7000 and 8000 Series animals are completed. Due to funding constraints, exposures of 9000 Series animals were temporarily discontinued. Instead, exposures of fewer rats to uranium ore dust alone (10,000 Series experiments; Table 4) was initiated, along with clinical chemistry measurements and urinalyses. The ore-dust studies address recent experimental data in rats (as well as epidemiological data) linking silica exposures to lung cancer. Because the silica content of the ore dust in the PNL animal studies exceeds 60%, this potential link in the response to combined ore-dust and radon-daughter exposures needed to be clarified.

TABLE 1. Exposure-Response Relationship Study for Radon-Daughter Carcinogenesis in Rats (6000 and 7000 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
64	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 1.3×10^5 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 an 80% incidence of carcinoma.

TABLE 2. Low-Exposure-Response Relationship Study for Radon-Daughter Carcinogenesis in Rats (8000 Series Experiments).

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
192	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.

(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 1.3×10^5 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 1 data.

The most significant lesions related to radon-daughter and carnotite-ore-dust exposures in the 6000 and 7000 Series experiments are neoplastic and non-neoplastic lesions of the respiratory tract. Histopathologic data for the 6000 Series animals were shown in the 1984 Annual Report; data for the 7000 Series animals are shown in Table 5.

The 7000 (100-WL) Series animals show an increase in lung tumors, compared to the 6000 (1000-WL) Series animals, at all exposure levels except 320 WLM (Figure 1). The ratios of percent lung tumors (7000 Series/6000 Series) progressively increase from 1.6 at 5120 WLM to 2.8 at 640-WLM

exposures. The experiments clearly demonstrate an inverse exposure rate effect, which increases with decrease in exposure level.

TABLE 3. Ultralow Exposure-Rate Study for Radon-Daughter Carcinogenesis in Rats (9000 Series Experiments).

Number of Animals ^(a)	Exposure Regimen ^(b)	Total Exposure, WLM ^(c)
64	10 WL Radon Daughters 15 mg/m ³ Uranium Ore Dust	320
64	10 WL Radon Daughters 3 mg/m ³ Uranium Ore Dust	320
352	10 WL Radon Daughters 15 mg/m ³ Uranium Ore Dust	80
352	10 WL Radon Daughters 3 mg/m ³ Uranium Ore Dust	80
512	10 WL Radon Daughters 15 mg/m ³ Uranium Ore Dust	20
512	10 WL Radon Daughters 3 mg/m ³ Uranium Ore Dust	20
192	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 (tumor incidence is approximately 16% at 640 WLM), and 0.13% spontaneous incidence.

(b) Exposure rate, 90 hr/wk; planned periodic sacrifice.

(c) Working level (WL) is defined as any combination of the short-lived 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.

TABLE 4. Control Study for Uranium Ore Dust Carcinogenesis in Rats (10,000 Series Experiments).

Number of Animals	Exposure Regimen ^(a)
96	15 mg/m ³ Uranium Ore Dust
64	Sham-Exposed Controls

(a) Exposures, 12-18 mo at 72 hr/wk; planned periodic sacrifice

TABLE 5. Summary of Primary Tumors of the Respiratory Tract (7000 Series Experiments).

Nominal Exposure, WLM	Extrathoracic Tumors		Lung Tumors						Animals with Lung Tumors
	Laryngeal	Nasal	Animals Examined	Adenoma	Adenocarcinoma	Epidermoid Carcinoma	Adenosquamous Carcinoma	Sarcoma(a)	
5120	1/18(b)	0/29	32	7	17	14	1	1	25
2560	0/22	0/32	32	3	13	15	3	0	22
1280	0/22	0/32	32	7	13	4	3	2	21
640	0/48	1/60	64	2	13	2	2	1	18
320	0/88	1/125	127	6	6	1	1	2	14
Controls	0/59	0/94	95	0	0	0	0	1	1

(a) Sarcomas include 4 malignant histiocytomas, 1 mesothelioma, 1 leiomyosarcoma, and 1 hemangiosarcoma

(b) No. of tumors/No. examined

FIGURE 1. Percent of Lung Tumors in Rats Versus Radon-Daughter Exposure Rate and Level. WLM = Working Level Months (see Text).

Large-Animal Studies

The remaining beagle dogs on study to determine the pathogenic role of carnotite uranium ore dust in inhalation exposures

were killed following a total of 9.1 years of exposure (20 hours/week) to 15 mg/m³ uranium ore dust concentrations; the uranium content ranged from 2 to 4%. The tissues await histopathological examinations.

Renal function was evaluated on four uranium-ore-dust-exposed and five sham-exposed dogs following 9 years of exposure. Tests conducted on 24-hour urine samples included: osmolality, specific gravity, glucose, creatinine, protein, sodium, potassium, chloride, alkaline and acid phosphatase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, gamma glutamyl transpeptidase, and a microscopic examination. There were no statistically significant differences (because of large standard deviations) in the mean values of those parameters in the two groups of animals; however, two relatively sensitive measures of renal damage, glucose and protein excretion, tended to be higher in the exposed than in the control animals. This nonsignificant trend suggests the possibility of some renal damage. Earlier measurements, reported in the 1983 Annual Report, showed significant elevation of glucose levels following 6-year exposures to uranium ore dust.

Hematology evaluations showed no statistically significant differences between exposed and control groups in total leukocytes or in differential counts, or in blood serum constituents. Although BUN and creatinine values are of value in assessing renal damage, in dogs, about 75% of the nephron must be destroyed before any change is seen in these parameters. In addition, no differences were observed in red cell indices: cell counts, hemoglobin concentrations, volumes of packed red cells, mean cell volumes, mean corpuscular hemoglobins, and mean corpuscular hemoglobin concentrations.

TABLE 2. Effect of temperature on the growth of *Staphylococcus aureus* in milk.

Temperature (°C)	Time (h)	Optical Density (600 nm)	Optical Density (660 nm)	Optical Density (700 nm)
4	0	0.00	0.00	0.00
4	24	0.05	0.02	0.01
4	48	0.10	0.04	0.02
4	72	0.15	0.06	0.03
10	0	0.00	0.00	0.00
10	24	0.20	0.10	0.05
10	48	0.40	0.20	0.10
10	72	0.60	0.30	0.15
18	0	0.00	0.00	0.00
18	24	0.30	0.15	0.08
18	48	0.50	0.25	0.12
18	72	0.70	0.35	0.18
25	0	0.00	0.00	0.00
25	24	0.40	0.20	0.10
25	48	0.60	0.30	0.15
25	72	0.80	0.40	0.20

Optical density was measured at 600, 660, and 700 nm. The values are the mean of three determinations.

were killed following a heat of 7.1 units of exposure. The results of the tests are shown in Table 3. The results show that the heat treatment was effective in killing the bacteria.

Heat treatment was evaluated on four different media. The results are shown in Table 4. The results show that the heat treatment was effective in killing the bacteria on all four media. The results also show that the heat treatment was more effective on the media that contained a higher concentration of bacteria.

The results of the heat treatment tests are shown in Table 5. The results show that the heat treatment was effective in killing the bacteria on all four media. The results also show that the heat treatment was more effective on the media that contained a higher concentration of bacteria.

FIGURE 1. Effect of temperature on the growth of *Staphylococcus aureus* in milk.

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• Toxicity of Thorium Cycle Nuclides

Principal Investigator: J. E. Ballou

Other Investigators: R. L. Buschbom, A. C. Case, G. E. Dagle, R. A. Gies, and J. L. Ryan

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, in rodents, of inhaled ^{233}U - ^{232}U nitrate and of ^{231}Pa , a major, long-lived, radioactive waste product. This project, initiated in 1975, was terminated in FY1986.

Male Wistar rats exposed to graded doses of $^{233}\text{UO}_2(\text{NO}_3)_2$ and $^{232}\text{UO}_2(\text{NO}_3)_2$ aerosols (0.6 to 53 nCi initial lung burden) have been observed for their life span. Retention kinetics in major tissues and dose-response relationships for malignant lung-tumor and bone-tumor induction have been presented in previous Annual Reports (1975-1985).

Cumulative radiation doses to lung and skeleton, the tissues most affected by ^{232}U inhalation, were calculated from the quantity of ^{232}U and ^{228}Th measured in the lung and skeleton at death. Generalized retention curves were constructed for the two radionuclides, using the pooled data for the three graded-dose groups. Retention curves were derived by fitting multiexponential functions to data for individual rats that died and were analyzed during the lifespan study. Lung and skeleton doses were estimated from the retention parameters, and the calculated radiation

doses were divided into dose ranges and plotted against the incidence of malignant tumors (Figure 1). Data observed in similar studies with other actinides are included for comparison. Radiation doses for ^{232}U -exposed rats were adjusted for the dose contribution associated with the ingrowth of decay progeny, based on the retention of the first daughter product, ^{228}Th , and assuming that subsequent decay progeny were in equilibrium with ^{228}Th . The overall dose adjustment amounted to 2.8 times the calculated ^{232}U dose to lung and 5 times the ^{232}U dose calculated for skeleton. This procedure is expected to yield a maximum dose estimate for lung and skeleton because no allowance was made for diffusion or respiratory losses of ^{220}Rn and sequelae from the tissues of interest. No dose correction was required for ^{233}U ($T_{1/2} = 1.63 \times 10^5 \text{ y}$), which decays to a comparatively long-lived daughter, ^{229}Th ($T_{1/2} = 7.34 \times 10^3 \text{ y}$).

FIGURE 1. Incidence of Malignant Lung Tumors in Rats Sustaining Lifetime Cumulative Radiation Doses (<10 to 5000 Rad) to Lung.

The dose-response for malignant tumors (Figure 1) agreed fairly well for all the actinides studied except for: 1) the unusually high value for ^{239}Pu between 10 and 100 rad (associated with a single rat that fell in this dose range and happened to develop a lung tumor), and 2) the low incidence with ^{253}Es between 1000 and 10,000 rad, due to the acutely lethal effects of this dose and dose rate, which resulted in early death before tumors had time to develop. Apparent differences in tumor incidence between ^{232}U and ^{233}U at doses >100 rad may be an artifact of the manner in which the data were grouped for plotting. That is, tumor incidence was grouped over a fairly wide range of radiation doses to include sufficient numbers of rats in each category for comparison purposes. Accordingly, the average dose to the ^{233}U -exposed rats in the 101- to 500-rad dose range was only 120 rad compared to twice that, or 240 rad, for the corresponding group of ^{232}U -exposed rats. Since the dose-response curve (Figure 1) appears to change very rapidly in this dose range, the twofold difference in radiation dose may account for the fourfold difference in lung-tumor incidence. In addition, the dose distribution within the lung is not necessarily the same for ^{233}U and ^{232}U and its decay progeny. That is, the decay products may be translocated to some extent and may not decay exactly at the site of uranium deposition and decay. This could result in the irradiation of comparatively more cells and a corresponding increased probability of tumor production with the ^{232}U decay series.

The malignant bone-tumor incidence, also shown in Figure 1, was of minor significance compared to the incidence of lung tumors in these rats. This has been a general observation in a series of studies with this rat strain when exposed to inhaled soluble actinides. Bone tumors have been a major factor in only two studies: 1) in rats administered large amounts of ^{253}Es by intratracheal instillation, and 2) in rats exposed to inhaled ^{238}Pu nitrate aerosols.

Results now available from other studies describe the distribution and retention of ^{232}U decay products in rats up to 147 days after intratracheal instillation of $^{232}\text{UO}_2(\text{NO}_3)_2$ plus decay progeny. Figures 2 and 3 show the retention of ^{228}Th , ^{224}Ra , ^{212}Pb and ^{212}Bi in lung and skeleton, respectively. These are the tissues of major deposition and retention and the recipients of the greatest radiation dose. The data for each of the decay products are plotted as a fraction of the amount of parent ^{232}U activity recovered in the tis-

sue at the indicated time after instillation. The figures illustrate the disparity between our results in rats and the assumed parent/daughter equilibrium proposed by ICRP-30 to estimate radiation dose for decay-chain nuclides. The more nearly the relationships in Figures 2 and 3 approach a value of unity, the more nearly the results conform to the proposals in ICRP-30, i.e., that decay-chain progeny are born and decay in situ with the historical parent radionuclide. The preponderance of retention fractions that are substantially greater than unity in Figures 2 and 3 is the result of metabolic differences between ^{232}U and ^{228}Th , which result in the rapid excretion of ^{232}U and the more avid retention of ^{228}Th . Subsequent radioactive decay of ^{228}Th results in the ingrowth of the more remote decay progeny, as illustrated by the general increase in ^{224}Ra , ^{212}Pb and ^{212}Bi fractions as they grow toward equilibrium with ^{228}Th .

FIGURE 2. Retention in Lung of ^{232}U Decay Progeny Expressed as Fraction of ^{232}U Parent Activity.

Figure 3. Retention in Skeleton of ^{232}U Decay Progeny Expressed as Fraction of ^{232}U Parent Activity.

The level of ^{228}Th exceeded ^{232}U activity levels in all tissues examined (lung, skeleton, kidney, liver, blood, muscle) after instillation of $^{232}\text{UO}_2(\text{NO}_3)_2$ plus daughters. The rapid clearance of ^{232}U via urine resulted in a transient excess in kidney of ^{232}U over ^{228}Th that disappeared after the first week. Thereafter, the ^{228}Th activity level was about five times higher than that of ^{232}U .

It is apparent from Figures 2 and 3 that ^{228}Th and ^{232}U levels in lung and skeleton would probably never attain equilibrium within the lifespan of the rats and that the ICRP-30 dose estimate would seriously underestimate the cumulative radiation dose. In a longer-lived animal, such as the beagle dog or man, equilibrium could conceivably be attained, and the ICRP-30 assumptions for dose estimation would be applicable for that portion of the cumulative radiation dose.

• Aerosol Technology Development

Principal Investigator: W. C. Cannon

Other Investigators: E. F. Blanton, W. C. Forsythe, B. J. Greenspan, O. R. Moss, and R. J. Weigel

In this project we have developed methods and apparatus for generation, characterization and delivery of aerosols for studying the biological effects of inhaled air pollutants. We report here on continued development of electric field nebulization apparatus, an improved aerosol chamber, and on lung deposition studies in rodents.

Aerosol Deposition in Rats

Although it has been the practice at PNL to train or acclimate dogs to the nose-only exposure apparatus for approximately 2 weeks prior to exposure, for economic reasons this practice was not extended to rats or other small animals, except in a few cases. In these cases, it appeared that more aerosol was deposited than in rats that encountered the apparatus for the first time. However, deposition was measured in only a few animals. This year we conducted an experiment to compare aerosol deposition in "trained" and "untrained" rats that were simultaneously exposed to the same aerosol in the same chamber. Eighteen of 36 male Fischer-344 cesarean-derived rats, 70 days of age and weighing approximately 250 g each, were placed in containment tubes for 30 minutes on the first day and for 40 minutes on each of the next 8 working days (trained group). This group and the remaining 18 rats (untrained group) were exposed simultaneously on the tenth working day. The untrained rats were handled only to the extent required for normal animal care during this period.

The exposure aerosol was plutonium dioxide tagged with ytterbium-169. Rats were sacrificed immediately after exposure, the lungs and trachea were excised and the lobes were removed from the trachea. The lung lobes of each rat were combined and counted in a gamma well counter along with calibrated standard samples of the same material used to generate the aerosols. This procedure essentially measured the initial alveolar and bronchial deposition (IABD). The mean IABD and standard deviation (SD) were 22.08 ± 1.78 nCi for the trained rats; for the untrained rats, 23.23 ± 1.80 nCi. Histograms of the lung burdens are shown in Figures 1 and 2. Since the difference between the mean values is less than the SD of either group, it appears that there was no significant effect of training on aerosol deposition.

FIGURE 1. Histogram of Initial Alveolar and Bronchial Deposition of Plutonium for Rats "Trained" in Inhalation Exposure Containment Tubes.

FIGURE 2. Histogram of Initial Alveolar and Bronchial Deposition of Plutonium for Rats that were not "Trained" in Containment Tubes. Animals were exposed simultaneously with those described in Figure 1.

Electric Field Nebulization (EFN)

We investigated EFN of liquids for aerosol production this year with the specific goal of neutralizing the high electric charge on the particles that resulted from this technique. Borrowing from vacuum tube technology, we then created a drift, or mixing zone, where the positively charged aerosol particles would have time to interact with the negative ions produced by a corona discharge. We constructed a device consisting of a high positive-field capillary, which ejected the charged droplets; a negatively charged wire brush as a negative ion source; and two control grids to establish the mixing zone. We used the vacuum tube terminology "aerosol tetrode" for the device (Figure 3). We successfully produced neutral, or nearly neutral, liquid droplet aerosols which could be transported and sampled by standard aerosol techniques. Without this treatment we were previously unable to treat the highly charged particles in this manner because of their strong attraction to grounded or low-potential surfaces. We have not, however, succeeded in producing the sub-micron-size monodisperse aerosols desired. Figure 4 shows polystyrene particles formed by evaporating methyl ethyl ketone droplets with dissolved polystyrene. Sixty-seven percent of the aerosol particles were small, but they contributed 0.8% of particle mass (mass median diameter [MMD], 0.28 μm); the large particles constituted only 33% of the aerosol, by count, but accounted for 99% of the particle mass (MMD, 1.8 μm).

FIGURE 3. Schematic Diagram of Aerosol Tetrode (Electric Field Nebulizer).

FIGURE 4. Polystyrene Particles Generated by Electric Field Nebulization from Methyl Ethyl Ketone Solution.

Improved Nose-Only Exposure Chamber

A nose-only exposure chamber for rodents has been designed, and a prototype has been built, tested and used in exposures of rats to radioactive aerosols. This chamber is an improved version of the flow-past chamber reported by Cannon et al. (*Am. Ind. Hyg. Assoc. J.* 44: 923-928, 1983). The original flow-past chamber had a fixed number of ports and could not be disassembled for cleaning. The improved chamber has a minimum of 16 ports, and tiers of eight ports each can be added as needed to accommodate different numbers of animals.

The chamber can be disassembled for cleaning between exposures to different materials. Although the improved chamber costs more to build because it is mechanically complex, we believe these higher costs are justified by the greater versatility and ease of operation.

The improved chamber is mounted on a turntable bearing, allowing the operator to rotate the chamber and load the animals from one location. This feature is especially important when a double containment system is used in which the only access to the chamber is through glove ports. Inlet and exhaust lines are attached to the chamber by swivel fittings. The animal containment tubes are sealed into the exposure ports by means of double O-rings. This feature not only provides a more positive seal between the chamber and the containment tubes than would a single O-ring, but holds the tubes more firmly in place so that they cannot be accidentally detached from ports during exposures.

• Fetal and Juvenile Radiotoxicity

Principal Investigator: M. R. Sikov

Other Investigators: R. L. Buschbom, G. E. Dagle, P. L. Hackett, and D. N. Rommereim

Technical Assistance: M. O. Carey, J. J. Evanoff, and T. T. Sherer

This project is obtaining comparative information on the deposition, distribution, retention, and toxicity of radionuclides in prenatal and juvenile mammals. Because quantitative data cannot necessarily be extrapolated to man, emphasis is on identifying patterns, phenomenologic interactions, and relationships which might be useful in radiological protection practices for rapidly growing infants or children and for pregnant women. It was found that about two-thirds of the osteogenic sarcomas that resulted from exposure of rats to ^{239}Pu during late gestation originated in the bones of the head, and only one-third developed in the axial and appendicular skeleton. The incidences of soft-tissue lesions, including tumors, were significantly greater in these prenatally exposed rats than in groups that were exposed postnatally or were not exposed to plutonium. In other studies, evaluation of radiation doses from ^{233}U indicated that the observed embryotoxic and teratogenic effects were attributable to chemical toxicity and maternal effects rather than to a radiation effect on the conceptus. Results of a pilot study with ^{233}U suggest that prenatal exposure affects the postnatal development of kidney function.

Histopathological evaluations were completed on tissues from a recently completed study on the influence of foster-rearing on the early and long-term postnatal effects produced by prenatal exposure to ^{239}Pu via intravenous injection of pregnant rats. As was described in previous Annual Reports (1981-1982), pregnant rats at 19 days of gestation (dg) were injected intravenously with 60 nCi/g body weight of a citrated (70-fold molar excess) ^{239}Pu solution or with an equivalent citrate solution (control). The offspring from some litters were fostered to other dams at 1 day of age to form the six experimental groups indicated in Table 1.

Radionuclide distribution patterns, postnatal growth and longevity, and overall

incidences of bone and mammary tumors have also been reported (Annual Reports, 1983-1985). The incidence of bone tumors was elevated in the three groups that were exposed to plutonium prenatally, but it was not influenced by the exposure of the dams that reared the offspring. These data have now been analyzed in terms of the anatomic location at which the osteogenic sarcomas developed. Whether results are considered independently for each group (Table 1) or as the sum of the three prenatally exposed groups (Figure 1), the head was the predominant site of tumor development; significantly fewer tumors originated in the axial or appendicular skeleton. This pattern confirms results of our previous experiments with exposure during the perinatal period but is in

TABLE 1. Effect of Prenatal ^{239}Pu Exposure on the Incidence and Anatomic Distribution of Tumors.

Prenatal Exposure of Offspring	Exposed	Exposed	Control	Exposed	Control	Control
Exposure of Foster Mother	NF(a)	Exposed	Exposed	Control	Control	NF
Number of Rats	75	80	75	81	88	78
Number of Rats with Osteogenic Sarcoma	13	13	0	12	0	1
Number in Indicated Anatomic Sites						
Head	10	8	0	8	0	0
Axial Skeleton	1	2	0	2	0	0
Appendicular Skeleton	2	3	0	2	0	1
Number of Rats with Pheochromocytoma	12	10	3	20	4	3
Malignant	6	5	1	3	3	0
Benign	6	5	2	17	1	3
Number of Rats with Adenomatous Hyperplasia (Liver)	10	10	2	14	4	3
Number of Rats with Hepatoma	2	5	0	3	0	0

(a) Not Fostered

sharp contrast with the pattern observed after injection of adult rats, i.e., tumor development mostly in the vertebral column and long bones. These differences in the anatomical distribution of bone tumors are consistent with the age-dependent patterns of radiation doses to the skeleton that we previously described.

FIGURE 1. Predominant Anatomical Sites of Osteogenic Tumor Development in Offspring of Rats Injected with ^{239}Pu at 19 Days of Gestation.

Increased incidences of several histopathological lesions of soft tissues, including tumors, were detected in the prenatally exposed groups relative to the groups that were not exposed or were exposed postnatally (Table 1). The incidences of these lesions, which include hepatomas, adenomatous hyperplasia of the liver and benign and malignant pheochromocytomas of the adrenal gland, did not differ among the three groups that were exposed prenatally. The induction of these lesions appears to be unique to prenatal or neonatal exposure and confirms preliminary observations, made in previous experiments, involving comparison of adult and perinatal rat responses to ^{239}Pu exposure. These hepatic changes may suggest a mechanism involved in developmental derangements, since the liver cells are postmitotic in adults, but they are mitotic and intermittent in neonatal rats.

We are also investigating the toxicity of uranium in juvenile and fetal animals because only limited prenatal distribution and teratogenicity data were available for this element. As previously reported (Annual Report, 1984), a pilot study with ^{233}U was performed in conjunction with a com-

parison of the embryotoxicity and fetoplacental dosimetry of ^{239}Pu and ^{241}Am , and the results led us to subsequently perform a more detailed experiment (Annual Report, 1985). Pregnant rats, at 9, 15, or 19 dg, were intravenously injected with 1.8, 3.3, 5.75, or 10 nCi/g of citrated ^{233}U or with a citrate solution. Rats from all dose groups were killed at 20 dg for teratological evaluations and rats from selected dose groups were killed at sequential times to collect tissues and fetoplacental samples for radioanalysis and autoradiography. Teratological effects observed included a variety of rib anomalies and cleft palate.

Markedly bent limbs were found in about half the fetuses from two of the three surviving litters from the group that was injected, in the pilot study, with the high dose at 9 dg. The automatic watering systems serving those animals malfunctioned during gestation, which suggests that alteration in maternal fluid balance, which is consistent with the known nephrotoxic effect of uranium, may have led to the bent limbs. By inference, altered fluid balance may be involved in the other changes detected in the detailed experiment, including edematous fetuses from litters exposed at the higher dose levels at 15 dg.

Increasing the dose injected at either 9 or 15 dg clearly increased the fraction of the dose in the fetuses, placentas, and membranes at 20 dg (Figure 2). A similar but weaker relationship was noted for rats evaluated at 16 dg after injection at 9 dg, but it was not found for the 15- to 16-dg or 19- to 20-dg intervals. It thus appears that this effect of mass administered may relate to increased amounts of nuclide available for translocation, rather than to the extent of development at the time of evaluation.

The pattern of relative concentrations in the fetoplacental compartments was similar to that observed with other actinides. Figure 3 presents average uranium concentrations (percent administered dose/kg wet weight) throughout the fetoplacental unit to allow comparisons and description of general patterns. The highest fetoplacental concentration, represented by the egg cylinder, was found 1 day after exposure at 9 dg. The concentrations in the conceptus (embryo or fetus) and the placenta progressively decreased thereafter, but the concentration in the fetal membranes remained relatively constant. After injection at 15 or 19 dg, the concentrations in the fetuses were 0.004 and 0.009%/g. The ratios of placental to fetal concentrations were 6 and 4 at those times, and the corresponding ratios for fetal membranes to fetus were 45 and 43.

FIGURE 2. Effects of Magnitude of Administered Dose on the Percentage Present in Components of the Fetoplacental Unit at 20 Days of Gestation (dg) After Injection at 9 or 15 dg. Vertical Bars Represent Standard Errors of the Means.

FIGURE 3. Concentrations (Mean Percent of Dose/kg Body Weight $\pm$ SE) of ^{233}U in Components of the Fetoplacental Unit of Rats at Various Days of Gestation (dg) After Intravenous Injection at Representative Stages of Gestation.

Radioanalytical data were used to calculate radiation doses to the three fetoplacental compartments. The highest (10-nCi/g) dose produced absorbed doses in the conceptus, placenta, and membranes, respectively, of about 1, 2, or 3 rad after injection at 9 dg, and 0.2, 1, and 7 rad after injection at 15 dg. Comparisons of these doses with the literature on radiation embryotoxicity and teratogenicity suggest that the developmental effects were attributable to chemical toxicity rather than to a radiation effect per se, but some of the effects on the conceptus may have been mediated through changes in the pregnant rat.

The known nephrotoxicity of uranium in adult animals raised the question of whether exposure during late gestation might adversely affect the development of the kidney in the neonatal period. A pilot study was performed to address this question. Three pregnant rats were intravenously injected at 19 dg or 20 dg, with 5.75 nCi/g body weight of ^{233}U citrate, and three were injected with citrate solution (controls). Neonatal rats from another litter were injected intraperitoneally with the mitotic inhibitor, colchicine, to serve as positive controls. At 1, 5, 8, and 12 days of age, the offspring were removed from their dam and placed in individual beakers in an incubator for a 6-hour period. Male offspring were used to assay the hydropenia response, an index of the concentrating ability of the kidneys. These animals were not provided with fluid. Their bladders were emptied at the

start of the test, the volume of urine collected at 3 and 6 hours was measured, and urine osmolarity was determined in the samples collected at 6 hours (Table 2). Females were used to examine the diuresis response (their ability to excrete an exogenous water load). Their bladders were voided, and they were gavaged with a volume of water equivalent to 5% of their body weight. Urine was collected, and volumes were measured at frequent intervals. Results were evaluated in terms of changes in the shape of the age-specific temporal excretion curves and total volumes over the 150-minute test period (Table 2).

Because of the small group sizes used in this pilot study, the same pups were tested at each age, contrary to usual practice. The detectable responses produced by the positive control material indicate that some confidence may be placed in the results. Both uranium and colchicine exposures decreased urine volumes in the assays performed at 5 and 12 days of age; only at the latter time was osmolarity significantly affected. Uranium exposure increased excretion of the water load at 1 day of age but did not have a significant effect at older ages. These differences may be of significance, because the distribution study had shown that the uranium concentrations in fetal kidneys (0.05%/g) at 1 day after exposure were only about 1% of those in their dams (5.8%/g). More detailed experiments have been initiated to better define these relationships.

TABLE 2. Effects of Prenatal Exposure of Rats to ^{233}U on the Postnatal Development of Kidney Function.^(a)

Hydropenia Study

Days of Age at Evaluation	6-hour Urine Volume, μl			Osmolarity, mOsm		
	Control	Uranium ^(b)	Colchicine ^(c)	Control	Uranium	Colchicine
1	10.2 $\pm$ 2.4	10.2 $\pm$ 4.4	--	492 $\pm$ 53	507 $\pm$ 59	--
5	10.4 $\pm$ 1.4	7.9 $\pm$ 2.6 ^(d)	7.2 $\pm$ 1.2 ^(d)	677 $\pm$ 65	697 $\pm$ 89	537 $\pm$ 12 ^(d)
8	7.6 $\pm$ 1.5	7.2 $\pm$ 1.5	7.4 $\pm$ 0.7	795 $\pm$ 66	796 $\pm$ 111	742 $\pm$ 88
12	10.4 $\pm$ 2.1	8.8 $\pm$ 1.4 ^(d)	9.1 $\pm$ 0.4 ^(d)	876 $\pm$ 132	903 $\pm$ 92	759 $\pm$ 137

Diuresis Study

Days of Age at Evaluation	Percent Administered Volume Excreted in 150 Minutes		
	Control	Uranium	Colchicine
1	64 $\pm$ 10	80 $\pm$ 10 ^(d)	--
5	75 $\pm$ 7	72 $\pm$ 14	65 $\pm$ 10 ^(d)
8	80 $\pm$ 5	82 $\pm$ 6	90 $\pm$ 6 ^(d)
12	81 $\pm$ 6	76 $\pm$ 8	87 $\pm$ 7

(a) Presented as Mean $\pm$ Standard Deviation

(b) Dam Injected IV with 5.75 $\mu\text{Ci/kg}$ at 19 or 20 Days of Gestation

(c) 60 $\mu\text{g/kg}$ IP at 1 Day of Age

(d) Denotes Groups with Statistically Significant Difference from control at $P < 0.05$

• Gut-Related Studies of Radionuclide Toxicity

Principal Investigator: M. F. Sullivan

Other Investigators: P. S. Ruemmler and R. P. Schneider

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.

Research on this project has been directed toward mechanism studies that might contribute to an understanding of the transport system involved in the absorption of actinides from the gastrointestinal (GI) tract. It is known from previous research in this laboratory that absorption increased substantially when plutonium was gavaged to iron-deficient rats and mice that were made anemic by dietary deficiencies of iron. Other investigators have shown that plutonium binds to transferrin, the carrier system utilized in the absorption of iron, but it is not known if absorption of the trivalent actinides americium or curium or the pentavalent actinide neptunium is also increased when they are gavaged to iron-deficient animals.

Our earlier research demonstrated that both fasting and an excess of ferric iron increased absorption of gavaged ^{233}U , ^{237}Np , ^{238}Pu , and ^{244}Cm , while ferrous iron decreased absorption of these elements in fasted adult rats. Absorption of ^{238}Pu decreased in 2-day-old rats gavaged with high doses of supplemented ferric iron, suggesting that the mechanism of absorption by the neonate differed from that seen in the adult rat.

Influence of Iron Deficiency on the GI Absorption of Uranium, Neptunium, Americium and Curium

Like plutonium, uranium binds to transferrin and probably utilizes the iron transport system for absorption from the GI tract. Americium and curium are trivalent actinides that do not bind as firmly to transferrin as either hexavalent uranium or quadrivalent plutonium. We investigated whether iron deficiency increased absorption of uranium, neptunium, americium or curium as has been shown for ^{238}Pu .

Weanling rats were placed on an iron-deficient diet either immediately after weaning, or blood was withdrawn and they were maintained on an iron-deficient diet until their volume of packed red cells indicated they were anemic.

Results of these experiments are shown in Table 1, where comparisons can be made between absorption by iron-deficient rats and controls maintained on an iron-replete diet. The data demonstrate that absorption and retention increased, at various rates over controls, in iron-deficient rats given the different actinides: for uranium, 3.4 times; ^{237}Np , 7.1 times; ^{241}Am , 2.7 times; and curium, 1.7 times. These findings suggest that pentavalent ^{237}Np and hexavalent ^{238}Pu utilized the iron transport system more efficiently than the trivalent actinides ^{241}Am and ^{244}Cm .

Influence of Intragastrically Administered Iron on GI Absorption of ^{238}Pu by Rats Fed an Iron-Deficient Diet

To determine if plutonium and iron utilized the same transport system, anemic rats were gavaged with low doses of iron immediately prior to intragastric administration of ^{238}Pu nitrate, pH 2. Data obtained by measuring retention in the liver and carcass showed an iron-dose-related effect on plutonium absorption (Table 2). The higher iron dose, 120 $\mu\text{M}/\text{kg}$, blocked 94% of the amount of ^{238}Pu absorbed by iron-deficient rats, indicating competition for the same transport mechanism. In our earlier research with neonatal rats, iron blocked 93% of the ^{238}Pu absorption, suggesting that the mechanism of transport may be the same for both age groups. However, the reduction is not as obvious in iron-replete adult animals as in neonates because plutonium absorption is usually very low (<0.01%).

TABLE 1. Absorption and Retention of ^{233}U , ^{237}Np , ^{241}Am and ^{244}Cm by Rats Fed an Iron-Deficient or an Iron-Replete Diet and Gavaged with Nitrate Solutions of the Actinides. Rats were Killed 7 Days after Gavage.

Actinide	Dose, MBq/kg	Number of Rats	Dietary Iron Status	Weight, g	VPRC, %	Percent of Gavaged Dose $\pm$ SEM $\times$ 100		
						Lung	Liver	Kidney
^{233}U	1	8	Deficient	227	35	0	0.09 ± 0.02	2.4 ± 0.6
	1	6	Replete	228	48	0	0.03 ± 0.02	0.6 ± 0.3
^{237}Np	2	6	Deficient	180	31	0.01 ± 0.003	1 ± 0.4	0.3 ± 0.1
	1.3	6	Replete	213	48	0	0.1 ± 0.04	0.04 ± 0.01
^{241}Am	0.8	7	Deficient	223	36	0.03 ± 0.01	1.4 ± 0.4	0.01 ± 0.004
	1.1	5	Replete	210	45	0.02 ± 0.01	0.15 ± 0.05	0.05 ± 0.02
^{244}Cm	0.9	6	Deficient	182	30	0	1.3 ± 0.2	0.06 ± 0.01
	0.7	6	Replete	255	47	0	0.3 ± 0.2	0.01 ± 0.004

Actinide	Percent of Gavaged Dose $\pm$ SEM $\times$ 100					
	Femur	Skeleton ^(a)	Carcass	Total Retained ^(b)	Urine	Absorption ^(c)
^{233}U	0.3 ± 0.1	7.0 ± 1.8	5.9 ± 1.7	8.6 ± 2.3	16.8 ± 1.7	25.4 ± 3.5
	0.1 ± 0.06	2.1 ± 0.1	1.7 ± 0.6	2.4 ± 0.6	5.0 ± 0.9	7.4 ± 1.2
^{237}Np	0.7 ± 0.1	16.1 ± 2.8	16.0 ± 2.9	17.2 ± 3.3	14.1 ± 2.9	31.3 ± 4.4
	0.1 ± 0.03	2.3 ± 0.7	2.4 ± 0.7	2.6 ± 0.8	1.8 ± 0.3	4.4 ± 1.0
^{241}Am	0.02 ± 0.01	0.4 ± 0.2	2.4 ± 0.9	4.3 ± 1.1	0.5 ± 0.1	4.8 ± 1.2
	0.002 ± 0.002	0.03 ± 0.03	0.8 ± 0.1	1 ± 0.2	0.8 ± 0.2	1.8 ± 0.2
^{244}Cm	0.07 ± 0.01	1.6 ± 0.2	2.2 ± 0.2	3.6 ± 0.5	1.7 ± 0.6	5.3 ± 1.3
	0.01 ± 0.01	0.3 ± 0.2	1.4 ± 0.3	1.7 ± 0.5	1.4 ± 0.1	3.1 ± 0.5

(a) Amount in skeleton calculated by formula: femur value $\times$ 23

(b) Retention equals sum of carcass, liver, kidney, femur, lung values

(c) Absorption = retention + urine values

TABLE 2. Retention of ^{238}Pu by Rats Fed an Iron-Deficient Diet and Gavaged with 60 or 120 $\mu\text{M/kg}$ of Ferric Nitrate and/or 3.26 MBq of ^{238}Pu . Animals were Killed 7 Days Later^(a).

Treatment	N	Percent of Gavaged ^{238}Pu Dose $\pm$ SEM $\times$ 100			
		Lung	Liver	Carcass	Total Retained
^{238}Pu	5	0.02 ± 0.01	0.40 ± 0.10	61.0 ± 34	61.4 ± 34
$^{238}\text{Pu} + 120 \mu\text{M Fe}^3/\text{kg}$	5	0.01 ± 0.01	0.18 ± 0.04	3.6 ± 0.6	3.8 ± 0.6
$^{238}\text{Pu} + 60 \mu\text{M Fe}^3/\text{kg}$	4	0.02 ± 0.01	0.35 ± 0.10	0.8 ± 1.2	7.1 ± 1.3

(a) Rats were 1 month old when gavaged and weighed 85 g when killed

• Mutagenicity of Complex Chemical Mixtures

Principal Investigator: R. A. Pelroy

Other Investigator: D. L. Stewart

Technical Assistance: E. J. Sass

Mutational synergism was demonstrated between highly aromatic chemical mixtures and several classes of mutagens, including amino polycyclic aromatic hydrocarbons (amino-PAH), nitroaromatic hydrocarbons (NO₂-PAH), and the DNA intercalating agent ICR191. For amino-PAH, synergism was partly due to differential effects on rate of degradation versus activation by P450/448 metabolizing enzymes. Synergism was observed at comparatively low concentrations of the complex mixtures (5-20 µg/ml exposure medium), where the mixtures were in excess (200- to 500-fold) on a weight/weight mixture-to-mutagen basis. For several amino-PAH, complex chemical mixtures also lowered the apparent threshold concentration of the amino-PAH capable of inducing mutagenesis. Complex mixtures capable of interacting synergistically with amino-PAH, NO₂-PAH, and ICR191 failed to stimulate, or slightly inhibited, the mutagenic potential of benzo[a]pyrene.

Previous work suggested that the mutagenicity of 6-aminochrysene (6-AC) in the presence of highly aromatic complex chemical mixtures was much greater than could be accounted for by the mutagenicity of these mixtures alone or the amino polycyclic aromatic hydrocarbon (amino-PAH) alone. Only interactions between the aromatic constituents of a complex chemical mixture (i.e., a coal-derived distillate) and 6-AC induced the nonadditive effect. The aliphatic constituents from these complex mixtures were without effect.

The present work is a detailed examination of the dose-response relationships for mutagenesis induced by 6-AC in the presence and absence of a complex aromatic chemical mixture. Similar experiments were done with 6-nitrochrysene (6-NC), which is the nitroaromatic hydrocarbon (NO₂-PAH) homolog of 6-AC. Results are also presented for several other amino-PAH, 1-nitropyrene (1-NP), and the direct mutagen ICR191, with and without complex aromatic mixtures.

The premutagen 6-AC is activated by oxidation to a proximal metabolite mutagen by P450/448 enzymes in the S9 homogenates. Sufficiently high concentrations of 6-AC induced strong mutagenic responses in *Salmonella typhimurium*. However, lower concentrations of 6-AC induced no measurable mutagenic response in the *S. typhimurium* mutagen tester strains. This is shown for *S. typhimurium* TA98 in the fluctuation bioassay system (Figure 1). For concentrations of 6-AC ranging from 0.001 to 0.02 µg/ml (initial concentration in the exposure medium), the mutational dose-response curve was flat, showing virtually no increase in the induced mutation frequency over the spontaneous or background mutation frequency. In this set of exper-

iments, the lowest level of 6-AC that induced a significant response in the absence of coal liquid (CL) was not reached until 0.04 µg/ml initial concentration of promutagen. However, in the presence of a highly aromatic CL, a strong mutational response was induced for initial concentrations of 6-AC as low as 0.001 µg/ml of exposure medium. One of the major effects of CL was, therefore, to lower the threshold concentration for 6-AC capable of inducing frameshift mutagenesis in *S. typhimurium* TA98.

In contrast to 6-AC, which is activated to a proximal mutagen externally by P450/448 mixed-function oxidases (MFO) in the S9-homogenates, 6-NC is converted to a mutagen by reductases present within the target cells, in this case, cells of *S. typhimurium* TA98. Activation of 6-NC is therefore independent of the P450/448 MFO in the S9-homogenates (Figure 2). If an actively metabolizing P450/448 system was present during exposure of *S. typhimurium* TA98 to 6-NC, little or no induced mutation was observed. This suggests deactivation of the NO₂-PAH by MFO in S9-homogenate. If CL was also present in the assay system along with S9-homogenate, a highly significant, dose-dependent increase in induced mutagenesis was observed even at the lowest levels of 6-NC (0.05 µg/ml initial concentration in the exposure medium). Therefore, CL acted in such a way as to prevent S9-dependent degradation of the mutagenic potential associated with 6-NC. A reasonable interpretation of these observations is that CL prevented the S9-catalyzed degradation of 6-NC, thus allowing the bacterial enzymes inside *S. typhimurium* to reduce this compound to the active mutagenic metabolite, and increasing its apparent mutagenic potency.

FIGURE 1. Mutagenicity of 6-Aminochrysene (6-AC), in the Presence or Absence of a Crude Aromatic Mixture (CL) from a High-Boiling Coal Liquid. Symbols: (●) indicates 6-AC with CL at an initial concentration in the exposure medium of 5 µg/ml; (○) indicates the mutagenic response induced by 6-AC alone. Numbers near open/closed circles represent results from independent experiments. Asterisks near solid circles indicate that the induced mutagenic response was significantly greater than the noninduced (spontaneous) response ($P < 0.001$) and that the response was significantly greater ($P < 0.001$) than could be accounted for based on an additive model for 6-AC and CL acting independently. Induced mutation in the fluctuation assay was assumed to follow Poisson statistics. Metabolic activation provided by Aroclor-induced microsomes from rat liver (S9) homogenates.

FIGURE 2. Mutagenicity of 6-Nitrochrysene (6-NC) with or without 5 µg/ml Coal Liquid (CL). Symbols: (●) indicates mutagenic response for 6-NC plus CL 5 µg/ml plus 0.5% v/v S9 homogenate. (▲) indicates induced response for 6-NC plus S9 homogenate without CL. (○) indicates induced response for 6-NC, without CL or S9 homogenate. Independent experiments indicated by numbers next to symbols. Double asterisks indicate that the induced mutagenic response for 6-NC with CL and S9 homogenate were significantly greater than spontaneous mutational responses ($P < 0.001$) and also significantly greater ($P < 0.001$) than could be accounted for in terms of independent effects. Single asterisks indicate that mutagenic responses induced by 6-NC alone were significantly greater ($P < 0.001$) than spontaneous mutation. Metabolic activation provided by 0.5% v/v Aroclor-induced S9 homogenate.

The effect of CL or its neutral components on the mutagenicity of ICR191 (a DNA intercalating agent that does not require metabolic activation), 1-nitropyrene (1-NP), and benzo[a]pyrene (BaP), is shown by the data in Figure 3. In the absence of S9-homogenate, 1-NP and ICR191 were mutagenic for *S. typhimurium* TA98. The BaP, an indirect mutagen like 6-AC, requires metabolic activation by the P450/448 MFO in S9-homogenate and was inactive in the absence of S9-homogenate (data not shown). In the presence of S9-homogenate (and co-factors required for metabolic activation by the MFO), the mutagenicity of 1-NP and ICR191 was significantly reduced relative to that of the compounds assayed for mutagenicity without S9-homogenate. Inclusion of CL in the exposure system protected (or enhanced) the mutagenicity of ICR191 and 1-NP but had a slightly inhibitory effect on the mutagenicity of BaP. At much higher concentrations of CL than those shown in Figure 3, CL inhibited the mutagenicity of BaP strongly (data not shown). Therefore, CL was capable of protecting (activating) the mutagenic potential of 1-NP and ICR191 but not that of BaP.

Several amino-PAH were assayed for their mutagenicity in the presence and absence of chemical class fractions derived from CL (Figure 4). As can be seen, the mutagenicity of the aminochrysenes (1-AC, and 6-AC), the aminopyrenes (1-APy and 4-APy) and the mutagenicity of 3-aminophenanthrene (3-APhe) and 3-aminofluoranthene (3-AFlu) were all strongly enhanced in a

dose-dependent way in the presence of the neutral PAH (A_2) fraction from CL. The aliphatic constituents (A_1 fraction) had little or no effect on the mutagenicity of these amino-PAH. These data confirm previous results with 6-AC in the standard Ames plate incorporation bioassay and demonstrate that, in each case, the effects on the mutagenicity of the amino-PAH were statistically nonadditive, i.e., they could not be accounted for in terms of the amino-PAH and complex aromatic fractions acting independently.

FIGURE 3. Effect of Coal (CL) on the Mutagenicity of ICR191, 1-Nitropyrene (1-NP), and Benzo[a]pyrene (BaP). Cells were exposed for 2 hours at initial concentrations indicated. Symbols: solid bars indicate exposure of target cells in the absence of S9 homogenate. Stippled bars indicate exposure of cells to mutagens in the presence of S9 homogenates (2% v/v for ICR191, 0.5% for 1-NP, and 10% for BaP). Initial concentration of CL in the exposure medium are indicated. Solid triangles above a bar indicate response significantly greater ($P < 0.001$) than spontaneous response. Asterisk above a bar indicates that induced response was statistically significant ($P < 0.001$) and nonadditive for mutagen with CL ($P < 0.001$). Target strains of *Salmonella typhimurium* as indicated.

In quantitative terms, the A_2 fraction had the strongest effects on the mutagenic activity of the four-ring 6-AC, the five-ring 3-AFlu and the four-ring 4-APy. The A_2 fraction also appeared to lower the threshold concentrations of the amino-PAH capable of inducing mutagens in *S. typhim-*

urium TA98. On the other hand, the mutagenicity of the three-ring 3-APhe was measurable at all concentrations assayed (0.005 to 0.02 µg/ml exposure medium) with or without the A_2 fraction, suggesting little or no effect on the threshold limit for this amino-PAH. However, the level of induced mutation was greater at all levels of 3-APhe if the A_2 fraction was also present in the exposure system.

The major effect of CL on the mutagenicity of amino-PAH, NO_2 -PAH and ICR191 appeared to be at the level of the P450/448 MFO reactions catalyzed by the S9-homogenates. A logical hypothesis to explain these results is that aromatic compounds in CL compete with the polycyclic mutagens for ring-oxidizing MFO. According to this hypothesis, once these mutagens undergo ring oxidation, their mutagenicity is reduced or abolished. The aromatic compounds in CL are highly enriched in polycyclic aromatic compounds containing three to five condensed rings, e.g., phenanthrenes, chrysenes, pyrenes and a variety of five-ring structures such as benzo-pyrenes. Most or all of these compound types are likely substrates for the P450/448 MFO which oxidize aromatic rings in PAH systems, producing a variety of diols, tetrols and related compounds.

Conversely, the amino-PAH and NO_2 -PAH are activated by oxidation and reduction, respectively, of their exocyclic nitrogens. For example, amino-PAH and NO_2 -PAH with the same PAH nucleus (such as 6-AC and 6-NC, 1-APy and 1-NP) are probably converted to the same metabolite mutagens, albeit by different biochemical routes. If these metabolite mutagens require an intact PAH nucleus for maximum mutagenic activity, then competition for the ring-oxidizing MFO by the aromatic constituents of CL would enhance the apparent mutagenic potency of the amino-PAH and NO_2 -PAH. To explain the results for ICR191, it must be assumed that an intact polycyclic ring is required for effective intercalation in the DNA of the target cell, and that ring oxidation can effectively prevent or retard such intercalation and, thus, mutagenesis.

The results for BaP are consistent with what is known about the metabolic activation of this compound by P450/448 mixed-function enzymes. Unlike the amino-PAH, NO_2 -PAH and ICR191 used in this study, BaP requires oxidation of the ring to generate metabolite mutagens. It would therefore be expected that the aromatic constituents of CL would inhibit rather than enhance the metabolic activation of BaP to its diol epoxide, metabolite mutagen form. Under the conditions of the experiments reported, CL was a weak inhibitor of mutagenicity by BaP.

FIGURE 4. Mutagenicity of Six Amino Polycyclic Aromatic Hydrocarbons (amino-PAH), With and Without Complex Chemical Mixtures. Symbols: (○) indicates mutational response for each of the amino-PAH alone. (●) indicates mutational response in the presence of the neutral polyaromatic (A₂) fraction of CL. The A₂ fraction was present at the following initial concentrations in the exposure medium; 5 µg/ml for 6-aminochrysene (6-AC), 3-aminofluoranthene (3-AFlu) and 3-aminophenanthrene (3-APhe); 20 µg/ml for 1-aminochrysene (1-AC), 4-aminopyrene (4-APy) and 1-aminopyrene (1-APy); (▲) indicates induced mutational responses for amino-PAH with the neutral aliphatic (A₁) fraction from CL. Initial concentrations of A₁ fraction in the exposure medium with the various amino-PAH same as for A₂ fraction. Decimals in parentheses indicate level of significance (P-value) for cells exposed for amino-PAH versus unexposed cells. Decimals in brackets indicate level of significance (P-value) for nonadditive, i.e., synergistic, effects between the complex mixtures and amino-PAH. Metabolic induction supplied by 0.5% v/v S9 homogenate (6-AC, 3-AFlu, 3-APhe) or 1% v/v S9 homogenate (1-AC, 4-APy, 1-APy).

The details of the mechanism(s) by which the aromatic components of CL and related complex chemical mixtures influence the mutagenicity of mutagens and promutagens is under investigation. However, the data strongly suggest that complex chemical mixtures that are highly enriched in aromatic constituents can strongly influence the mutagenic potency of individual compound types, when they are either present

in the mixtures or added simultaneously to the bioassay system. The data thus far favor the hypothesis that most of the effects are exerted at the level of metabolic activation, i.e., external to the target cells and their DNA-targets. However, more direct effects at the level of DNA-repair/induced mutagenesis cannot be excluded.

• Complex Mixtures Biostudies

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The objective of the project is to identify potential adverse biological activities associated with human exposures to complex organic mixtures (COM) from energy-related industries. Studies to identify the influence of chemical class fractions from a COM on the initiating activity of a known carcinogen, benzo(a)pyrene (BaP), demonstrated that the polycyclic aromatic hydrocarbons (PAH) and nitrogen-containing polycyclic aromatic compound (NPAC) fractions were the most effective inhibitors of initiation. The aliphatic and hydroxylated PAH were the least effective, indicating that compounds with structures similar to that of BaP were the most effective inhibitors. In an effort to determine the contribution of BaP to the initiating activity of the COM, binding of radiolabeled BaP to mouse skin DNA was measured. Results indicated that binding of BaP to DNA decreased in the presence of the COM so that at initiating COM doses, BaP binding was near the limit of detection. Addition of unlabeled BaP to the COM at an amount similar to that originally present in the COM did not significantly increase the binding. Studies to determine the rates of disappearance of carcinogenic PAH from the site of application on the skin indicated that half-lives for PAH differed by a factor of about 2; that the half-life for BaP was near the middle of the range for the PAH evaluated; and that the PAH fraction increased the half-life of BaP relative to its half-life when administered alone. Analytical methods developed to identify PAH from COM which covalently bind to DNA demonstrated that the lower level of detection is approximately 200 picograms. Developmental studies demonstrated that both pregnant rats and mice treated dermally with a high-boiling COM developed fetuses with major malformations including cleft palate, small lungs, edema, and sagittal suture hemorrhages. These effects occurred at doses not unlike those that may be anticipated from occupational exposures. Adverse cardiovascular effects were also observed following dermal exposure to a high-boiling COM but not after treatment with a middle-boiling-range material. Histopathological examination of organs from mice exposed by inhalation to a high-boiling COM demonstrated that this material caused lung and skin tumors after either 4 or 12 weeks exposure.

Since occupational exposures are nearly always to mixtures of organic materials, the Department of Energy has a critical need to develop fundamental information to predict the toxic effects of materials when they are presented to humans as mixtures. Because of this need, we are conducting multi-level studies to develop a database from which toxicological principles can be derived for evaluating complex organic mixtures (COM). For most of this work the end-point is carcinogenicity, but other effects are also considered. Since previous work in our laboratory identified several coal-derived COM, which produce adverse health effects in rodents, we are using these as positive-control materials. The advantages of using these materials include: 1) certain of them are carcinogenic, others are not; 2) they are well-characterized chemically; 3) they are composed of several classes of compounds and can be readily separated into these chemical classes; and 4) there is a substantial database on the effects of many of these materials both in *in vitro* and *in vivo* systems.

INITIATION/PROMOTION STUDIES

A substantial body of information has accumulated indicating that the mouse skin tumor-initiating activity of known carcinogens is not fully expressed when the carcinogen is present in a complex mixture. We reported last year on the effects of distillates that had discrete boiling ranges on the tumor-initiating activity of benzo(a)pyrene (BaP). Those data showed that distillates boiling above 700°F decreased BaP initiating activity by 40-70%. For the current study we fractionated one of these distillates (boiling range 750-800°F) in an attempt to identify the chemical classes responsible for the inhibition of BaP activity. Twenty-five- μ g doses of BaP in methylene chloride or in methylene chloride containing 5 mg of the 750-800°F distillate or its chemical class fractions (aliphatic, polycyclic aromatic hydrocarbons [PAH], nitrogen-containing polycyclic aromatic compounds [NPAC], and hydroxylated PAH [HPAH]) were applied to the dorsal surface of female Charles River CD-1 mice. The chemical class fractions

were administered in the same proportion in which they were found in the parent distillate. Beginning 2 weeks after initiation, the mice were treated dermally with twice weekly applications of 5 µg of 12-O-tetradecanoylphorbol-13-acetate for 24 weeks.

The results of this experiment are shown in Table 1. BaP (25 µg) induced tumors in 97% of the mice, with a total of 216 tumors/group of 30. The aliphatic, NPAC and HPAH fractions did not exhibit greater activity than the vehicle controls. Both the 750-800°F distillate and its PAH fraction had significant initiating activity, but much less than for BaP alone. The initiating activity of BaP was significantly lower when it was co-administered with the 750-800°F distillate or with its PAH or NPAC fractions. The aliphatic and HPAH fractions had minimal effect on expression of BaP initiating activity. These results indicate, again, that the carcinogenic activity of known carcinogens may be inhibited when they are present in a COM. Furthermore, this inhibition may be associated with certain aromatic constituents of the mixture. However, hydroxylated aromatic compounds apparently do not participate, in any major way, in the inhibition.

TABLE 1. Effect of Chemical Class Fractions on Benzo[a]pyrene (BaP) Skin-Tumor-Initiating Activity.

Initiator	% Incidence	Number of Tumors/30 Mice
Methylene Chloride	21	7
BaP	97	216
750-800°F Distillate	48	21
Aliphatic Fraction	13	4
PAH ^(a)	53	20
NPAC ^(b)	23	8
HPAH ^(c)	20	7
BaP + 750-800°F Distillates	83	67
BaP + Aliphatic	97	172
BaP + PAH	73	75
BaP + NPAC	78	84
BaP + HPAH	100	193

(a) Polycyclic aromatic hydrocarbons

(b) Nitrogen-containing polycyclic aromatic compounds

(c) Hydroxylated PAH

DNA Binding and Adducts

As indicated above, co-administration of complex organic mixtures with a known carcinogen (BaP) altered the skin-tumor-initiating activity of BaP with certain mixtures but not with others. Previously, we determined the influence of two COM

(both contained approximately 50 µg of endogenous BaP) on the DNA binding of BaP. Twenty-four hours after dosing, binding to mouse-skin DNA was inhibited by about 98% when it was present in the COM, relative to the binding for BaP administered alone. These data, along with the carcinogenicity results, suggest that the BaP contribution to COM initiating activity is small.

The quantity of BaP adducts formed may be influenced by the effect of the COM on the rate of BaP disappearance from the site of application. Because of this, mice were treated dermally with radiolabeled BaP, and we determined the time course for disappearance of BaP from the skin. Results from this study demonstrated that the times required for 50% of the applied dose to disappear for BaP, for BaP + an 800-850°F distillate and for BaP + >850°F distillate were 4, 12, and 29 hours, respectively (Figure 1). Since the length of time that BaP was present at the site of application increased in the presence of the COM, binding studies were conducted at 48 and 72 hours after dosing. Under these conditions, BaP binding was inhibited to about the same extent as was observed at 24 hours after treatment.

FIGURE 1. Percent of Administered Dose of Radiolabeled Benzo[a]pyrene Remaining at the Dermal Application Site from the Time of Treatment Through 72 Hours After Exposure. BaP = benzo[a]pyrene.

Currently, studies are underway to determine the influence of the COM on the DNA adducts profile and to identify the PAH in the mixtures that bind to DNA. For the

profile studies, mice were treated with tritium-labeled BaP; 24 hours later, the DNA was isolated and extensively purified. The DNA was then enzymatically hydrolyzed to nucleosides, and adducted nucleosides were isolated by LH-20 chromatography. These samples are being further separated by high-performance liquid chromatography to provide the adducts profile. They will then be compared to standard BaP adducts prepared from reactions of the syn and anti-isomers of BaP-diol-epoxide (BPDE) with calf thymus DNA. We anticipate that results from these studies will be available in the near future.

Studies to identify the carcinogenic PAH present in the COM which covalently bind to DNA have been initiated. Our procedure is to isolate BaP-adducted DNA from mouse skin and to cleave the hydrocarbon moiety from the DNA by acid hydrolysis. The released hydrocarbons (primarily tetraols) are derivatized to increase their volatility, then separated and characterized by gas chromatography (GC)/mass spectrometry (MS) procedures. Using this method, the lower level of sensitivity was about 200 femtomoles, indicating that sensitivity is adequate to evaluate unlabeled PAH adducts (those other than BaP). Currently, we are preparing unlabeled adducts from reactions of diol-epoxides of chrysene and benz[a]anthracene with calf thymus DNA as a first step toward obtaining standards for determining the PAH from COM which covalently bind to DNA.

Metabolism

High-boiling COM are carcinogenic, with the neutral PAH fraction being the most active as a skin-tumor initiator. However, these carcinogenic COM have been shown to suppress the skin tumorigenicity of added BaP. Thus, the carcinogenicity of COM are not additive with that of BaP but, rather, appear to be inhibitory. The COM inhibit the covalent binding of BaP to mouse-skin DNA *in vivo* and are inhibitors of BaP metabolism *in vitro*. The mechanism of these effects has not been definitively established, but it is consistent with the "mass-action" inhibition of PAH-induced mutagenesis previously observed with COM.

Recent information in the literature indicates that, using an *in vitro* skin culture system, the rate of BaP uptake from mouse skin may be at least partially limited by the oxidation rate of BaP by the mixed-function-oxidase enzymes in mouse skin. This led us to hypothesize that the co-application of BaP and COM to mouse skin would reduce the rate of BaP uptake and metabolic activation by skin mixed-function oxidases, which may account, at least partially, for the suppression of BaP carcinogenicity by COM. Therefore, we exam-

ined the effects of several COM on the rate of disappearance of BaP from mouse skin, using either radioactive tracer techniques or gas-liquid chromatographic procedures. In addition, we have determined the individual rates of disappearance from mouse skin of 12 compounds in the PAH fraction isolated from a high-boiling COM.

Female CD-1 mice (four/group) were shaved 1-2 days prior to dosing, and the shaved areas were treated with either 25 or 50 μ g of BaP, or with 8 mg of the PAH fraction isolated, as previously described, from the 800-850°F COM. Since the 8-mg dose of the PAH fraction from the 800-850°F material contained 27 μ g BaP, unlabeled BaP was added so that the 8-mg PAH dose contained 50 μ g BaP.

Twelve compounds from the PAH fraction of the 800-850°F COM were selected for quantitative analysis: pyrene, benz[a]anthracene, benzo[b]fluorene, BaP, chrysene, 4- or 6-methylchrysene, two additional methylchrysenes, a C_4 -substituted pyrene, benzo[j or b]fluoranthene, a C_2 -substituted chrysene, and benzo[e]pyrene. At intervals of 0, 1, 2, 4, 8, 16, or 24 hours after dosing, the mice were killed, the dosed areas of skin removed and digested with proteinase K, then extracted with hexane. The hexane fractions were concentrated under a stream of nitrogen and analyzed by GC. Recoveries for the PAH were determined at time 0 by comparing the PAH recovered from mouse skin with those in the original dosing solution. The biological half-lives of the individual PAH were determined from their slopes on a semi-log plot of percent recovered versus time. In addition, the unmetabolized BaP in the skin digests of mice treated with ^{14}C -labeled BaP was determined by liquid scintillation counting of the hexane extract. Total metabolites of BaP were determined by counting aliquots of the aqueous phase.

Samples designated for GC were "spiked" with two internal standards because of the occasional co-chromatography of endogenous substances with the internal standards. The chromatographic separation of the PAH fraction was carried out using a Hewlett-Packard gas chromatograph, model 5880, equipped with a 30-m DB-5 fused silica capillary column and flame ionization detector. Calibration was performed with NBS standard PAH mixture, SRM 1647, plus individual PAH of four-six rings. Selected samples were also analyzed by GC/MS to verify the compound identifications. The recoveries of the 12 selected PAH at time 0 ranged from 77-92%. The recovery of a 25- μ g dose of BaP alone (i.e., not in the presence of a COM) at time 0 was $95 \pm 3\%$, versus $84 \pm 4\%$ in the presence of the PAH

fraction. The biological half-lives on mouse skin of the 12 selected PAH at 1-24 hours after dosing are shown in Table 2. At 1 hour after dosing the recoveries of the individual PAH ranged from 46-57% and declined to between 3 and 12% at 24 hours after dosing. The half-lives of the 12 PAH on mouse skin ranged from 5.3-10.0 hours. The skin half-life of a 25- μ g dose of BaP applied alone (not in the presence of a complex mixture) was 3.0 hours, as opposed to 7.7 hours for the same amount of BaP in the presence of the PAH fraction of the COM, indicating that BaP was reasonably representative of the group.

TABLE 2. Recoveries and Biological Half-Lives, in Mouse Skin, of Benzo[a]pyrene and 12 Selected Polycyclic Aromatic Hydrocarbons from a Complex Organic Mixture.

Compound	% Recovery ^(c)	Skin Half-Life, ^(d) hours
Benzo[a]pyrene ^(a)	95 $\pm$ 3	3.0 (2.8- 3.3)
Pyrene ^(b)	92 $\pm$ 10	5.3 (4.6- 6.1)
Benz[a]anthracene ^(b)	83 $\pm$ 2	7.1 (6.0- 8.5)
Benzo[b]fluorene ^(b)	84 $\pm$ 3	7.4 (6.2- 9.1)
Benzo[a]pyrene ^(b)	84 $\pm$ 4	7.7 (6.1- 9.7)
Methylchrysene ^(b)	83 $\pm$ 2	7.7 (7.3- 8.1)
Chrysene ^(b)	87 $\pm$ 2	8.1 (6.7-10.2)
4 or 6-Methylchrysene ^(b)	81 $\pm$ 3	8.4 (7.1-10.2)
Methylchrysene ^(b)	80 $\pm$ 1	8.4 (7.1-10.4)
C ₄ -Pyrene ^(b)	83 $\pm$ 8	8.8 (7.4-11.0)
Benzo[j] or b]fluoranthene ^(b)	81 $\pm$ 8	9.4 (7.7-12.1)
C ₂ -Chrysene ^(b)	77 $\pm$ 3	9.9 (8.2-12.5)
Benzo[e]pyrene ^(b)	81 $\pm$ 2	10.0 (7.9-13.7)

(a) Benzo[a]pyrene applied dermally, 25 μ g per mouse (with out complex mixture)

(b) Identified as a component of the polycyclic aromatic hydrocarbon fraction isolated from a 800-850°F boiling-range complex organic mixture applied dermally at 8 mg per mouse

(c) Mean $\pm$ standard error of mean

(d) Mean and lower and upper bounds of 95% confidence interval

Within this limited data set there appears to be some association of half-life with the number of rings in the parent PAH. Two of the three four-ringed parent PAH identified, pyrene and benz[a]anthracene, had the shortest half-lives of the compounds identified, while a five-ringed PAH, benzo[e]pyrene, had the longest. However, chrysene (four rings) had a longer skin half-life than BaP (five rings), suggest-

ing either that chrysene is unique or that the correlation between number of rings and skin half-life is imperfect. The three identified methylchrysenes all had approximately the same skin half-life as the parent chrysene, therefore the effect of methylation on half-lives of PAH on skin appears to be minimal.

The inclusion of BaP within either the 800-850°F COM, the >850°F COM (Figure 1) or the PAH fraction markedly increased the skin half-life of the BaP relative to its half-life when applied alone. This may be due to the competitive inhibition of BaP metabolism by the other components of the applied complex mixture. It may also be related to the inhibition of skin-tumor-initiating activity and the inhibition of BaP binding in skin by COM. Further research will be required to clarify this point.

Oncogene Studies

Studies have been initiated to determine the types of oncogenes activated in tumors produced from exposure to COM. Several independent reports have shown that point mutations occurring in the 12th and 59-61st codons of ras genes, following exposure to specific carcinogens, can produce dominant-acting oncogenes. Results from the same studies also suggest that a specific carcinogen/mutation causes exactly the same mutation in >90% of the tumors from these animals. It may therefore be possible to determine the nature (chemical class) of the agent responsible for inducing the effect by identifying the mutations that are produced.

In order to test this hypothesis, we have isolated DNA from about 30 papillomas induced with BaP or with the PAH or NPAC fractions from the >850°F distillate from solvent refined coal (SRC)-II. These preparations will be digested with the restriction enzyme (Xba), then probed with viral Ha-ras gene. Results will provide information on the relationship between activation of specific oncogenes and tumors produced by COM, their chemical class fractions and individual carcinogens.

Antibodies to DNA Adducts

The purpose of these studies was to develop immunological reagents of broad specificity for detecting DNA adducts, using monoclonal or polyclonal antibodies coupled with radioimmune, ELISA and/or fluorometric indicators. These studies included selecting appropriate carrier molecules, preparing methods for coupling the hapten molecule to the carrier, and preparing appropriate antigens for immuniza-

tion. BaP was selected as a model PAH carcinogen and poly-L-lysine (PLL) as the carrier molecule. Initially, 0.15 μ moles of BPDE, dissolved in 25 μ l tetrahydrofuran, was incubated with 1 mg of PLL suspended in 200 μ l of 0.1 M sodium acetate buffer, pH 7.3. Under these conditions, approximately 8% of the BPDE was bound to PLL, indicating that reactive PAH metabolites could be coupled to the weakly antigenic carrier molecule, PLL. Next, we will prepare larger quantities of the antigen, administer them to mice, isolate the antibody and determine the binding characteristics for the antibody and BaP-adducted DNA.

Inhalation Studies

Previously, we reported on a subchronic study in which CD-1 mice (Charles River) were exposed by inhalation to SRC-II heavy distillate (HD; 550-850°F). In addition to animals for the subchronic study, other mice (30/group) were exposed for 6 hours/day, 5 days/week, for either 1, 4 or 12 weeks to aerosol concentrations of 0.69 ± 0.03 , 0.14 ± 0.01 , 0.03 ± 0.003 or 0.0 mg HD/L of air for the high, middle, low or control groups, respectively. Particle sizes ranged between 1.7 and 1.8 μ m mass median aerodynamic diameter, with a geometric standard deviation of approximately 2.0. After exposure, these animals were observed daily, weighed weekly for several weeks, then monthly for the remainder of their lives. Statistical evaluation of survival data indicated significant decreases in the survival curves for 12-week-exposed animals from all three dose groups and for 4-week-exposed animals in the high-dose group. Decreases were also observed in body-weight curves for exposed mice, compared to those of controls, with the most pronounced effects in the group exposed for 12 weeks to the high dose.

Results of light microscopic examination of organ samples indicated that a significant number of mice exposed to the high and middle doses for 12 weeks developed alveolar epithelial hyperplasia, alveolar/bronchiolar adenomas and alveolar/bronchiolar carcinomas (Table 3). Similar lesions were also observed for 4-week-exposed, high-dose animals, although the incidence and frequency of these lesions were lower than for animals exposed to the same concentration for 12 weeks. In addition, a significant number of high-dose animals from the 4- and 12-week exposure groups developed skin tumors, both papillomas and carcinomas. Squamous cell papillomas of the forestomach were observed in 3 of 26 12-week-exposed, high-dose-group animals. Although not statistically significant, this incidence suggests that the forestomach may be a target organ after long-term exposures.

TABLE 3. Incidence of Lung Tumors, as Observed by Microscopic Examination of Tissue Sections, from Mice Exposed for 12 Weeks to Solvent Refined Coal-II Heavy Distillate and Evaluated at the End of Their Life Span.

	Control	Low	Middle	High
Alveolar Epithelial Hyperplasia	4/27	2/27	15/30	20/29
Alveolar/Bronchiolar Adenoma	4/27	4/27	18/30	19/29
Alveolar/Bronchiolar Carcinoma	5/27	4/27	15/30	19/29
Alveolar/Bronchiolar Adenoma + Alveolar/Bronchiolar Carcinoma	8/27	8/27	23/30	25/29

In addition to the carcinogenic responses described above, we also observed noncarcinogenic changes in the nasal cavity, including atrophy of the olfactory epithelium and suppurative inflammation. Incidence of these lesions were higher than those in controls only in 12-week-exposed, high-dose animals.

Results from this study indicate that relatively short-term inhalation exposures to a high-boiling coal liquid resulted in carcinogenic lesions in tissues that had direct contact with the test material.

Developmental Studies

Previous studies have shown that oral administration or inhalation exposure of high-boiling COM to pregnant rats from 11 to 16 days of gestation results in embryolethality, reduced growth and a number of fetal abnormalities, including cleft palates and small lungs. Since exposure by the dermal route is one of the most likely routes to be encountered in the work place, we determined the responses of both rats and mice after topical application of HD. For this study, pregnant Sprague-Dawley rats and CD-1 mice were treated dermally at doses of 500 or 1500 mg/kg body weight on 11-15 days of gestation. The rats were killed on day 20 of gestation, and the mice on day 18 of gestation. The uterus was removed, and the fetuses were weighed and examined for gross, visceral, and skeletal abnormalities.

Maternal weight gains in rats were inhibited by both dose levels of HD; however, for mice the decrease was not statistically significant. For the rat, the smaller weight gain during gestation reflected a lower weight for the products of conception as well as lower maternal

extragestational weight gain. The lower weight for the products of conception accounted for all of the decrease in weight gain in the mice. Maternal liver and kidney weights increased and thymus weights decreased, in a dose-dependent manner, for both species.

The incidence of resorptions in rats, occurring during middle and late gestation, increased significantly in both treatment groups; for mice, they were limited to the midgestational period. Overall, the number of live fetuses/litter decreased in a dose-dependent manner in both species, although the effect was more pronounced for rats.

Fetal weights for mice were unaffected by HD exposure; however, there was a dose-related decrease in fetal rats' weights. Placental weights followed a similar pattern. The most dramatic difference between rats and mice was in lung response. Fetal lung weights for rats decreased in a dose-dependent manner, and lungs for fetuses from the high-dose group weighed about 75% less than those from control animals. For mice, there were no significant differences in lung weights between treated and control fetuses. The incidence of small lungs was estimated: when lung weights were two standard deviations below those of controls on both a relative and absolute weight basis, they were considered small (Tables 4 and 5). When evaluated in this manner, 100% of the rat fetuses from both dose groups were small, but for mice, the incidence was substantially less (i.e., 25% for the high-dose group).

The incidence of cleft palate in the high-dose group was about 50% for both rats and mice; it was less than 10% in the low-dose group. Other, less dramatic abnormalities included renal pelvic cavitation and dilated ureters in mice, and edema and a midcranial surface lesion in rats.

Results from this study demonstrate considerable similarity between rats and mice with regard to prenatal effects resulting from dermal exposure to a high-boiling COM. For both species, growth retardation and cleft palates were observed; however, small lungs in mice were observed at substantially lower incidences than for rats. These species differences may indicate that: 1) constituents responsible for cleft palate are different from those that induce small lungs; 2) there are differences in the metabolite profiles between the two species; 3) lung development in the rat is more sensitive than in mice to constituents or metabolites from the mixture, or 4) there are other, more generalized metabolic differences between the two species.

TABLE 4. Summary of Morphological Findings in Rat Fetuses from Dams Dermally Exposed to a Complex Organic Mixture on Days 11-15 of Gestation.

Observation	Control	Dose Group	
		Low	High
Number Examined ^(a)	220/16	157/17	40/14
Gross/Visceral Lesions			
Small Lungs	0/0	157/17 ^(b)	39/14 ^(b)
Cleft Palate	0/0	8 ^(b) /4	22/10 ^(b)
Edema	0/0	17/7 ^(b)	24/9 ^(b)
Midcranial Lesion	0/0	23/5 ^(b)	18/7 ^(b)
Dilated Ureter	6/6	0/0 ^(b)	0/0 ^(b)
Number Skeletons Examined	16/91	59/15	16/11
Reduced Cranial Ossification	2/1	59/15 ^(b)	16/11 ^(b)

(a) Expressed as number of fetuses/number of litters

(b) Significantly different from control ($P < 0.05$)

TABLE 5. Summary of Morphological Findings in Mouse Fetuses from Dams Dermally Exposed to a Complex Organic Mixture on Days 11-15 of Gestation.

Observation	Control	Low	High
Number Examined ^(a)	89/7	62/7	52/7
Gross/Visceral Lesions			
Small Lungs	1/1	1/1	13 ^(b) /3
Cleft Palate	0/0	5 ^(b) /3	26/7 ^(b)
Renal Pelvic Cavitation	4/3	13 ^(b) /4	24/7 ^(b)
Dilated Ureter	1/1	12 ^(b) /4	15 ^(b) /4

(a) Expressed as number of fetuses/number of litters

(b) Significantly different from control ($P < 0.05$)

CARDIOVASCULAR STUDIES

Over one-half the total annual mortality in the United States is due to cardiovascular disease. Other than the overwhelming evidence linking cigarette smoking to a greater risk of developing cardiovascular disease, correlations between environmental exposure to toxic materials and cardiovascular disease have not been extensively examined. Recent evidence indicates that PAH induce cardiovascular diseases. Both dimethylbenzanthrene and BaP treatment have been shown to result in significant increases in the incidence and size of atherosclerotic lesions in the chicken. We have previously observed a dramatic increase in blood pressure of rats exposed by whole-body inhalation to HD. In a preliminary experiment, we found evidence suggesting that the blood pressure of anesthetized, dermally exposed rats may also be elevated following HD treatment. Therefore, a study was con-

ducted to investigate the effects of dermal exposure to HD and middle distillate (MD) on blood pressure and heart rate of anesthetized, conscious and captopril-treated rats.

Nine-week-old, male Fischer rats were exposed to 400 $\mu\text{g/kg}$ of HD or MD by dermal application (5 days/week for 6 weeks). The material, in acetone, was applied on the shaved dorsal region of the animal. After a 10-day recovery period, each rat was anesthetized with sodium pentobarbital, and the femoral artery was cannulated. Blood pressure was measured directly, using a Statham pressure transducer and a Grass polygraph. One day later, after the rats had recovered from the anesthesia, a second blood-pressure measurement was recorded for conscious rats, before and 30 minutes after the injection of 5 mg/kg of captopril.

The HD exposure significantly ($P < 0.05$) increased blood pressure of dermally exposed anesthetized rats compared to those of control and vehicle control animals (Figure 2). Blood pressures of HD-exposed and control rats were not significantly different in the conscious rat, although these results approached significance ($P = 0.07$). Captopril, an angiotensin inhibitor, did not significantly alter blood pressure in the conscious rats. Although these data suggest that the anesthesia may have differentially affected blood pressure in the HD-exposed rat compared to the control, the difference between anesthetized and conscious rats may be due to the small number of animals evaluated ($N = 7/\text{group}$). Nevertheless, these results suggest that HD does not act directly on the pulmonary system to produce cardiotoxic effects.

Exposure to MD did not significantly alter blood pressure in the anesthetized or the conscious rat compared to controls (Figure 2). These results suggest that the observed effect from HD exposure is not a general phenomenon and that the active material responsible for the elevated blood pressure is a component of HD rather than of MD.

Exposure to HD increased the heart rate of anesthetized rats compared to control or vehicle control animals, whereas MD exposure had no effect on heart rate (Figure 3). Although the heart rate of conscious HD-exposed rats tended to exceed that of the control groups, this trend was not statistically different. Heart rate was increased in the captopril-treated (control) rats compared to the anesthetized or conscious control rats, but no significant differences were observed among the various treatment groups given captopril.

FIGURE 2. Blood Pressure Following Dermal Exposure of Rats to Two Complex Organic Mixtures ($\bar{X} \pm \text{SEM}$; *, $P < 0.05$). MD = middle distillate; HD = heavy distillate.

FIGURE 3. Heart Rate Following Dermal Exposure of Rats to Two Complex Organic Mixtures ($\bar{X} \pm \text{SEM}$; *, $P < 0.05$). MD = middle distillate; HD = heavy distillate.

The results from these studies suggest that the cardiovascular system could be detrimentally affected by exposure to complex mixtures, but further studies are required to demonstrate that a health problem exists. We are currently attempting to develop an atherosclerotic model in the mouse to study the effects of complex mixtures. We also plan to investigate the effects of the chemical class fractions of HD on blood pressure in rats.

Fig. 1. Effect of different concentrations of the substance on the measured variable. The y-axis represents the measured variable (0-100). The x-axis represents the concentration levels (Control, 10%, 20%, 30%, 40%, 50%).

Fig. 2. Effect of different concentrations of the substance on the measured variable. The y-axis represents the measured variable (0-100). The x-axis represents the concentration levels (Control, 10%, 20%, 30%, 40%, 50%).

The results from the present study show that the concentration of the substance has a significant effect on the measured variable. The effect is dose-dependent, with higher concentrations leading to a greater decrease in the measured variable. This suggests that the substance may have a inhibitory effect on the process being measured.

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• Teratology of Complex Mixtures

Principal Investigator: T. J. Mast

Other Investigators: P. L. Hackett, R. L. Rommereim, and D. L. Springer

The dermal exposure of pregnant rats to SRC-II heavy distillate results in a highly significant incidence of fetal abnormalities. Exposure to four different chemical class fractions of this mixture indicated that nearly 100% of the activity lay in the polycyclic aromatic hydrocarbon fraction.

Previous studies on coal-derived complex mixtures have demonstrated that these materials produce dose-related teratogenic effects in rats when administered to dams by either an inhalation or an oral route. It has also been established that the higher boiling components ($>700^{\circ}\text{C}$) are more teratogenic than the lower boiling ranges. However, further attempts to isolate an active fraction by obtaining cuts of narrower boiling ranges resulted only in a smearing of the teratogenic activity across the fractions. Since the teratogenically active components could not be isolated on the basis of boiling point ranges, an alternative approach, based on chemical class separation by liquid chromatography, was used.

The SRC-II heavy distillate (HD), previously shown to be teratogenic when administered orally to rats, was chromatographically separated into four chemical class fractions. The major components of each of the four fractions obtained from alumina column chromatography, were: 1) aliphatic hydrocarbons (AH), 2) polycyclic aromatic hydrocarbons (PAH), 3) nitrogen-containing polycyclic aromatic compounds (NPAC), and 4) hydroxylated PAH (HPAH). Seven treatment groups of pregnant rats were dermally exposed to whole HD, to each of the four chemical class fractions individually, to a mixture consisting of the recombined fractions, or to an acetone control. The dermal rather than the oral route was chosen because of solubility problems with both the whole material and the fractions, as well as to alleviate toxicity to the gastric system.

The mixtures were applied in acetone at a constant dosing volume (1.0 ml/kg body weight) to the shaved backs of the dams on 11-15 days of gestation (dg). The whole HD and recombined mixtures were administered at 500 mg/kg, a dose previously shown to produce a significant incidence of terata but no maternal deaths and a low number of fetal deaths. Dose levels for the chemical class fractions were adjusted so that the dosage of each fraction was equivalent to its proportion by weight in the original

mixture (Table 1). Dams were weighed on 0, 6, and 11-15 dg, and again at the time of sacrifice on 20 dg.

TABLE 1. Chemical Class Fractions of a Coal-Derived Complex Mixture, Solvent Refined Coal (SRC)-II Heavy Distillate (HD),^(a) Obtained from Alumina Column Chromatography.

	% of Total	Dose, mg/kg
Aliphatic Hydrocarbons	20	98
Polycyclic Aromatic Hydrocarbons	56	280
Nitrogen PAH ^(b)	17	86
Hydroxylated PAH	10	48
	103	500 ^(c)

(a) Dose of whole SRC-II HD = 500 mg/kg body weight

(b) PAH = polycyclic aromatic hydrocarbon

(c) Dose of recombined mixture = 500 mg/kg

In the case of continuous variables such as weight, statistical significance on a per-litter basis was determined by Duncan's multiple range *t*-test. The statistical significance of the incidence data was determined by this same test after an arcsin transformation of the data.

The whole HD and the recombined mixture were both fetotoxic when administered by the dermal route, but no maternal deaths occurred. Results for the four individually administered chemical class fractions clearly demonstrate that the fetotoxicity of whole HD lies in the PAH fraction. This was also the only chromatographic fraction that caused a significant reduction in maternal body weight, gravid uterine weight and extragestational weight gain, compared to those values for controls (Table 2). Only the PAH fraction resulted in a significant reduction in litter size and an increased number of resorptions, although the latter was not statistically significant (Table 3).

TABLE 2. Effect on Maternal Weight Parameters of Dermal Exposure of Pregnant Rats to Solvent Refined Coal (SRC)-II Heavy Distillate.

Treatment(a)	Body Weight, g	Gravid Uterine Weight, g	Extagestational Weight Gain, g
Control	367 ± 34	73 ± 22	52 ± 14
Whole HD	330 ± 29(c)	58 ± 13	30 ± 12(c)
AH	358 ± 31	64 ± 28	53 ± 15
PAH	324 ± 31(c)	51 ± 16(d)	35 ± 18(d)
NPAC	354 ± 25	66 ± 17	50 ± 25
HPAH	355 ± 25	74 ± 21	45 ± 21
Recombined Mixture	340 ± 36	54 ± 20(d)	39 ± 14(d)

(a) HD = heavy distillate, AH = aliphatic hydrocarbon, PAH = polycyclic aromatic hydrocarbon, NPAC = nitrogen-PAH, HPAH = hydroxylated PAH

(b) Average ± standard deviation

(c) Significantly less than that of control at $P < 0.01$

(d) Significantly less than that of control at $P < 0.05$

TABLE 3. Effect on Fetal Status of Dermal Exposure of Pregnant Rats to Solvent Refined Coal (SRC)-II Heavy Distillate.

Treatment(a)	N	Live Fetuses		Late Resorptions	
		Number	Percent	Number	Percent
Control	15	13.3 ± 4.3(b)	93 ± 9	1.0 ± 0.0	6.6 ± 0.5
Whole HD	15	12.9 ± 3.4	85 ± 13(c)	2.4 ± 1.8	15.8 ± 11.7
AH	11	11.6 ± 5.3	95 ± 6	0.0 ± 0.0	0.0 ± 0
PAH	11	9.5 ± 4.1(c)	84 ± 18(c)	3.8 ± 2.2	26.2 ± 16.3
NPAC	13	13.1 ± 3.8	96 ± 7	0.0 ± 0.0	0.0 ± 0
HPAH	10	14.1 ± 4.0	96 ± 5	1.0 ± 0.0	5.4 ± 0
Recombined Mixture	15	11.7 ± 4.6	89 ± 9	1.4 ± 1.1	10.3 ± 7.0

(a) HD = heavy distillate, AH = Aliphatic hydrocarbon, PAH = polycyclic aromatic hydrocarbon, NPAC = nitrogen-PAH, HPAH = hydroxylated PAH

(b) Average ± standard deviation

(c) Significantly less than that of control at $P < 0.05$

Several maternal organs were affected by exposure to whole HD and its chemical class fractions, as indicated by changes in organ:body-weight ratios (BWR; Table 4). Maternal adrenal BWR were higher than those of controls in the groups treated with whole HD or with the PAH fraction; however, the increase was significant only for the whole-HD group. Liver BWR were significantly higher for groups given whole HD, the PAH fraction, or the recombined mixture, a response indicative of the presence of mixed-function oxidase inducers. Spleen BWR were also higher for groups given whole HD, the PAH fraction, or the NPAC fraction. Exposure to whole HD, the PAH fraction, or the recombined mixture induced maternal thymic atrophy, resulting in a 40% decrease of maternal thymus BWR for these groups compared to controls.

Exposure to whole HD or to the PAH fraction resulted in a significant incidence of cleft palate, cutaneous syndactyly and edema in the offspring (Tables 5A and 5B). These same defects were present in the group treated with the recombined mixture, however the incidence was not statistically significant. A large percentage of the pups from the whole-HD, PAH-fraction, and recombined-mixture treatment groups exhibited hemorrhages in the area of the occipital and frontal fontanelles. These hemorrhages did not appear to involve the cranial cavity but may be associated with abnormalities in ossification and closure of the fontanelles that were present in many of the fetuses from these treatment groups.

TABLE 4. Effect of Dermal Applied Solvent Refined Coal (SRC)-II Heavy Distillate on Organ Weight of Pregnant Rats.

Treatment(a)	Adrenal Weight, g	Adrenal to Body Weight Ratio, %	Kidney Weight, g	Kidney to Body Weight Ratio, %	Liver Weight, g	Liver to Body Weight Ratio, %
Control	0.082 ± 0.019(b)	0.028 ± 0.007	2.346 ± 0.209	0.800 ± 0.061	17.23 ± 2.02	5.86 ± 0.44
Whole HD	0.097 ± 0.015	0.036 ± 0.005(c)	2.292 ± 0.198	0.845 ± 0.052	18.12 ± 1.81	6.67 ± 0.46(d)
AH	0.092 ± 0.025	0.031 ± 0.007	2.227 ± 0.179	0.757 ± 0.048	16.15 ± 1.86	5.48 ± 0.54
PAH	0.090 ± 0.016	0.033 ± 0.005	2.228 ± 0.168	0.821 ± 0.086	17.56 ± 1.85	6.46 ± 0.70(d)
NPAC	0.080 ± 0.021	0.028 ± 0.007	2.110 ± 0.391(c)	0.733 ± 0.128	17.06 ± 1.22	5.94 ± 0.42
HPAH	0.087 ± 0.025	0.031 ± 0.009	2.238 ± 0.088	0.797 ± 0.065	16.21 ± 1.42	5.77 ± 0.58
Recombined Mixture	0.086 ± 0.016	0.030 ± 0.007	2.315 ± 0.196	0.810 ± 0.065	18.55 ± 2.03	6.50 ± 0.45(d)

Treatment(a)	Spleen Weight, g	Spleen to Body Weight Ratio, %	Thymus Weight, g	Thymus to Body Weight Ratio, %
Control	0.61 ± 0.07(b)	0.206 ± 0.022	0.250 ± 0.062	0.085 ± 0.020
Whole HD	0.64 ± 0.08	0.236 ± 0.022(c)	0.093 ± 0.022(d)	0.034 ± 0.008(d)
AH	0.60 ± 0.05	0.204 ± 0.022	0.277 ± 0.075	0.094 ± 0.023
PAH	0.65 ± 0.07	0.237 ± 0.029(c)	0.093 ± 0.024(d)	0.034 ± 0.007(d)
NPAC	0.67 ± 0.06	0.233 ± 0.019(c)	0.184 ± 0.052(c)	0.064 ± 0.017(d)
HPAH	0.62 ± 0.08	0.220 ± 0.029	0.224 ± 0.061	0.076 ± 0.020
Recombined Mixture	0.64 ± 0.12	0.227 ± 0.044	0.097 ± 0.027(d)	0.034 ± 0.010(d)

(a) HD = heavy distillate, AH = aliphatic hydrocarbon, PAH = polycyclic aromatic hydrocarbon, NPAC = nitrogen-PAH, HPAH = hydroxylated PAH

(b) Mean ± standard deviation

(c) Significantly different from that of control at P < 0.05

(d) Significantly different from control at P < 0.01

TABLE 5A. Summary of the Incidence of Fetal Abnormalities Produced as a Result of Exposure of Pregnant Rats to Solvent Refined Coal (SRC)-II Heavy Distillate and its Chemical Class Fractions.

Treatment(a)	Fetal Basis, % affected							Litter Basis, % affected						
	Control	HD	AH	PAH	NPAC	HPAH	Recombined Mixture	Control	HD	AH	PAH	NPAC	HPAH	Recombined Mixture
Number Rats Treated:	200	193	127	104	170	141	175	15	15	11	11	13	10	15
Major Abnormalities														
Cranial Defects														
Cleft Palate		7(b)		13(b)			1		47		36			13
Subcutaneous Hemorrhage		26(b)		19(b)	1		17(b)		80(b)		55(b)	8		53(b)
Unilateral Cerebral Meningocele				1							9			
Internal Hydrocephaly		1							7					
Craniofacial Abnormalities		2		3			1		7		18			7
Circulatory Defects														
Major Vessel Malformation					1		2(b)					8		13(b)
Ventricular Septal Defect				1							9			
Limb Abnormalities														
Arthrogryposis		1					1		7					7
Club Foot		1		2					7		9			
Micromelia				1			1				9			7
Cutaneous Syndactyly		4(b)		6(b)			1		40(b)		36(b)			13
Other Abnormalities														
Anal Atresia				1			1				9			7
Edema		10(b)		6(b)			2		60(b)		27			13
Open Eye				1							1			

(a) HD = heavy distillate, AH = aliphatic hydrocarbon, PAH = polycyclic aromatic hydrocarbon, NPAC = nitrogen-PAH, HPAH = hydroxylated PAH

(b) Significantly greater than control at P < 0.01

TABLE 5B. Summary of Percent Incidence of Fetal Abnormalities Produced as a Result of the Exposure of Pregnant Rats to Solvent Refined Coal (SRC)-II Heavy Distillate and its Chemical Class Fractions.

Treatment(a)	Fetal Basis, % affected							Litter Basis, % affected						
	Control	HD	AH	PAH	NPAC	HPAH	Recombined Mixture	Control	HD	AH	PAH	NPAC	HPAH	Recombined Mixture
Number Rats Treated:	200	193	127	104	170	141	175	15	15	11	11	13	10	15
Reduced Ossification Sites														
Pelvis	4	8(c)		7	1		5	33	33		45	8		20
Phalanges		1		2			1		7		18			7
Rib				1							9			
Skull		45(b)		38(b)	18(b)	1	46(b)		93(b)		91(b)	62(b)	10	93(b)
Sternebrae 1-4	8	46(b)	3	32(b)	8	9	33(b)	33	80(c)	27	64	54	70	67
Vertebrae	12	35(b)	7	52(b)	23(b)	18	31(b)	60	73	36	100	77	80	80
Minor Anomalies or Variations														
Bent Limb				1							9			
Dilated Ureter	4	1	9	5	4	5	3	20	13	55	27	38	50	27
Renal Pelvic Cavitation	1		1		1		2	7		9		15		13
Small Genitalia		1		1			1		7		9			7
Supernumary Rib	6	3	1		2	1	2	27	27	9		15	10	7
Supernumary Rudimentary Rib	1	1		1	1		1	7	13		9	8		7
Short Tail				1							9			
Missing Innominate		1	1	2			1		13	9	18			13

(a) HD = heavy distillate, AH = aliphatic hydrocarbon, PAH = polycyclic aromatic hydrocarbon, NPAC = nitrogen-PAH, HPAH = hydroxylated PAH

(b) Significantly greater than control at $P < 0.01$

(c) Significantly greater than control at $P < 0.05$

The most prevalent abnormality associated with the whole-HD, recombined-mixture, and PAH-fraction groups was pulmonary hypoplasia (Table 6). A lung:BWR greater than or equal to two standard deviations below the mean for control fetuses or litters was used as the criterion for identifying a hypoplastic lung. When examining the incidence calculated on a fetal basis, groups treated with whole HD, the recombined mixture, or the PAH fraction had incidences of pulmonary hypoplasia of approximately 90% or greater. There may be a link between the mechanism producing cleft palate and that causing pulmonary hypoplasia: cleft palates were found only in treatment groups that also contained fetuses with small lungs. Furthermore, fetuses with cleft palates had mean lung:BWR significantly lower than those for other pups in their respective dose groups, suggesting that cleft palate may be a more severe manifestation of a syndrome of abnormalities (Figure 1).

TABLE 6. Incidence of Pulmonary Hypoplasia in the Offspring of Pregnant Rats Exposed to Solvent Refined Coal (SRC)-II Heavy Distillate and its Chemical Class Fractions.

Treatment(a)	Incidence of Lung Hypoplasia			
	Fetuses		Litters	
	Number(b)	Percent	Number(b)	Percent
Control	3/200	2	2/15	13
Whole HD	188/193	97(c)	15/15	100(c)
AH	4/127	3	4/11	36
PAH	99/104	95(c)	11/11	100(c)
NPAC	11/170	6	7/13	54(d)
HPAH	2/141	1	1/10	10
Recombined Mixture	156/175	89(c)	15/15	100(c)

(a) HD = heavy distillate, AH = aliphatic hydrocarbon, PAH = polycyclic aromatic hydrocarbon, NPAC = nitrogen-PAH, HPAH = hydroxylated PAH

(b) Number with hypoplastic lungs/total number in group

(c) Significantly different from that of control at $P < 0.01$

(d) Significantly different from that of control at $P < 0.05$

FIGURE 1. Fetal Lung-to-Body-Weight Ratios (%) Versus Groups of Pregnant Rats Treated with Solvent Refined Coal (SRC)-II Heavy Distillate or its Chemical Class Fractions. HD = heavy distillate, AH = aliphatic hydrocarbons, PAH = polycyclic aromatic hydrocarbons, NPAC = nitrogen-PAH, HPAH = hydroxylated PAH.

With the exception of the cranial hemorrhages, these abnormalities are characteristic of those induced in offspring of pregnant rats exposed to synthetic glucocorticoids. Another fetotoxic effect which may also involve the endocrine system is the significant decrease in the male:female sex ratio of the offspring in the PAH-fraction treatment group (Table 7). However, it is hard to reconcile

that hypothesis with the fact that the sex ratio is different from that of controls only in the PAH-fraction group, but not in either the whole-HD or the recombined-mixture treatment groups.

TABLE 7. Fetal Sex Ratios of Offspring of Pregnant Rats Treated with Solvent Refined Coal (SRC)-II Heavy Distillate and its Chemical Class Fractions.

Treatment(a)	N/ Litter	Male:Female Sex Ratio, average per litter $\pm$ SD	No. Fetuses	Male:Female Sex Ratio, on fetal basis
Control	15	1.15 $\pm$ 0.49	200	0.96
Whole HD	15	1.03 $\pm$ 0.48	193	0.95
AH	11	1.06 $\pm$ 0.65	127	0.98
PAH	11	0.66 $\pm$ 0.55(b)	104	0.51(b)
NPAC	13	0.95 $\pm$ 0.40	170	0.87
HPAH	10	1.03 $\pm$ 0.55	141	0.93
Recombined Mixture	15	0.93 $\pm$ 0.38	175	0.90

(a) HD = heavy distillate, AH = aliphatic hydrocarbon, PAH = polycyclic aromatic hydrocarbon, NPAC = nitrogen-PAH, HPAH = hydroxylated PAH

(b) Significantly less than that of control at $P = 0.05$

We conclude from this study that compounds present in the PAH fraction are teratogenic in rodents and produce a syndrome of defects that closely resembles that found in animals treated with synthetic glucocorticoids. However, it is not possible at this time to directly implicate PAH as the causative agents, since other classes of compounds are present (i.e., the sulfur-containing hydrocarbons), albeit in lesser amounts. Future work will concentrate on identifying the mechanism of action of these teratogenic mixtures and on further defining the class(es) of compounds responsible for the observed effects.

• Perinatal Effects of Complex Mixtures

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To study the effects of complex organic mixtures (COM) as promoters of preneoplastic changes in rat liver, it was necessary to develop dose-response information for initiation by diethylnitrosamine (DEN). For this purpose, 1-day-old rat pups were injected with either 4, 8, or 16 μg DEN/g body weight and promoted by twice-weekly applications of a COM beginning at 3 weeks of age. Liver samples collected after 8 weeks of promotion were analyzed for foci of phenotypically changed cells by exclusion of iron and/or increased gamma glutamyl transpeptidase (GGT) activity. Results indicate that female rats were more sensitive to COM promotion than males and that the number of altered foci increased with larger doses of DEN. At higher DEN doses the phenotypic alterations did not require promotion, indicating that the changes were induced by DEN initiation alone. Furthermore, the greatest number of phenotypic changes involved foci of cells which either stained positive for GGT activity, or stained for GGT and excluded iron. These results demonstrate that female rats are preferable for studies using this assay system and that the selection of the DEN initiator dose is critical.

Treatment of animals with hepatocarcinogens results in a number of phenotypic alterations that can be detected by biochemical, morphological or proliferative changes. These phenotypic changes are present in groups of closely associated cells and can be identified by several methods. For example, exposure to either diethylnitrosamine (DEN) or benzo[a]pyrene (BaP) results in foci of cells which stain positive for gamma glutamyl transpeptidase (GGT).

Previously, we reported the results of a study to determine the influence of complex organic mixtures (COM) on the development of preneoplastic foci in rat liver. The end-point for these studies was foci that stained positive for GGT. For the first study, animals were treated with two high-boiling COM that were known to initiate skin tumors in mice. Results demonstrated that these COM were not very effective as initiators of preneoplastic changes. When a known carcinogen was co-administered with these two COM, the initiating activity was inhibited. In addition, dermal application of a broad-boiling-range COM to rats preinitiated with a known carcinogen resulted in the promotion of preneoplastic changes. In an effort to extend these studies to determine the chemical class(es) responsible for the tumor-promoting activity, it became necessary to better define the dose-response relationship for the initiating agent, DEN.

For this study, pregnant CD Sprague-Dawley rats were obtained from Charles River. Solutions of DEN were prepared in 0.9%

NaCl, and 1-day-old pups were injected intraperitoneally with DEN at doses of either 4, 8, or 16 μg DEN/g body weight. Pups from each litter were distributed over treatment groups so that the influence of each litter was minimized within a treatment group. Positive-control animals of both sexes received 4 μg DEN/g body weight, and foci development was promoted by dietary phenobarbital (0.05%). All animals were fed pelleted synthetic diet containing 30% casein (Teklad). To determine whether dermal treatment with a high-boiling COM promoted foci development, weanling animals initiated with DEN were dermally administered the COM at doses of 400 μg /g body weight, twice weekly for 8 weeks. On 3 consecutive days prior to sacrifice, animals were injected with 1.0 ml of $\text{Fe}(\text{OH})_3$ containing 100 mg of elemental iron/ml. Animals were euthanized with nembutal, their livers were immediately excised, and a 1.6-cm-diameter plug removed from the left lobe. Liver samples were shipped on dry ice to Argonne National Laboratory for analysis. After sectioning, the slices were histochemically stained and the number of foci per section was determined by direct microscopic examination. The foci were classified either as staining positive for GGT activity, excluding iron, or both.

Data at weaning indicated that DEN treatment did not result in significant changes in body weight; however, at sacrifice, body weights for several rat groups exposed dermally to the COM were significantly below those of corresponding controls (Table 1). Similarly, liver-to-body-weight ratios were increased for ani-

TABLE 1. Body and Liver Weights and Survival Rate for Rats Initiated with Diethylnitrosamine (DEN; $x \pm \text{SEM}$) and Promoted with Either Phenobarbital or a Complex Organic Mixture (COM).

Group No.	Sex, N	DEN Dose, μg	Promoter	Body Weight		Liver to Body Wt. Ratio, %	Survival, %
				At 3 Weeks of age	At 11-Week Sacrifice		
1	M,15	4	PB(a)	56.0 $\pm$ 1.5	496 $\pm$ 7.9	5.2 $\pm$ 0.12	100
2	M,15	4	None	57.5 $\pm$ 1.3	487 $\pm$ 9.3	5.0 $\pm$ 0.11	100
3	M,15	4	COM(b)	56.1 $\pm$ 1.2	409 $\pm$ 11.6(c)	6.0 $\pm$ 0.29(c)	100
4	M,15	8	None	55.2 $\pm$ 1.3	487 $\pm$ 15.0	5.0 $\pm$ 0.08	100
5	M,15	8	COM	57.1 $\pm$ 1.3	422 $\pm$ 16.6(c)	5.6 $\pm$ 0.14(c)	100
6	M,15	16	None	56.9 $\pm$ 1.3	480 $\pm$ 8.3	5.0 $\pm$ 0.09	100
7	M,13	16	COM	55.9 $\pm$ 1.9	448 $\pm$ 16.2	5.8 $\pm$ 0.09(c)	87
8	F,14	4	PB	55.6 $\pm$ 2.5	285 $\pm$ 13.4	4.8 $\pm$ 0.17	100
9	F,14	4	None	55.7 $\pm$ 2.2	299 $\pm$ 12.4	4.5 $\pm$ 0.12	100
10	F,14	4	COM	56.2 $\pm$ 1.2	274 $\pm$ 8.5	5.9 $\pm$ 0.17(c)	93
11	F,15	8	None	56.7 $\pm$ 1.8	306 $\pm$ 8.7	4.8 $\pm$ 0.09	100
12	F,15	8	COM	57.7 $\pm$ 1.4	272 $\pm$ 6.6(c)	5.8 $\pm$ 0.15(c)	100
13	F,15	16	None	54.1 $\pm$ 1.7	299 $\pm$ 6.4	4.6 $\pm$ 0.13	100
14	F,14	16	COM	53.4 $\pm$ 1.3	256 $\pm$ 9.1(c)	5.8 $\pm$ 0.11(c)	93

(a)Phenobarbital

(b)Complex organic mixture

(c)Significantly different from that for corresponding DEN-initiated group, $P \leq 0.05$

mals receiving the COM, and survival decreased for animals treated with the high dose of DEN followed by repeated applications of COM.

When liver sections were evaluated and phenotypes identified, several changes became apparent (Table 2). For both males and females initiated with DEN (4 $\mu\text{g/g}$) followed by dietary phenobarbital, the number of foci staining positive for GGT increased three- to fourfold over those administered DEN only. Dermal treatment with COM resulted in small increases in the numbers of GGT foci for animals initiated with 8 $\mu\text{g/g}$ DEN and promoted with COM; at doses of either 4 or 16 $\mu\text{g/g}$ DEN, increased numbers of phenotypic changes were not present. Foci that excluded iron accounted for a small portion of the total number of altered foci; however, a significant number of foci were detected that both excluded iron and stained positive for GGT activity. Comparisons based on total numbers of foci and those based on

total GGT-positive foci provided similar results for COM promotion. These data demonstrate that there was a clear increase in the number of phenotypically altered hepatic foci in females initiated with either 4 or 8 μg DEN/g. However, at 16 μg DEN/g, the number of foci for females given DEN only was nearly identical to those receiving the same dose of DEN followed by COM promotion. Similarly, COM promotion was not observed with males.

Results from this study indicate that female rats were more sensitive to COM promotion than males and that the number of altered foci increased with increasing dose of DEN. Since, at higher doses, the changes did not require promotion, it appears that these phenotypic changes can be induced by DEN initiation alone. Furthermore, the data indicate that the greatest number of phenotypic changes involved foci of cells which either stained positive for GGT activity or stained for GGT and excluded iron.

TABLE 2. Preneoplastic Foci that Stained Positive for Gamma Glutamyl Transpeptidase (GGT), or Iron, or Both, for Animals Exposed to Diethylnitrosamine (DEN) and Promoted with Either Phenobarbital (PB) or a Complex Organic Mixture (COM).

Group No.	Promoter	Number of Foci/cm ³				
		GGT ^(a)	Iron	GGT-Iron	Total Foci ^(b)	Total GGT ^(c)
1	PB	26.4 ± 4.2	1.54 ± 1.54	9.61 ± 2.14	37.5 ± 4.9	36.0
2	None	6.67 ± 2.29	1.40 ± 1.40	2.21 ± 1.24	10.3 ± 3.0	8.88
3	COM	5.60 ± 2.61	4.57 ± 2.89	3.26 ± 1.82	13.4 ± 4.3	8.86
4	None	9.23 ± 3.40	7.39 ± 3.09	2.40 ± 1.80	19.0 ± 4.9	11.6
5	COM	14.1 ± 3.75	0	2.95 ± 1.53	17.0 ± 4.1	17.0
6	None	14.8 ± 3.65	0.77 ± 0.77	6.11 ± 2.04	21.7 ± 4.2	20.9
7	COM	11.7 ± 5.68	1.48 ± 1.07	2.70 ± 1.74	15.9 ± 6.0	14.4
8	PB	33.3 ± 7.60	1.80 ± 0.99	49.0 ± 7.6	84.1 ± 10.8	82.3
9	None	9.67 ± 2.17	1.74 ± 1.07	4.16 ± 1.38	15.6 ± 3.8	13.8
10	COM	11.83 ± 3.46	3.44 ± 1.17	28.1 ± 4.3 ^(d)	43.4 ± 5.6 ^(d)	39.9
11	None	19.7 ± 3.32	5.57 ± 1.80	20.4 ± 5.6	45.7 ± 6.7	40.1
12	COM	27.1 ± 3.90	3.71 ± 1.54	44.9 ± 7.5 ^(d)	75.7 ± 8.6 ^(d)	72.0
13	None	41.8 ± 7.79	13.7 ± 3.5	36.9 ± 9.5	92.4 ± 12.8	78.7
14	COM	40.3 ± 7.6	12.1 ± 3.2	35.4 ± 5.9	87.8 ± 10.1	75.6

^(a)Gamma glutamyl transpeptidase

^(b)Columns 1 + 2 + 3

^(c)Columns 1 + 3

^(d)Significantly different from that for corresponding DEN-initiated Group. $P \leq 0.05$

• Tissue Dose in Complex Mixture Exposures

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ABSTRACT

The disposition of several complex mixture components in experimental animals has been studied as a function of several variables. The presence of the complex mixture was found to reduce the rate of removal of individual compounds from the blood, to decrease the rate of exhalation of these compounds, and to reduce the breath/blood partition coefficients for these compounds. The complex mixture did not alter the distribution of components within the animal.

The purpose of these experiments is to develop a predictive capability to estimate levels of complex mixture constituents in target organs as they are related to the exposure regime and the time after exposure. We are developing the capability to predict exposures to complex organic mixtures by analyzing exhaled breath. As part of this effort, we have investigated the disposition of complex mixture components in female Sprague-Dawley rats as a function of route of exposure, time after exposure and nature of the mixture. We have also determined the levels of several aromatic components from a coal liquid in rats' blood and breath.

Experiments were performed using either a simple mixture of four polycyclic aromatic hydrocarbons (PAH; benzene, toluene, naphthalene, and phenanthrene) or a complex material composed of the PAH mixture and a mid-boiling-range coal liquid (solvent refined coal [SRC]-II middle distillate [MD]). The four PAH selected are common constituents of light distillate (LD) and MD coal liquids and represent a range of vapor pressures. One mg of each of the compounds was administered in the PAH mixture by intravenous (IV) injection into cannulated jugular veins. In the PAH mixture co-administered with the MD, the quantities of each of the four compounds were: benzene, 1 mg; toluene, 1 mg; naphthalene, 2.3 mg; and phenanthrene, 1.2 mg. Immediately after dosing, individual rats were placed in a glass metabolism cage; all exhaled vapors were collected and passed over Tenax® to trap exhaled parent compounds. Ten concurrent samples of blood, exhaled vapors, feces and urine were collected during the 24-hour period following dosing. At the termination of the experiment, selected organs were removed from the animals for further chemical analysis. The concentrations of the four PAH and several of the MD components were determined in the blood,

breath, and tissue samples. Identification and quantitation of components were performed by gas chromatography (GC) and GC/mass spectrometry.

The mid-boiling-range coal liquid (the MD) used as the representative complex mixture is composed predominantly of two-ring and substituted two-ring aromatic and hydroaromatic compounds. Most components were present in the blood at a level too low for quantitation. Table 1 lists compounds which could be identified and quantified in blood and exhaled breath samples; they are, principally, substituted naphthalenes. The time-course for blood levels of naphthalene is presented in Figure 1 as an example of the concentrations observed for the blood components. Data in this figure are for naphthalene administered in two different matrices: 1) in the simple mixture of four PAH, and 2) in the complex MD material. The presence of the MD in the dosing mixture both decreased the rate of naphthalene removal and also caused a higher level of naphthalene to be maintained in the blood. The curve in Figure 1 can be resolved into two components, each representing a different rate of naphthalene removal from the blood. These rates of removal are listed in Table 2 for both naphthalene and phenanthrene, under two different dosing regimes. The addition of the MD to the PAH mixture decreased the initial rate of removal of both naphthalene and phenanthrene by approximately an order of magnitude. The slower removal rate was also reduced.

Breath/blood partition coefficients (exhaled air concentration/blood concentration) were calculated for those complex mixture components which could be quantified in both blood and breath samples (Table 3). The partition coefficient for the two-ring and substituted two-ring components ranged from approximately 10^{-6} to 10^{-7} . The partition coefficients for the more

polar, substituted phenol and for the three-ring phenanthrene were approximately 6×10^{-8} . In general, these partition coefficients decreased with increasing molecular weight of the compound. For naphthalene and phenanthrene, a comparison of partition coefficients calculated for different methods of sample administration is given in Table 4. Since the partition coefficients determined for other single compounds (specifically, benzene and toluene) administered by intraperitoneal (IP) injection agree with available literature data, the IP data are taken as representative of true breath/blood partition coefficients. The IV administration of either the simple PAH mixture or the PAH mixture co-administered with the MD yielded significantly reduced values for the calculated partition coefficients relative to those for naphthalene and phenanthrene when administered alone.

TABLE 1. Complex Mixture Constituents Identified in Blood and Exhaled Breath of Rats.

Compound	Blood	Exhaled Breath
Indan		X
o-Cresol		X
m + p-Cresol/C ₁ -Indan		X
C ₁ -Indan		X
Tetralin/C ₁ -Indan	X	X
C ₂ -Phenol	X	X
Naphthalene	X	X
2-Methylnaphthalene	X	X
1-Methylnaphthalene		X
C ₂ -Naphthalene	X	X
2,6-Dimethylnaphthalene	X	X
C ₃ -Naphthalene	X	X
C ₃ -Naphthalene		X
C ₄ -Naphthalene	X	X
Phenanthrene	X	X

FIGURE 1. Blood Concentration ng/ μ l, in Rats, of Naphthalene after Intravenous Administration of a Mixture of Polycyclic Aromatic Hydrocarbons (PAH) or a Complex Mixture.

TABLE 2. Rates of Removal (ng/ μ l/hr) from Blood of Individual Polycyclic Aromatic Compounds Administered to Rats.

Route of Administration ^(a)	Naphthalene		Phenanthrene	
	Initial Rate	Slower Rate	Initial Rate	Slower Rate
IV, DMSO, PAH Mixture	26.8	1.18	21.2	1.36
IV, DMSO, PAH Mixture + Middle Distillate	2.77	1.02	2.24	1.05

(a) IV = intravenous; DMSO = dimethylsulfoxide; PAH = polycyclic aromatic hydrocarbons

TABLE 3. Breath/Blood Partition Coefficients for Complex Mixture Components Administered to Rats.

Compound	Breath/Blood Partition Coefficient
Tetralin/C ₁ -Indan	1.1×10^{-6}
C ₂ -Phenol	6.5×10^{-8}
Naphthalene	4.9×10^{-7}
2-Methylnaphthalene	9.6×10^{-7}
C ₂ -Naphthalene	3.9×10^{-7}
2,6-Dimethylnaphthalene	3.8×10^{-7}
C ₃ -Naphthalene	8.1×10^{-7}
C ₄ -Naphthalene	2.0×10^{-7}
Phenanthrene	5.8×10^{-8}

TABLE 4. Effect of Route of Administration on Breath/Blood Partition Coefficients in Rats that Received Complex Mixtures.

Route of Administration ^(a)	Partition Coefficient	
	Naphthalene	Phenanthrene
IP, DMSO, Single Compound	6.2×10^{-5}	1.0×10^{-6}
IV, DMSO, PAH Mixture	9.2×10^{-6}	2.8×10^{-7}
IV, DMSO, PAH Mixture + Middle Distillate	4.9×10^{-7}	5.8×10^{-8}

(a) IP = intraperitoneal; DMSO = dimethylsulfoxide; IV = intravenous; PAH = polycyclic aromatic hydrocarbons

The concentration of the two representative PAH, naphthalene and phenanthrene, in four selected organ samples are listed in Table 5 for two different mixtures administered. In general, there are no major differences in the distribution of these two compounds when administered in the simple PAH mixture versus when administered as PAH mixture plus MD. Most of these data are for animals that lived from 4 to 9 hours after dosing. Some of the data for the concentrations of these compounds in fat are for animals that received the simple PAH mixture and survived the entire 24-hour experiment. The fatty tissue appears to be a short-term reservoir for these PAH since, after 24 hours, the concentrations have decreased to represent only a small fraction of the original dose.

Application of breath analysis to dosimetry for personnel exposed to complex mixtures containing PAH has been evaluated. Using the partition coefficient data listed in Table 4 as representative, analysis of a 10-minute breath-collection sample would detect blood levels of approximately 1 ppm of two- and three-ring PAH. Because of the method of administration, these partition coefficients are lower, by 10- to 100-fold, than those expected for personnel exposed by a more typical route (inhalation or dermal ab-

sorption). The actual limit of detection for blood concentrations of two- and three-ring PAH in humans, using a 10-minute breath sampling, would be approximately 10-100 ppb. These data suggest that this may be a useful approach to evaluating workplace exposure to PAH.

TABLE 5. Concentrations of Polycyclic Aromatic Hydrocarbons in Organs of Rats 4 to 9 Hours after Administration of Complex Mixtures.

Route of Administration(a)	Organ	Naphthalene		Phenanthrene	
		$\mu\text{g/g}$	% of Dose	$\mu\text{g/g}$	% of Dose
IV, DMSO, PAH Mixture	Muscle	0.30	4.7	--	--
	Lung	0.25	0.1	0.1	0.2
	Liver	0.36	0.5	--	--
	Fat	8.0 0.66(b)	18 1.6(b)	7.1 1.0(b)	13 2.1(b)
IV, DMSO, PAH Mixture + Middle Distillate	Muscle	0.43	4.0	0.74	6.3
	Lung	1.0	0.2	2.3	0.5
	Liver	1.3	0.8	1.5	0.8
	Fat	23.0	18.0	7.5	8.2

(a) IV = intravenous; DMSO = dimethylsulfoxide;
PAH = polycyclic aromatic hydrocarbons

(b) Animals sacrificed after 24 hr

Medical
Applications of
Nuclear Technology

• Blood Irradiator Development for Medical Applications

Principal Investigators: F. P. Hungate and R. E. Weller

Other Investigators: L. R. Bunnell and W. F. Riemath

Histopathological evaluation of renal tissues obtained from dogs that have received transplants revealed a difference in the appearance of the rejection response between irradiated and non-irradiated animals, indicating that the irradiator may positively affect the outcome of transplants. Also during this year, the irradiator connector design was modified to better fit the newly developed radiation shielding.

The dogs used in the renal transplantation study were genetically very diverse to ensure that the donor and recipient were as DLA-incompatible as possible. In some of the transplants, the donor contributed kidneys to both the irradiated and non-irradiated recipient. Irradiated dogs exhibited a subacute rejection response characterized by varied degrees of mononuclear cell infiltrate and vacuolization of tubular epithelial cells, while nonirradiated animals showed consistent evidence of hyperacute rejection with infarction. These observations suggest differences in both the mechanism and time-course of the rejection responses.

Looking forward to eventual human trials of the vitreous carbon blood irradiator, some design modifications were made for the primary purpose of maximizing shielding efficiency and minimizing overall size and fragility. In the first modification, we tried to eliminate the thin vitreous carbon leading edge which interfaced with a standard Quinton Instruments female connector; this edge has been the site of every breakage we have experienced. Figure 1 is a sketch of the original design, for illustrative purposes. In the first modification, the projecting tube was replaced by a male thread on each end, as shown in Figure 2. Several different types of silicone rubber were tried before finding one with sufficient stiffness to allow the threads to tightly engage the shunt tubing to the irradiator without excessive deformation. When this modification was tested, the tightness of fit was not sufficient to prevent thrombus induction.

FIGURE 1. The Blood Irradiator Used on Animals.

FIGURE 2. First Attempt at a New Connector for the Blood Irradiator.

The second modification was developed more fully but was not tested. This modification also eliminated the projecting carbon tube, but in this case an interface to a Quinton Instruments male connector was made part of the irradiator. This approach was avoided earlier because of the close fits required, but the finished coupling is simple in design and the most compact to date, an advantage for shielding. Figure 3 shows the concept. In practice, a simplified irradiator is produced as usual, then the end fitting is machined, using a specially sized counterbore (0.205 in. diameter). This counterbore is sized so that the finished irradiator, after about 22% shrinkage, is a snug fit with a standard Quinton Instruments male connector, which has an outside diameter of 0.164 in. It was difficult to consistently produce the necessary tight fit; a fit which was tight enough to prevent blood leakage required some forcing during assembly. The final version of the second modification used a standard tapered reamer to enlarge the terminal portion of the counterbore, but the end portion is of a diameter that is a tight fit with the Quinton Instruments male connector (Figure 3). Several irradiators produced with this modification allowed secure connections with the Quinton Instruments hardware, but they have not yet been tested in a shunt.

FIGURE 3. Final Design of the End Connector for the Blood Irradiator.

- **List of DOE Radioisotope Customers with Summary of Radioisotope Shipments, FY 1985**

Principal Investigator: D. A. Baker

The annual list of DOE radioisotope customers (PNL-5948) was published in August 1986. The document contains the various radioisotopes sold by the DOE laboratories to domestic, foreign, and other DOE facilities. A list of suppliers is given along with the isotopes sold or transferred. Lists of customers are given, with their addresses and the isotopes purchased. Customers are also cross-referenced by isotope and state, or country for foreign sales. A table of isotope sales by number of shipments, quantity, and dollar value is given for each customer type (domestic, DOE, and foreign). Total value of sales of DOE radioisotopes for FY 1985 were \$11.4 million, an increase of 39% from last year.

Appendix

APPENDIX

- **Dose-Effect Studies with Inhaled Plutonium in Beagles**

On the following pages data are presented for all dogs assigned to current life-span dose effect studies with inhaled $^{239}\text{PuO}_2$, $^{238}\text{PuO}_2$, and ^{239}Pu nitrate. Information is presented on the estimated initial lung deposition, based on external thorax counts and on estimated lung weights ($0.011 \times$ body weight) at time of exposure. Information is also provided on the current interpretation of the most prominent clinical-pathological features associated with the death of animals. These data represent information presently available, and are presented as reference material for scientists who desire to follow in detail the progress of these experiments.

INHALED PLUTONIUM NITRATE IN DOGS

DOSE GROUP	DOG IDENT	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		9/30/86	DEATH	
D-6 HIGH	1420 M	3840	30.36	333.91	11.5	23.3	06/23/76	07/12/78	24.6		Radiation Pneumonitis
D-6 HIGH	1517 F	5185	49.62	545.79	9.5	20.6	06/23/76	11/02/77	16.3		Radiation Pneumonitis
D-6 HIGH	1510 F	6969	55.09	606.02	11.5	20.9	06/23/76	11/09/77	16.6		Radiation Pneumonitis
D-6 HIGH	1424 M	7681	69.83	768.12	10.0	23.2	06/23/76	08/31/77	14.3		Radiation Pneumonitis

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

INHALED PLUTONIUM NITRATE IN DOGS

DOSE GROUP	DOG IDENT	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		9/30/86	DEATH	
D-4 MEDIUM	1404 M	260	1.48	16.25	16.0	21.5	04/20/76	02/03/84		93.5	Pleuritis
D-4 MEDIUM	1521 F	205	1.49	16.37	12.5	21.3	07/22/76	06/07/85		106.5	Bone Tumor, Lung Tumor
D-4 MEDIUM	1656 M	211	1.54	16.90	12.5	18.4	11/07/77		106.7		
D-4 MEDIUM	1379 M	278	1.74	19.16	14.5	19.1	01/20/76			128.3	
D-4 MEDIUM	1362 M	267	1.87	20.54	13.0	20.2	01/20/76			128.3	
D-4 MEDIUM	1639 F	248	2.05	22.57	11.0	18.5	11/07/77			106.7	
D-4 MEDIUM	1647 M	294	2.05	22.58	13.0	18.5	11/07/77			106.7	
D-4 MEDIUM	1640 M	307	2.06	22.71	13.5	18.5	11/07/77	03/20/84		76.4	Lung Tumor
D-4 MEDIUM	1645 F	257	2.13	23.39	11.0	18.5	11/07/77	08/07/86		105.0	Lung Tumor
D-4 MEDIUM	1534 M	295	2.14	23.57	12.5	20.8	07/22/76	05/26/85		106.1	Congestive Heart Failure
D-4 MEDIUM	1414 F	233	2.35	25.86	9.0	18.2	01/20/76	08/14/86		126.8	Processing
D-4 MEDIUM	1618 F	277	2.40	26.36	10.5	20.3	11/07/77		106.7		
D-4 MEDIUM	1385 M	373	2.42	26.63	14.0	19.0	01/20/76	07/12/84		101.7	Bone Tumor, Lung Tumor
D-4 MEDIUM	1408 F	331	2.62	28.77	11.5	18.5	01/20/76	10/12/83		92.7	Bone Tumor
D-4 MEDIUM	1428 F	378	3.12	34.36	11.0	21.1	04/20/76	10/28/85		114.3	Processing
D-4 MEDIUM	1535 F	345	3.13	34.48	10.0	20.7	07/22/76		122.3		
D-4 MEDIUM	1446 F	354	3.22	35.40	10.0	21.0	04/20/76	08/10/86		123.7	Processing
D-4 MEDIUM	1364 M	463	3.24	35.65	13.0	20.2	01/20/76	08/02/84		102.4	Lung Tumor
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	07/11/85		92.1	Bone Tumor, Lung Tumor
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	7.32	80.51	12.3	18.3	11/07/77	08/19/83		69.4	Bone Tumor
D-5 MED-HIGH	1636 M	1212	8.48	93.25	13.0	18.9	11/07/77	05/03/83		65.8	Bone Tumor
D-5 MED-HIGH	1621 M	1334	8.66	95.26	14.0	20.3	11/07/77	11/19/84		84.4	Bone Tumor, Lung Tumor
D-5 MED-HIGH	1646 F	1061	8.77	96.45	11.0	18.5	11/07/77	11/11/82		60.1	Bone Tumor
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	06/28/85		91.7	Lung Tumor
D-5 MED-HIGH	1660 M	1518	10.22	112.41	13.5	18.3	11/07/77	09/05/84		81.9	Bone Tumor, Lung Tumor
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	03/18/85		88.3	Bone Tumor, Lung Tumor
D-5 MED-HIGH	1652 F	1320	12.00	131.95	10.0	18.4	11/07/77	07/20/83		68.4	Bone Tumor, Lung Tumor
D-5 MED-HIGH	1619 F	1490	12.32	135.50	11.0	20.3	11/07/77	01/21/83		62.5	Bone Tumor
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	10/22/82		76.0	Bone Tumor, Lung Tumor
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, Lung Tumor
D-6 HIGH	1518 M	3565	29.46	324.09	11.0	20.6	06/23/76	12/18/79		41.8	Rad. Pneumonitis, Lung Tumor

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

INHALED PLUTONIUM NITRATE IN DOGS

DOSE GROUP	DOG IDENT	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		9/30/86	DEATH	
D-2 LOW	1484 F	11	0.10	1.08	10.0	20.5	05/20/76		124.4		
D-2 LOW	1599 F	10	0.10	1.14	9.0	19.2	04/19/77	03/12/86		106.7	Adrenal Tumor
D-2 LOW	1490 F	16	0.15	1.65	9.5	20.5	05/20/76		124.4		
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	0.23	2.36	14.5	22.0	04/20/76	01/04/86		116.5	Processing
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	03/01/85		109.3	Malignant Lymphoma
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		114.5		
D-3 MED-LOW	1595 M	50	0.29	3.23	15.5	18.0	03/15/77		114.5		
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	07/22/85		111.0	Thyroid Tumor, Lung Tumor
D-3 MED-LOW	1587 M	53	0.31	3.40	15.5	18.1	03/15/77	01/14/86		106.0	Hemangiosarcoma, Lung Tumor
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		122.3		
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		114.5		
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		125.3		
D-3 MED-LOW	1439 F	53	0.42	4.61	11.5	21.0	04/20/76		125.3		
D-3 MED-LOW	1523 F	55	0.42	4.60	12.0	21.3	07/22/76		122.3		
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		128.3		
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		114.5		
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		114.5		
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		125.3		
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		128.3		
D-3 MED-LOW	1604 M	85	0.74	8.10	10.5	18.0	03/15/77		114.5		
D-3 MED-LOW	1530 F	72	0.76	8.41	8.5	20.8	07/22/76	09/17/86		121.9	Processing
D-3 MED-LOW	1456 F	61	0.79	8.68	7.0	20.5	04/20/76		125.3		
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		128.3		
D-4 MEDIUM	1637 M	192	1.45	15.99	12.0	18.9	11/07/77		106.7		

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

INHALED PLUTONIUM NITRATE IN DOGS

DOSE GROUP	DOG IDENT	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		9/30/86	DEATH	
VEHICLE	1620 M	0	0.00	0.00	11.0	21.1	12/01/77		106.0		
VEHICLE	1634 F	0	0.00	0.00	10.5	19.6	12/01/77		106.0		
VEHICLE	1651 F	0	0.00	0.00	11.0	19.2	12/01/77		106.0		
D-1 LOWEST	1416 M	0	0.00	0.00	12.0	22.1	05/20/76		124.4		
D-1 LOWEST	1458 F	0	0.00	0.00	10.5	21.5	05/20/76		124.4		
D-1 LOWEST	1489 F	0	0.00	0.00	8.0	20.5	05/20/76	08/04/84		98.5	Esophageal Tumor
D-1 LOWEST	1501 M	0	0.00	0.00	14.0	20.4	05/20/76	01/03/84		91.5	Thyroid Tumor
D-1 LOWEST	1515 M	0	0.00	0.00	13.5	19.8	05/20/76		124.4		
D-1 LOWEST	1573 M	0	0.00	0.00	11.5	19.4	04/19/77		113.4		
D-1 LOWEST	1581 M	0	0.00	0.00	16.5	19.3	04/19/77	07/31/86		111.4	Processing
D-1 LOWEST	1596 M	0	0.00	0.00	14.0	19.2	04/19/77		113.4		
D-1 LOWEST	1600 F	1	0.01	0.11	11.0	19.2	04/19/77		113.4		
D-1 LOWEST	1603 M	2	0.01	0.12	14.0	19.2	04/19/77		113.4		
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		124.4		
D-1 LOWEST	1570 F	2	0.02	0.18	10.0	19.4	04/19/77		113.4		
D-1 LOWEST	1465 F	4	0.03	0.35	12.0	21.0	05/20/76		124.4		
D-1 LOWEST	1470 F	3	0.03	0.29	10.5	21.0	05/20/76	04/09/84		94.7	Meningioma
D-1 LOWEST	1507 M	4	0.03	0.32	14.0	19.8	05/20/76		124.4		
D-1 LOWEST	1592 F	4	0.03	0.29	13.5	19.2	04/19/77		113.4		
D-1 LOWEST	1607 M	5	0.03	0.35	13.0	19.0	04/19/77		113.4		
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		124.4		
D-1 LOWEST	1583 F	4	0.04	0.40	9.5	19.2	04/19/77		113.4		
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	09/28/85		101.3	Hemangiosarcoma
D-2 LOW	1513 M	0	0.00	0.00	11.5	19.8	05/20/76		124.4		
D-2 LOW	1520 M	1	0.01	0.12	10.5	19.5	05/20/76		124.4		
D-2 LOW	1415 M	2	0.02	0.20	11.5	22.2	05/20/76		124.4		
D-2 LOW	1575 M	3	0.02	0.19	14.0	19.4	04/19/77		113.4		
D-2 LOW	1466 F	5	0.03	0.37	14.0	21.0	05/20/76		124.4		
D-2 LOW	1606 F	5	0.04	0.42	12.5	19.0	04/19/77		113.4		
D-2 LOW	1579 M	8	0.05	0.59	14.0	19.3	04/19/77		113.4		
D-2 LOW	1590 F	6	0.05	0.51	12.0	19.2	04/19/77		113.4		
D-2 LOW	1585 F	8	0.06	0.68	12.0	19.2	04/19/77		113.4		
D-2 LOW	1580 F	9	0.07	0.82	11.0	19.3	04/19/77		113.4		
D-2 LOW	1591 M	11	0.07	0.76	15.0	19.2	04/19/77		113.4		
D-2 LOW	1417 M	11	0.08	0.89	12.0	22.1	05/20/76		124.4		
D-2 LOW	1423 M	10	0.08	0.87	11.0	22.1	05/20/76		124.4		
D-2 LOW	1567 M	10	0.08	0.83	12.0	19.4	04/19/77		113.4		
D-2 LOW	1472 F	10	0.09	1.01	10.0	21.0	05/20/76		124.4		
D-2 LOW	1503 F	9	0.09	1.03	8.5	19.8	05/20/76	12/13/84		102.8	Thyroid Tumor
D-2 LOW	1602 M	15	0.09	1.03	14.5	19.2	04/19/77	08/10/86		111.7	Processing

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

INHALED PLUTONIUM NITRATE IN DOGS

DOSE GROUP	DOG IDENT	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		9/30/86	DEATH	
CONTROL	1356 M	0	0.00	0.00					148.7*		
CONTROL	1365 M	0	0.00	0.00					148.6*		
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					147.3*		
CONTROL	1405 M	0	0.00	0.00			08/13/84			121.3* Heart Base Tumor	
CONTROL	1409 M	0	0.00	0.00					146.8*		
CONTROL	1418 M	0	0.00	0.00					146.5*		
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					145.8*		
CONTROL	1483 F	0	0.00	0.00					144.9*		
CONTROL	1509 M	0	0.00	0.00					144.1*		
CONTROL	1516 F	0	0.00	0.00					143.8*		
CONTROL	1525 M	0	0.00	0.00					143.6*		
CONTROL	1526 M	0	0.00	0.00					143.6*		
CONTROL	1528 F	0	0.00	0.00					143.0*		
CONTROL	1543 M	0	0.00	0.00			08/12/86			141.3* Processing	
CONTROL	1563 F	0	0.00	0.00					132.8*		
CONTROL	1572 F	0	0.00	0.00					132.7*		
CONTROL	1577 M	0	0.00	0.00					132.7*		
CONTROL	1584 F	0	0.00	0.00					132.6*		
CONTROL	1594 F	0	0.00	0.00					132.6*		
CONTROL	1608 M	0	0.00	0.00					132.3*		
CONTROL	1633 F	0	0.00	0.00					125.6*		
CONTROL	1638 F	0	0.00	0.00					125.3*		
VEHICLE	1361 M	0	0.00	0.00	8.5	21.0	02/13/76			127.5	
VEHICLE	1381 F	0	0.00	0.00	8.5	19.8	02/13/76			127.5	
VEHICLE	1392 M	0	0.00	0.00	13.0	22.0	04/22/76			125.3	
VEHICLE	1406 M	0	0.00	0.00	13.5	21.6	04/22/76			125.3	
VEHICLE	1412 F	0	0.00	0.00	9.0	19.0	02/13/76			127.5	
VEHICLE	1421 M	0	0.00	0.00	13.0	23.3	06/23/76			123.2	
VEHICLE	1457 F	0	0.00	0.00	12.0	20.6	04/22/76			125.3	
VEHICLE	1491 F	0	0.00	0.00	8.0	21.6	06/23/76			123.2	
VEHICLE	1504 F	0	0.00	0.00	10.0	20.9	06/23/76			123.2	
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			122.1	
VEHICLE	1531 F	0	0.00	0.00	9.0	20.9	07/27/76			122.1	
VEHICLE	1542 M	0	0.00	0.00	12.0	20.8	07/27/76			122.1	
VEHICLE	1566 M	0	0.00	0.00	14.0	18.3	03/15/77			114.5	
VEHICLE	1578 M	0	0.00	0.00	10.5	18.2	03/15/77			114.5	
VEHICLE	1593 F	0	0.00	0.00	11.0	18.0	03/15/77			114.5	
VEHICLE	1601 F	0	0.00	0.00	8.5	18.0	03/15/77			114.5	

* 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 IDENT	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		9/30/86	DEATH	
D-4 MEDIUM	1220 F	518	4.28	47.09	11.0	19.0	04/18/74		149.4		
D-4 MEDIUM	992 F	555	4.39	48.26	11.5	18.8	12/19/72	07/26/84		139.2	Bone Tumor
D-4 MEDIUM	983 M	617	4.67	51.42	12.0	19.0	12/19/72	12/29/83		132.3	Adrenal & Pituitary Tumor
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	03/02/84		123.8	Bone Tumor
D-5 MED-HIGH	1035 F	571	5.46	60.11	9.5	18.2	02/22/73	03/04/84		132.3	Bone Tumor, Cushing's Disease
D-5 MED-HIGH	1192 F	754	6.53	71.81	10.5	18.1	02/26/74	03/29/83		109.0	Bone Tumor
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	01/06/83		110.0	Bone Tumor, Lung Tumor
D-5 MED-HIGH	1047 M	900	8.61	94.74	9.5	18.1	02/22/73	10/05/82		115.4	Vertebral Disk Herniation
D-5 MED-HIGH	1109 F	1119	8.85	97.30	11.5	16.4	05/31/73	08/06/80		86.2	Bone & Lung Tumor, 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 Disease, 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	Lung Tumor, Osteoarthritis
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 Disease
D-6 HIGH	1175 F	6201	75.16	826.80	7.5	16.6	11/06/73	02/24/78		51.6	Lung Tumor

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

DOSE GROUP	DOG IDENT	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		9/30/86	DEATH	
D-3 MED-LOW	1165 M	76	0.43	4.75	16.0	17.3	11/06/73	07/21/86		152.4	Processing
D-3 MED-LOW	1309 M	60	0.44	4.80	12.5	18.5	03/04/75		138.9		
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		138.9		
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	09/22/83		123.7	Delayed Radiation Pneumonitis
D-3 MED-LOW	1190 F	71	0.54	5.92	12.0	18.1	02/26/74	05/09/85		134.4	Lung Tumor
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	01/29/86		157.3	Processing
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	08/08/83		125.5	Malignant Lymphoma
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		160.0		
D-3 MED-LOW	1000 F	70	0.71	7.78	9.0	18.7	12/19/72		165.4		
D-3 MED-LOW	1056 M	97	0.71	7.76	12.5	17.9	02/22/73	06/17/86		159.8	Processing
D-3 MED-LOW	1004 M	116	0.73	8.00	14.5	19.6	01/18/73		164.4		
D-3 MED-LOW	1026 M	116	0.78	8.59	13.5	19.2	01/18/73	11/13/85		153.8	Processing
D-3 MED-LOW	1043 F	98	0.89	9.80	10.0	18.1	02/22/73	09/21/81		102.9	Empyema, Pituit.T., Cushing's
D-3 MED-LOW	1031 F	76	0.92	10.13	7.5	19.1	02/22/73	05/04/84		134.3	Pneumonia
D-3 MED-LOW	1212 F	111	1.12	12.33	9.0	17.6	02/26/74		151.1		
D-4 MEDIUM	1176 M	129	0.87	9.56	13.5	16.6	11/06/73	12/12/85		145.2	Hemangioma, Spleen
D-4 MEDIUM	1221 F	124	1.13	12.40	10.0	19.0	04/18/74		149.4		
D-4 MEDIUM	1195 M	228	1.38	15.20	15.0	18.1	02/26/74		151.1		
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	02/02/85		143.3	Cushing's Disease
D-4 MEDIUM	997 M	203	1.60	17.65	11.5	19.6	01/18/73	05/08/86		159.6	Processing
D-4 MEDIUM	991 F	194	1.76	19.40	10.0	18.8	12/19/72	06/20/83		126.0	Urinary Bladder & Ovarian Tmr
D-4 MEDIUM	1177 M	262	1.76	19.41	13.5	16.6	11/06/73	03/12/85		136.1	Bone Tumor
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	04/08/83		118.2	Bone Tumor, Lung Tumor
D-4 MEDIUM	973 F	271	2.24	24.64	11.0	19.2	12/19/72	10/08/84		141.6	Bone Tumor
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		160.0		
D-4 MEDIUM	1114 M	430	2.70	29.66	14.5	16.4	05/31/73	04/23/85		142.8	Bone Tumor,Bile Duct Carcinom
D-4 MEDIUM	1062 M	435	2.93	32.22	13.5	17.8	02/22/73	05/30/84		135.2	Bone Tumor, Lung Tumor
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	04/14/83		121.7	Pneumonia, Rad. Pneumonitis
D-4 MEDIUM	1198 M	539	3.50	38.50	14.0	17.9	02/26/74	09/14/86		150.6	Processing
D-4 MEDIUM	952 F	365	3.69	40.56	9.0	19.2	12/19/72	06/03/83		125.4	Bone Tumor
D-4 MEDIUM	1166 M	673	4.08	44.87	15.0	17.3	11/06/73	06/23/84		127.5	Malignant Lymphoma

* 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 IDENT	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		9/30/86	DEATH	
D-1 LOWEST	1069 F	2	0.02	0.24	8.5	18.1	05/31/73	06/24/83		120.8	Malignant Lymphoma
D-1 LOWEST	1095 F	2	0.02	0.19	10.5	16.6	05/31/73		160.0		
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		151.1		
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	07/01/86		162.4	Processing
D-1 LOWEST	1106 F	5	0.05	0.50	10.0	16.4	05/31/73	03/14/83		117.4	Adrenal Tmr, Osteoarthropathy
D-2 LOW	1065 F	6	0.05	0.60	10.0	18.3	05/31/73	04/10/86		154.3	Processing
D-2 LOW	1082 M	11	0.06	0.69	16.0	18.0	05/31/73	12/04/79		78.1	Paralysis, Spinal Cord Degen.
D-2 LOW	1188 M	11	0.06	0.71	15.5	18.4	02/26/74	01/15/84		118.6	Metastatic Lnq Tmr, Prim. Unk
D-2 LOW	1084 M	13	0.07	0.76	17.0	17.5	05/31/73		160.0		
D-2 LOW	1090 F	10	0.08	0.83	12.0	17.3	05/31/73		160.0		
D-2 LOW	1222 M	15	0.10	1.07	14.0	19.0	04/18/74	03/19/86		143.0	Maliq (mediast) Mesothelioma
D-2 LOW	971 F	13	0.11	1.24	10.5	19.2	12/19/72	05/04/83		124.5	Hemangiosarcoma, Spleen
D-2 LOW	999 F	11	0.11	1.16	9.5	18.7	12/19/72	01/31/86		157.4	Processing
D-2 LOW	1229 M	16	0.11	1.19	13.5	16.8	02/26/74	05/25/84		122.9	Pneumonia, Thyroid Tumor
D-2 LOW	1070 M	22	0.12	1.33	16.5	18.1	05/31/73	12/13/83		126.4	Round Cell Sarcoma: Kidney
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		165.4		
D-2 LOW	1033 M	17	0.14	1.55	11.0	19.1	02/22/73	12/17/85		153.8	Processing
D-2 LOW	1036 F	16	0.14	1.52	10.5	18.2	02/22/73		163.2		
D-2 LOW	1216 M	23	0.16	1.77	13.0	19.3	04/18/74		149.4		
D-2 LOW	1060 F	22	0.18	2.00	11.0	17.8	02/22/73	12/21/84		141.9	Pneumonia
D-2 LOW	981 M	30	0.21	2.31	13.0	19.0	12/19/72		165.4		
D-2 LOW	1046 M	27	0.22	2.45	11.0	18.1	02/22/73		163.2		
D-2 LOW	1050 F	22	0.22	2.44	9.0	18.1	02/22/73	05/14/86		158.7	Processing
D-2 LOW	1078 F	29	0.22	2.42	12.0	18.0	05/31/73	11/09/83		125.3	Meningioma, Malignant
D-2 LOW	1207 F	22	0.24	2.59	8.5	17.6	02/26/74		151.1		
D-2 LOW	1196 F	28	0.25	2.80	10.0	17.9	02/26/74		151.1		
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	06/21/83		120.7	Malignant Lymphoma
D-3 MED-LOW	972 F	40	0.33	3.64	11.0	19.2	12/19/72	03/04/86		158.5	Processing
D-3 MED-LOW	1089 F	41	0.34	3.73	11.0	17.3	05/31/73		160.0		
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		149.4		
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		154.8		

* 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 IDENT	INITIAL ALVEOLAR DEPOSITION			INHALATION EXPOSURE			DATE OF DEATH	MONTHS SINCE INHALATION		COMMENTS ON DEAD DOGS
		NCI	NCI/G LONG	NCI/KG	WEIGHT (KG)	AGE* (MO)	DATE		9/30/86	DEATH	
CONTROL	939 M	0	0.00	0.00				10/01/82		136.9*	Urinary Bladder Tumor
CONTROL	949 F	0	0.00	0.00				10/30/84		161.7*	Malignant Lymphoma
CONTROL	978 M	0	0.00	0.00					184.5*		
CONTROL	990 F	0	0.00	0.00				07/08/79		97.4*	Pyometra
CONTROL	996 F	0	0.00	0.00				07/06/84		157.2*	Malignant Lymphoma
CONTROL	1005 M	0	0.00	0.00					184.0*		
CONTROL	1007 F	0	0.00	0.00					184.0*		
CONTROL	1024 M	0	0.00	0.00					183.5*		
CONTROL	1038 M	0	0.00	0.00					181.4*		
CONTROL	1045 M	0	0.00	0.00				06/08/86		177.6*	Processing
CONTROL	1054 F	0	0.00	0.00					181.1*		
CONTROL	1061 F	0	0.00	0.00				07/07/81		118.2*	Malignant Lymphoma
CONTROL	1093 M	0	0.00	0.00				11/04/83		142.4*	Pituitary Tumor, Cushing's
CONTROL	1097 F	0	0.00	0.00					176.6*		
CONTROL	1112 M	0	0.00	0.00					176.4*		
CONTROL	1116 F	0	0.00	0.00					176.1*		
CONTROL	1186 F	0	0.00	0.00				07/26/85		155.3*	Urinary Bladder Tumor
CONTROL	1197 M	0	0.00	0.00					169.0*		
CONTROL	1209 M	0	0.00	0.00					168.7*		
CONTROL	1225 F	0	0.00	0.00					167.9*		
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			158.7	Processing
D-1 LOWEST	1003 M	0	0.00	0.00	14.0	19.6	01/18/73		164.4		
D-1 LOWEST	1023 F	0	0.00	0.00	12.5	19.2	01/18/73		164.4		
D-1 LOWEST	1039 M	0	0.00	0.00	11.0	17.0	01/18/73			161.5	Processing
D-1 LOWEST	1044 F	0	0.00	0.00	11.5	17.0	01/18/73		164.4		
D-1 LOWEST	1055 M	0	0.00	0.00	13.0	16.8	01/18/73		164.4		
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	02/08/85		140.3	Malignant Lymphoma
D-1 LOWEST	1194 F	0	0.00	0.00	10.5	19.8	04/18/74	12/03/85		139.5	Malignant Lymphoma
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	09/30/86		149.4	Processing
D-1 LOWEST	951 M	2	0.01	0.14	14.0	19.3	12/19/72	02/14/83		121.9	Anesthetic Death
D-1 LOWEST	1008 M	2	0.01	0.15	13.5	19.6	01/18/73	10/24/85		153.2	Processing
D-1 LOWEST	1193 F	2	0.01	0.16	12.5	19.8	04/18/74	01/22/86		141.2	Immune Hemolytic Anemia
D-1 LOWEST	959 M	3	0.02	0.22	13.5	19.2	12/19/72	06/22/84		138.1	Liver Abscess

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DOSE-EFFECT STUDIES WITH INHALED PU-239 OXIDE IN BEAGLES

DOSE GROUP	DOG IDENT	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		9/30/86	DEATH	
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	Lung Tumor, Osteoarthropathy	
D-5 MED-HIGH	783 M	1394	10.14	111.52	12.5	19.0	02/10/71	12/03/75	57.7	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. Pneumonitis	
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	15.7	02/10/71	09/17/75	55.2	Lung Tumor, 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	02/10/71	06/26/75	52.5	Lung Tumor, 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	11/09/71	11/22/72	12.5	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	

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DOSE-EFFECT STUDIES WITH INHALED PU-239 OXIDE IN BEAGLES

DOSE GROUP	DOG IDENT	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		9/30/86	DEATH	
D-3 MED-LOW	797 F	85	0.70	7.73	11.0	16.4	03/04/71	05/16/86		182.4	Processing
D-3 MED-LOW	848 F	75	0.72	7.94	9.5	21.3	10/07/71		179.8		
D-3 MED-LOW	827 F	89	0.74	8.09	11.0	16.7	04/26/71	01/06/85		164.4	Acute Pneumonitis
D-3 MED-LOW	697 M	140	0.85	9.33	15.0	19.5	10/30/70	05/08/80		114.3	Cardiac Valve Insufficiency
D-3 MED-LOW	750 M	118	0.93	10.26	11.5	19.6	01/20/71	06/28/84		161.2	Lung Tmr, Malignant Lymphoma
D-3 MED-LOW	884 M	123	1.12	12.30	10.0	17.8	10/08/71	09/12/84		155.2	Lung Tumor
D-3 MED-LOW	844 F	135	1.17	12.86	10.5	17.6	06/08/71	08/08/85		170.0	Nephropathy, Lung Tumor
D-3 MED-LOW	905 F	127	1.36	14.94	8.5	15.9	11/10/71	02/07/83		134.9	Malignant Lymphoma
D-4 MEDIUM	866 M	200	1.35	14.81	13.5	17.4	07/06/71	06/27/84		155.7	Lung Tumor
D-4 MEDIUM	809 F	157	1.36	14.95	10.5	15.3	03/04/71	05/28/81		122.8	Liver Cirr, Thy T., Addison's
D-4 MEDIUM	764 F	158	1.37	15.05	10.5	18.2	12/21/70	07/07/82		138.5	Lung Tumor
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	02/03/86		177.3	Lung Tumor, Bile Duct Carcinom
D-4 MEDIUM	814 F	140	1.50	16.47	8.5	15.1	03/04/71	10/17/79		103.5	Lung Tumor, 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	08/20/85		170.4	Nephropathy, Lung Tumor
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	04/04/83		143.3	Metritis, Adrenal & Thy Tumor
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 Tumor, Cushing's
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		138.9	Lung Tumor
D-4 MEDIUM	805 F	257	3.12	34.27	7.5	18.5	06/08/71	07/22/82		133.5	Esophageal & Lung Tumor
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	04/25/71	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 Tumor, Intestinal Tumor
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 & Lung & Adr Tumor
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

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DOSE-EFFECT STUDIES WITH INHALED PU-239 OXIDE IN BEAGLES

DOSE GROUP	DOG IDENT	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		9/30/86	DEATH	
D-1 LOWEST	788 M	8	0.06	0.62	13.0	18.8	02/10/71	04/13/84		158.1	Chronic Nephropathy
D-1 LOWEST	850 F	5	0.06	0.63	8.0	21.3	10/07/71	06/06/83		140.0	Bone Tmr, Chronic Nephropathy
D-1 LOWEST	893 M	9	0.06	0.61	14.0	14.9	10/07/71	07/01/86		176.8	Pneumonia
D-1 LOWEST	807 F	8	0.07	0.73	11.0	14.6	02/10/71	07/24/81		125.4	Pituitary Tumor, Cushing's
D-1 LOWEST	841 F	6	0.07	0.75	8.0	17.7	06/08/71	04/01/86		177.8	Malignant Lymphoma
D-1 LOWEST	908 M	9	0.07	0.77	11.0	15.9	11/10/71	04/01/80		100.7	Unknown, Pulmon. Hyalinos
D-2 LOW	776 M	10	0.07	0.74	13.5	20.2	03/04/71	09/19/84		162.6	Bronchopneumonia
D-2 LOW	842 M	10	0.07	0.77	13.5	18.6	07/06/71	05/01/85		165.8	Lung Tmr, Chronic Nephropathy
D-2 LOW	767 M	10	0.08	0.83	12.0	18.2	12/21/70	12/09/85		179.6	Valvular Endocardopathy
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	06/25/83		144.6	Peritonitis
D-2 LOW	871 M	13	0.09	0.96	13.5	16.9	07/06/71	07/24/86		180.6	Processing
D-2 LOW	874 M	16	0.11	1.24	13.0	16.8	07/06/71	04/09/85		165.1	Chronic Nephropathy
D-2 LOW	754 M	22	0.15	1.69	13.0	19.5	01/19/71	01/10/78		83.7	Epilepsy
D-2 LOW	845 F	19	0.15	1.63	11.5	17.6	06/08/71	08/09/84		158.1	Urinary Bladder Tumor
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	15.7	02/10/71	08/29/74		42.6	Sacrificed
D-2 LOW	826 F	19	0.17	1.90	10.0	19.1	07/06/71	04/17/84		153.4	Hemangioma, Spleen
D-2 LOW	831 F	21	0.18	2.00	10.5	17.9	06/08/71	05/14/84		155.2	Pneumonia
D-2 LOW	881 F	19	0.19	2.09	9.0	17.7	10/07/71		179.8		
D-2 LOW	780 F	24	0.22	2.40	10.0	18.2	01/19/71	04/08/82		134.6	Pheochromocytoma
D-2 LOW	859 M	35	0.22	2.41	14.5	18.2	07/06/71	04/22/84		153.6	Urinary Bladder Tumor
D-2 LOW	757 M	36	0.23	2.57	14.0	18.5	12/21/70		189.3		
D-2 LOW	876 F	19	0.24	2.69	7.0	17.9	10/07/71	05/05/86		174.9	Nephropathy, Lung Tumor
D-2 LOW	806 F	26	0.25	2.74	9.5	15.3	03/04/71	10/29/82		139.9	Palate: Malignant Melanoma
D-2 LOW	813 F	32	0.29	3.20	10.0	15.1	03/04/71	12/15/83		153.4	Multilobular Sarcoma, Skull
D-2 LOW	877 F	34	0.29	3.24	10.5	17.9	10/07/71	05/06/86		174.9	Lung Tumor
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	12/28/84		164.1	Pneumonia
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	11/02/83		153.4	Lung Tumor
D-3 MED-LOW	782 M	62	0.42	4.59	13.5	19.0	02/10/71	05/27/83		147.5	Neurofibrosarcoma, Brachial P
D-3 MED-LOW	786 M	62	0.42	4.59	13.5	19.5	03/04/71	05/29/86		182.8	Processing
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	05/24/84		156.9	Urinary Bladder Tumor
D-3 MED-LOW	883 M	63	0.44	4.85	13.0	17.7	10/07/71		179.8		
D-3 MED-LOW	778 M	74	0.46	5.10	14.5	20.2	03/04/71	08/26/79		101.7	Pulmonary Thromboembolism
D-3 MED-LOW	838 M	56	0.46	5.09	11.0	17.8	06/08/71	07/20/84		157.4	Malignant Lymphoma, Lung Tmr
D-3 MED-LOW	795 F	54	0.49	5.40	10.0	15.0	01/20/71	09/06/83		151.5	Lung Tumor
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		179.8		
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 IDENT	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		9/30/86	DEATH	
CONTROL	738 F	0	0.00	0.00				08/11/83		171.5*	Hemangiosarcoma, Heart
CONTROL	740 F	0	0.00	0.00				06/18/83		169.8*	Malignant Lymphoma
CONTROL	749 F	0	0.00	0.00				09/14/84		183.4*	Adrenalitis
CONTROL	755 M	0	0.00	0.00				12/10/82		162.2*	Status Epilepti, Nephrosclero
CONTROL	766 M	0	0.00	0.00				06/26/84		180.3*	Lung Tumor
CONTROL	775 F	0	0.00	0.00				10/05/81		147.3*	Pulmonary Thromboembolism
CONTROL	785 M	0	0.00	0.00					206.4*		
CONTROL	789 M	0	0.00	0.00			07/25/83			167.9*	Malignant Lymphoma
CONTROL	792 M	0	0.00	0.00			04/28/76			79.5*	Oral Tumor
CONTROL	800 F	0	0.00	0.00					203.3*		
CONTROL	801 M	0	0.00	0.00			02/23/82			148.1*	Lung Tumor
CONTROL	811 F	0	0.00	0.00			02/24/85			183.1*	Oral cav.: Malignant Melanoma
CONTROL	846 M	0	0.00	0.00			04/08/83			159.6*	Nephrosclerosis
CONTROL	861 M	0	0.00	0.00					201.0*		
CONTROL	868 F	0	0.00	0.00					199.7*		
CONTROL	872 F	0	0.00	0.00			11/05/82			152.8*	Lung Tumor
CONTROL	878 M	0	0.00	0.00			01/22/85			177.4*	Chronic Nephropathy
CONTROL	882 M	0	0.00	0.00			11/06/81			138.7*	Hemangiosarcoma, Liver
CONTROL	885 F	0	0.00	0.00			02/18/83			153.5*	Lung Tumor
CONTROL	903 F	0	0.00	0.00			01/30/85			174.6*	Malignant Lymphoma
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	04/21/83		147.0	Epilepsy
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	01/23/85		162.6	Kidney Failure
D-1 LOWEST	858 M	0	0.00	0.00	13.5	18.2	07/06/71		182.8		
D-1 LOWEST	865 F	0	0.00	0.00	9.0	17.4	07/06/71	09/16/86		182.4	Processing
D-1 LOWEST	879 M	0	0.00	0.00	14.5	17.9	10/07/71	07/27/84		153.7	Hemangiosarcoma, Liver, Spleen
D-1 LOWEST	886 F	0	0.00	0.00	10.5	18.2	11/10/71	04/04/84		148.8	Meningioma, Malignant
D-1 LOWEST	907 F	0	0.00	0.00	11.5	15.9	11/10/71	05/10/86		174.0	Pneumonia
D-1 LOWEST	825 F	1	0.01	0.12	11.5	18.1	06/08/71	11/17/82		137.3	Hemangiosarcoma, Spleen
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	9.5	15.9	11/10/71	12/19/83		145.3	Chondrosarcoma, Nasal
D-1 LOWEST	832 F	2	0.02	0.22	9.0	16.5	04/26/71	03/03/86		178.2	Malignant Lymphoma
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	05/04/84		154.0	Pneumonia
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	02/07/86		175.1	Malignant Lymphoma
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	853 M	8	0.05	0.51	15.0	21.3	10/07/71	12/12/84		158.2	Bronchopneumonia
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
D-1 LOWEST	770 F	6	0.06	0.63	9.5	19.1	01/19/71	11/29/84		166.3	Glomerulosclerosis

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

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