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INHALED ^{239}PU OXIDE IN DOGS:
Experimental Protocol and Individual Animal Summary Information

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Introduction

Purpose and Scope

The long term effects of low levels of inhaled $^{239}PuO_2$ in beagle dogs were studied. The dogs were given a single exposure to a plutonium oxide aerosol in 1970-71. Since 1988, when the last dog died, the extensive clinical and histopathological records have been reviewed, radioanalysis of stored samples has been completed, and the experimental protocol has been up-dated to incorporate modifications in the 18 year period of life-span observation. This report summarizes those aspects of the study; it does not focus on the results of the study.

The document has two major sections. First, there is a description of the experiment, summarizing the background, design, dosimetry procedures, animal care protocol, necropsy protocol, and data management systems. Second, there is the tabular summary which is introduced by a description of each tabulated item. This summary is a more detailed report on individual beagle dogs assigned to the study than we have presented previously. Brief summaries (limited to one line per dog) have long been a feature of our annual reports. These summaries contained **provisional** descriptions of significant findings at the death of each dog. We have completed our review of the clinical and histopathologic records, and present in this document the **final** description of the cause of death. In addition to the rather restricted dosimetric information in the annual reports, we have added report fields which summarize the initial exposures or lung deposition and distribution of plutonium in major organs at death. The report which comprises the bulk of this report has been expanded to a six line summary for each of the 153 dogs.

Finally, the document includes two appendices, one listing annual reports, the other listing papers and presentations about our life span beagle studies.

Conventions used in this Document

Compilation of this report was complicated by the fact that the names and definitions of the units of measure for radioactivity and radiation dose have changed since the study was initiated. Our method

of dealing with this is outlined below.

Units of Radioactivity

This study was designed, and the data was collected, using the traditional measure of activity of a radionuclide, the Curie (Ci). Dogs inhaled from 0.001 to 5 μCi (micro Curie, 10^{-6} Ci). Tissue and excreta samples contained between less than 1 to several hundred nCi (nano Curie 10^{-9} Ci). Records in the study archives (written and electronic) use the Curie as the unit of measure.

However, our journal publications summarizing the study report the SI unit, the becquerel (Bq) or kilo becquerel (KBq) in accordance with international usage. The two measures are related by the expression;

$$1\text{Ci} = 3.7 \text{ X } 10^{10} \text{ Bq.}$$

Both units are reported in the body of this report, with the conventional unit first, followed by the SI value and unit within parenthesis. However, the tables summarizing individual dogs are reported strictly in nCi and reflect the organization of underlying databases.

Since the conversion to SI units of activity is not in even multiples, the reader is cautioned that values expressed as kBq in this report appear to be more precise than those in μCi. This is strictly an artifact in unit conversion. For example, we report in Table 2 that the target alveolar deposition for the highest exposure-level group was 3 μCi (111.111 kBq). If the study were being designed today, the target would have been 100 kBq.

Units of Radiation Dose

Detailed dosimetry is not included in this report, however there are occasional references to radiation dose in general. At the conception of this study, radiation dose was reported as radiation absorbed dose (rad) or roentgen equivalent man (rem). The corresponding SI units are the gray (Gy) and sievert (Sv). These are related by a factor of 100 as follows:

1 rad = 0.001 Gy

1 rem = 0.001 Sv

Background

The US Department of Energy (DOE), and its predecessor agencies, the Atomic Energy Commission (AEC) and the Energy Research and Development Administration (ERDA), recognizes the need for increased knowledge and has supported research regarding the biological effects of internally deposited radionuclides since its inception. The biological effects of internally deposited radionuclides were studied in experimental animals in order to help predict the risks of accidental exposures to workers or the general to general population. In addition to the wide variety of radionuclides studied, the pathways of exposures needed to be studied: ingestion, inhalation, or through absorption from the skin or wounds.

The first emphasis of biological effects research in the 1940s was on rodent studies to determine the acute effects of high levels of exposures. Later studies were performed on the long term effects of lower exposure levels of radionuclides in experimental animals. The dog was chosen as an experimental animal for studies on the biological effects of plutonium since dogs were large enough for the observation of clinical signs for correlation of effects with clinical signs in humans.

The earliest studies with ^{239}Pu in beagles began at the University of Utah in 1952. These studies involved a single intravenously injected dose of plutonium citrate in young dogs to compare the bone cancer risk to dogs similarly injected with ^{226}Ra. The plutonium/radium toxicity ratio would then be applied to the risk of humans exposed to radium in order to predict the effect of plutonium on bones. These studies were designed to parallel the exposure experience of the Radium Dial Painters; brief exposures at an early age. In another study, also started in 1952, at the University of California at Davis researchers examined the effects of external exposure to x rays; a possible exposure scenario for crews of nuclear-powered vessels (primarily aircraft).

Reason for Interest in Plutonium

The 1959-1962 $^{239}PuO_2$ inhalation life span studies incorporated relatively high levels of plutonium; results indicated a high incidence of pulmonary tumors in beagles exposed at these levels. These studies provided the first evidence that inhaled plutonium could cause lung tumors in beagles at moderately high exposure levels. The plutonium burdens at necropsy ranged from 0.4 to 2.7 μCi. (Park, *et al.* 1970) It became apparent to researchers that studies were needed at much lower exposure levels to obtain dose–effect relationships information that could be extrapolated to the human population. As a potential occupational health hazard, many radionuclides were an ingestion hazard because of environmental contamination of soil and food supply. However, occupational exposure to plutonium would most likely be through inhalation. ?????????? see page 6 of Park's comments to june 7 version.

~~^{239}Pu and ^{238}Pu were potential inhalation hazard concerns and the comparison of the effects of each radioisotope was expected to provide fundamental data on the influence of specific activity and spatial distribution of dose in the lung on cancer incidence. Both isotopes have "similar radiation characteristics, but ^{238}Pu has specific activity 280 times greater than ^{239}Pu." (Park, *et al* 1970) Although the original proposal called for a parallel study of the two nuclides, the $^{239}PuO_2$ study started in 1970 while the $^{238}PuO_2$ study was started 2 years later.~~

Choice of Experimental Animal

Mice and rats were commonly used as the experimental animals in early radiobiology studies to determine acute responses to radiation exposure. For studies investigating the life span effects of $^{239}PuO_2$ and $^{238}PuO_2$, a larger, longer–lived animal than the mouse was needed to extrapolate results to human exposure dose–effect relationships. Also, plutonium was not retained in the rodent liver and lung as long as it was expected to be retained in people or in dogs. The mouse is a relatively short–lived animal; the mouse average two–year life span is less than the latent period between exposure and the formation of lung tumors in what species?. An animal model was needed for these inhalation studies that would closer reflect

the life span of humans.

Several species of laboratory animals were considered for use in the life span experiments, and many were easily eliminated due to cost of maintenance, handling of contaminated animals, and were either too short–lived or too long–lived. Mice were too short–lived. Non–human primates were considered, but lived too long and were difficult, as well as expensive, to maintain and handle.

In 1950, the American Foxhound was proposed as ideal for use in this type of research, but an adequate was not readily available. By the time the $^{239}PuO_2$ life span research began at PNL, the beagle dog had already been used as the life span experimental animal in several laboratories and had been specifically bred for research. The continued use of the beagle in subsequent research was necessary for comparing data in the same animal model across laboratories. (Thompson, 1989)

Thompson (1989) summarized other justifications for choosing the beagle; they were readily available, easy to manage, had an optimum size (an average weight of 10 kg), an optimum life span of 12–15 years, and had physiologic and anatomic similarities to humans (particularly in regards to the hematopoietic, skeletal, and pulmonary systems). Furthermore, acute studies with inhaled plutonium had been completed at two separate laboratories for comparison with the proposed PNL studies.

Beagle Studies at PNL

The first $^{239}PuO_2$ inhalation life span studies began at the Pacific Northwest Laboratory (PNL) in 1959. Two separate studies, under the leadership of WJ Bair, involving 35 and 22 beagles respectively, began to determine the biological late effects of toxic levels of inhaled ^{239}Pu. The exposed animals in these experiments had nearly a 100% incidence of lung tumors (1988, Thompson); this was the first observation of the induction of lung tumors by inhaled plutonium in dogs. As time passed, the research emphasis shifted to the long term

effects of occupational exposures at lower dose levels and through "real life" exposure pathways. A worker's exposure would most likely be to breathe contaminated particles over a long period of time, resulting in a low–level, chronic exposure. This closely resembled the uranium miners exposures; a population which also had a high incidence of lung tumors.

This document describes the third beagle life span study conducted at PNL, "Inhaled ^{239}Pu Oxide in Dogs." (Studies were assigned sequential numbers by the Biology Department; this study was known internally as Experiment 140). Animals were exposed on this study between 1969 and 1975, and the last one died in January 1988. Table 1 places the ^{239}Pu Oxide study in perspective with the other PNL life span studies.

Table 1. PNL Life span Beagle Studies

PNL Experiment Number	Insult	Exposure Dates	Number of Dogs	Death of Last Dog	Investigator
32	$^{239}PuO_2$	1959—66	35	1971	Bair
110	$^{238}PuO_2$	1967—70	22	1979	Bair
140	$^{239}PuO_2$	1970—72	153	1988	Park
158	$^{238}PuO_2$	1972—75	165	1989	Park
182	$^{239}Pu(NO_3)_4$	1975—77	176	1992	Dagle

PNL Beagle Colony

A breeding colony of purebred beagles was developed at PNL especially for radiobiology research in 1961. The PNL dog breeding colony was started with combined stock from the University of California at Davis, Washington State University, commercial suppliers, and private breeders. The breeding program was based on separate–generation, and random–mating that prohibited full– and half–sibling matings. This breeding program minimized genetic drift and maintained a stable gene pool. Dogs were selected for breeding based on conformation, disposition, fertility, and lack of endemic canine epilepsy. They were primarily tri–colored (with mixtures of black, brown, and white) with predominantly white haircoat in approximately 20% of the dogs.

A lineage chart was developed for each animal and became part of the dog's permanent clinical record. Each animal represented on a lineage chart was letter–coded with a combination of D's (Davis), P's (Pullman), and H's (Hanford) to represent how many times each breeding line contributed to the animal's genetic makeup. For example, a male with an lineage–code with heavy representation from the Davis and Pullman lines (i.e. DDPPH) would be mated to a female with a genetic line under represented in the Davis and Pullman lines and heavy representation in the Hanford line (i.e DPHHH). These lineage codes were used to facilitate the choice of breeding animals. Our goal was to minimize genetic drift and to eliminate undesirable physiologic traits such as parrot mouth in the colony.

The breeding colony was originally located at the 100–F area of the AEC Hanford Site, about 30 miles North of Richland, Washington. New beagle colony housing and exposure facilities were constructed as part of the 331 building, and the colony was moved in 1970. Thus, while all exposures were performed in the 331 building, many of the animals were born at 100–F.

At the initiation of this study the colony had produced about 900 animals. Dogs, including

those which were stillborn, were assigned a number at birth as a routine colony management task. This unique sequential number was tattooed on the medial aspect of the pinna of both ears at what age?. The beagle colony was in a barrier maintained facility that required clean coveralls and shoe covers worn by all entering personal. An Institutional Animal Care and Use Committee, appointed by the Director of the Laboratory, reviewed the animal facilities and procedures at least annually.

Experimental Design

The original experimental design (Park *et al.*, 1969) included parallel inhalation exposures to both $^{239}PuO_2$ and $^{238}PuO_2$. The $^{238}PuO_2$ portion of the study, commonly known at PNL as Experiment 158, began in 1972, (Thompson 1989) and will be described in a subsequent document of similar format.

Plutonium Oxide Aerosol

$^{239}PuO_2$ aerosol was generated by nebulizing in water from crushed $^{239}PuO_2$. Crushed $^{239}PuO_2$ was prepared by calcining the oxalate at 750°C for 2 hours. The resulting aerosol had the Activity Mean Aerodynamic Diameter (AMAD) of 2.3μm and a mean Geometric Standard Deviation (GSD) of 1.9.(Park, *et al*, 1976)

Unanesthetized dogs can be subjected to nose–only inhalation exposures for only a limited time—a duration of approximately 5 – 50 minutes. (Craig, *et al*, 1972) In order to expose the dogs to different levels of $^{239}PuO_2$, a variation of the quantity of Pu inhaled by the dogs had to be achieved by changing the aerosol concentrations, and "the most important factor in determining the percentage of inhaled material that was deposited in the alveolar regions of the lung was the aerodynamic size distribution of the aerosol." (Craig, *et al*, 1972) In the early years, aerodynamic size, distribution, and concentrations of the aerosols were monitored in the laboratory by classification of particles on samples suitable for optical or electron microscopic examination. (Craig, *et al.* 1972)

The actual aerosol concentrations only became available when the filter paper samples taken during dog exposures were analyzed, usually after a delay of several days. An alpha aerosol monitor was developed which could determine the aerosol concentrations immediately preceding or during exposure. During exposures, two continuous samples [were] drawn through membrane filters that

were later counted on a separate counting system. This provided a value for the average concentration during the actual exposure. (Decker, *et al*, 1972)

Original Experimental Design

In 1969, a low level study was proposed to follow the high level studies. In the proposed experimental design, six exposure-level groups were planned for each nuclide: 3.0, 1.25, 0.25, 0.05, 0.01, 0.002 μCi respectively (110, 50, 10, 2, 0.4, 0.08 kBq). 10 dogs were assigned to each of the highest exposure-level groups, and 20 to each remaining group. Twenty dogs were designated as control animals. Twelve dogs were designated as sacrifice dogs to obtain plutonium distribution and pathology data, and 108 were assigned to lifespan dose–effect studies. The original experimental design is shown in Table 2. (Park, *et al*, 1970)

The two high–level exposure groups in the experimental design were to overlap the levels in the 1959–1962 inhalation studies. The evidence from the early studies predicted a high incidence of pulmonary tumors in these groups. The lowest level exposure group was corresponded to the then established permissible 16 nCi (~600 Bq) lung burden; i.e., a burden which would result in an average dose (as calculated by the International Commission on Radiological Protection) of 15 rem (150 mSv)/year to the lung. (Park, 1970)

Table 2. ^{238}Pu–^{239}Pu Oxide Study Design Proposed in 1969

Initial Alveolar Deposition Level		***Number of Dogs to be Exposed***	
µCi	***kBq***	***$^{239}PuO_2$ (Experiment 140)***	***$^{238}PuO_2$ (Experiment 158)***
3.0	111.111	10	10
1.25	46.296	10	10
0.25	9.259	20	20
0.05	1.852	20	20
0.01	0.370	20	20
0.002	0.074	20	20
Controls		20	

Experimental Implementation

In practice, it was not possible to follow the original design precisely and the original design was changed. Internally the $^{239}PuO_2$ portion became Experiment 140 (begun in 1970), and $^{239}PuO_2$ became Experiment 158 (began in 1972).

It was soon apparent that there would be a span of 5 to 10 years between the first and last exposures of the two experiments. This lead to the addition of separate control groups (20 dogs) for each nuclide. At weaning, dogs were randomly assigned to experiment groups based on animal number. Approximately an equal number of males and females were assigned to each plutonium exposure level. An experimental assignment plan was developed based on an expected average of six puppies per litter, if more than six were alive at weaning, the least healthy were culled (removed from the study). Culled dogs, and those in excess of study needs, were transferred to other studies. The breeding of dogs for these

studies and their entry into the inhaled $^{239}PuO_2$ study took several years. The lifespan experiment continues through the normal lifespan of the beagles, with a median of 13 years for the controls in this experiment. The oldest dog was 18 years of age at the time of death. The first exposure was done on 10/9/70 and the final exposure was completed on 7/19/72. Exposures were done on animals born of 42 litters, the earliest birth date was 7/21/64 and the final litter was born on 2/8/71. The first dosimetry animal was sacrificed on 10/16/70, and the last lifespan animal in this experiment was euthanized on 1/25/88.

Plutonium inhalation exposure at such low levels was difficult to detect in an intact animal, it was difficult to predict the breathing pattern of individual dogs, and the only way to confidently determine the quantity of plutonium delivered to the lung was through post mortem radioanalysis.

A combination of *in vivo* low energy x–ray detection of life span dogs and *post mortem* radioanalysis of serial sacrifice dogs was employed to determine the placement of animals into dose groups. The final experimental groups are shown in the Table 3 illustrating the exposure groups, number of dogs of each sex assigned each group, and lung activity expressed as both activity and concentration.

Table 3. ^{239}Pu Oxide Study as Implemented

Group	*Number of Dogs*		*Average Initial Lung Deposition*[1]		*Average Initial Lung Concentration*[2]	
	♀	♂	***µCi***	***kBq***	***µCi/g***	***Bq/g***
0CON	10	10	0	0	0	0
0SAC	1	2				
1LST	11	10	3.5±1.3	0.12±0.05	0.029±0.011	0.93±0.39
1SAC	1	1				
2LOW	11	11	22±4	0.69±0.14	0.18±0.04	6.2±1.3
2SAC	1	1				
3MLO	10	11	79±14	2.7±0.5	0.66±0.13	23±4
3SAC	0	2				
4MED	12	12	300±62	11±2	2.4±0.4	95±17
4SAC	0	2				
5MHI	10	10	1100±170	41±6	9.3±1.4	349±46
5SAC	2	2				
6HST	5	3	5800±3300	213±120	50±22	2130±1160
6SAC	1	1				
Life Span	69	67				
Sacrifice	6	11				
Total Dogs	153					

[1]Mean ± Standard Deviation estimated by averaging external thorax counts at 14 and 30 days post-exposure; for some dogs (shown in table 11) the final body burden was used in lieu of the thorax count.
[2]Mean ± Standard Deviation estimated from initial lung depositin and estimated lung weight (0.11 x body weight)

The three highest deposition levels overlapped those done in earlier studies and lead to predictably high incidence of lung and/or bone tumors. The lowest level corresponded with the then recommended (16 nCi) maximum permissible human lung burden (2 nCi in the dog lung ÷ by 110 g dog lung = .018 nCi/g, and 16 nCi in the human lung ÷ by 1000 g human lung = .016 nCi/g). Seventeen dogs were exposed for periodic sacrifice to obtain information on deposition, retention, translocation, and excretion of inhaled plutonium, and on the pathogenesis of dose–related effects.

Post Mortem Exposure-Level Group Adjustment

The composition of the exposure-level groups changed slightly in 1992 when seven dogs were re-assigned because their estimated Initial Lung Deposition (ILD) was within the range of another group. The seven dogs which were re-assigned are shown in Table 4. Because this re-assignment occurred so late in the study (after all animals were dead), one must be careful in the use of information published prior to 1992. For example, plots of serial lymphocyte values published in our annual reports prior to 1992 are slightly different from subsequent plots which reflect the new group composition. All references to these dogs, including the appended individual animal summary, in this document are based on their final group assignment.

Table 4. ^{239}Pu Oxide Dogs Re-assigned from one Exposure-Level Group to Another in 1992

Dog	*Group prior to 1992*	*Group after 1992*
787	5MHI	4MED
802	2LOW	3MLO
841	1LST	2LOW
877	2LOW	3MLO
893	1LST	2LOW
905	3MLO	4MED
908	1LST	2LOW

Unique Experimental Design Decisions/Techniques

Similar life-span studies were conducted at other laboratories. In this section, we point out differences in our approach to animal husbandry and experimental protocol which may confound comparisons of our study with others.

Prophylactic Mammectomy

Female dogs assigned to the ^{239}Pu Oxide study were mammectomized at approximately 12 months. Beagle dogs, especially aged females, have been reported to have a 50% mammary tumor incidence at age 10 years. (Anderson 1970) Since this was a lifespan study, the mammary glands were removed before assignment to reduce the number of dogs lost for long term follow–up due to early mammary tumor death or to avoid confusion with metastic disease in the lung versus primary lung tumors that might develop from $^{239}PuO_2$ exposure.

Pre–exposure Training

Dogs were gradually trained for the inhalation exposures in a simulated (mock up) exposure chamber each working day for 2 weeks prior to actual exposure. This was done to familiarize the animal to exposure protocol, handling, and to accustom the dog to wearing its form fit mask with minimal stress. Respiration rate and volume was measured during pre–exposure training and was used to estimate the volume of air inhaled by each animal during exposure. (Craig, *et al* 1972) These values were compared to the respiration rate and volume parameters gathered

during actual inhalation exposure. This was done to ensure the accuracy of the measurements during exposure, to estimate duration of exposure, and also to establish the average respiration rate and volume of each animal in case of instrument failure during exposure.

Two types of respiratory measurement instruments were used in the ^{239}Pu Oxide study, the earlier dogs were exposed using a Wet Test Meter (WTM) while later dogs used a Tidal Volume Air Monitor (TVAM).

LOOK UP IN CLINICAL FOLDERS TO DETERMINE WHICH COHORTS OF DOGS WERE MEASURED WHICH WAY AND WHEN DID THE METHOD CHANGE.

During non–exposure training using the WTM, the animal was placed in a metabolism cage with no mask the first day, the second day the "nose only" inhalation mask was applied, and on the sixth through the tenth training day, the rubber insert for the mask was employed. Respiration rate and volume was measured on days six through ten. (Craig, *et al*, 1972)

During non–exposure training with the TVAM, the rubber insert was required from the first day, and respiration rate and volume was measured all ten days. The TVM was developed to measure instantaneous air volume inhaled and exhaled by the dogs during exposure. (Craig, *et al,* 1972) The system consisted of a dog inhalation mask, venturi air velocity meter, two differential pressure transducers with associated carrier pre amps, a venturi transducer to digital integrator interface signal conditioning system, analog strip chart recorder, voltage to frequency converter, digital clock, and a digital integrator and printer. (Decker, *et al*, 1972)

The velocity profile was recorded by the analog strip chart recorder, and the tidal volume was automatically printed out in digital form at the end of each breath. (Decker, *et al*, 1972)

This lengthy and labor intensive training procedure was not conducted with the control animals.

Exposure in Cohorts

Dogs were exposed in small "cohort" groups. Cohorts were established to break the multi–dose animal exposures into manageable combinations. These smaller combinations made more efficient use of limited exposure chambers, manpower, and metabolism units. The ^{239}Pu Oxide study cohorts were identified with letters A—F, H, J—L, and Z. The cohort identification is reflected in the animal assigned identification number in the latest version of the electronic database. The letter inserted between the PNL experiment number and the dog number is the cohort number. For example, the assigned identification number for animal 870 is 140–J–870; this animal was in cohort group J. This was done to identify the animals exposed at roughly the same time. Animals from the same litter were generally exposed and assigned to the same cohort group, but at different exposure levels. Control dogs were assigned to the same cohort group as their litter mates. Control dogs were not sham exposed. This cohort letter also identified the animals that needed to have excreta, blood chemistry, or whole body counting done at the same time post–exposure. Tables 5 and 6 show the number of dogs in each cohort at each exposure dose level for the ^{239}Pu Oxide study.

There is approximately a five week gap in dates between cohorts. This reflects the

amount of time required to prepare a group of dogs for exposure, the exposure itself, and the time spent in metabolism units until release to a regular run. Before exposure, each animal had a baseline whole body skeletal and thoracic radiographic survey, a whole body count to determine pre–exposure background levels, and excreta and blood samples taken. After exposure, each animal was confined to a metabolism cage where excreta was collected daily for 7 days, weekly for 3 weeks; 30-day excreta and blood samples were taken and the animals were whole body counted. If the excreta radioanalysis indicated that the excreta did not exceed 2X background radiation, the animal was released to the general indoor/outdoor run.

Table 5. Sequence by which Cohorts of Dogs were added to the ^{239}Pu Oxide Study Design for Lifespan Observation

Exposure Level	***Exposure-Level Groups***	***Number of Dogs in Cohort Groups***											
		A	*B*	*C*	*D*	*E*	*F*	*H*	*J*	*K*	*L*	*Z*	*No*
0	0CON			4		4		4	4		4		
	0SAC												
1	1LST			2	2		1	1	6	3	6		
	1SAC			1						1			
2	2LOW		3	3		3		4	5	3	1		
	2SAC	3			1								1
3	3MLO	1	2	3	1	3	3	3		5			
	3SAC						1						1
4	4MED		1		1	6	5	6	1	2	2		
	4SAC	1					1						
5	5MHI	1	1	2	3			2	6	2	3		
	5SAC											4	
6	6HST		1	1					2		4		
	6SAC	1											1
	Total Life Span Dogs	2	8	15	7	16	9	20	24	15	20		
	Total Sacrifice Dogs	5		1	1		2			1		4	3
	Grand Total	7	8	16	8	16	11	20	24	16	20	4	3

Table 6. ^{239}Pu Oxide Study Cohort Group Exposure Dates

Year	*Month*	*Cohort*	*Dogs*	*Remarks*
1970	October	A	7	October 9—November 5
	November	Z	4	November 10—Sacrifice only
	December	B	8	December 21
1971	January	C	16	January 19—20
	February	D	8	February 10
	March	E	16	March 4
	April	F	11	April 25—26
	May			
	June	H	20	June 8
	July	J	24	July 6—7
	August			
	September			
	October	K	16	October 7—8
	November	L	20	November 9—10
	December			
1972	January			
	February			
	March			
	April			
	May			
	June	None	3	June 8—July 19—Sacrifice only

Genetic Contribution

The Table 7 summarizes the presence of sires, dams, littermates, and siblings in each exposure-level group of the life span beagles. Many practical factors prevented implementation of an experimental design which featured groups of 10 female and 10 male exposure dogs from 20 litters from 20 dams.

Table 7. Genetic Contribution to ^{239}Pu Oxide Study Exposure-Level Groups

Exposure-Level Group	*Total Number of Dogs in Group*	*Litters*	*Litters Represented More than Once*	*Littermates*	*Parents*	*Parents Represented More than Once*	*Siblings*
0CON	20	18	2	4	15	5	10
1LST	21	17	4	8	13	5	13
2LOW	22	19	3	6	16	5	11
3MLO	21	14	6	13	13	6	14
4MED	24	18	3	9	15	5	14
5MHI	20	16	3	7	14	4	10
6HST	8	7	1	2	5	2	5

Exposure Age

The protocol called for exposure of "young adult" beagles to the plutonium aerosol as close to 14 months (420 days) age as practical. Exposure ages are summarized in Table 8.

Table 8. Average Age and Weight at Exposure of Dogs in ^{239}Pu Oxide Study

Level	***Exposure-Level Group***	***Number***		***Age***	***Weight***
		♀	♂	***Days***	***kg***
0	0CON[1]	10	10	579.6±79.1	11.2±1.6
	0SAC[1]	1	2	593.0±17.3	12.7±2.7
1	1LST	11	10	536.5±53.5	11.0±1.6
	1SAC	1	1	617.5±43.1	11.5±1.8
2	2LOW	11	11	538.4±43.4	10.3±2.1
	2SAC	1	1	481.5±6.4	10.6±1.4
3	3MLO	10	11	558.1±47.7	10.7±1.9
	3SAC	0	2	499.0±18.4	11.8±0.4
4	4MED	12	12	526.6±49.3	10.8±1.9
	4SAC	0	2	1230.0±1016.8	10.2±0.0
5	5MHI	10	10	551.3±47.6	10.7±1.6
	5SAC	2	2	587.0±17.0	10.0±2.3
6	6HST	5	3	533.1±52.2	9.6±2.5
	6SAC	1	1	1414.5±1249.5	11.3±0.8
Total Dogs		153			

[1]Controls were not sham exposed, exposure date taken from exposure date of litter mates.

Post-exposure Housing in Metabolism Cage

In the experimental design, some animals were designated to have excreta continuously collected, and others had periodic samples collected for 1 week each year for the lifetime of the animal. Lifespan observation dogs with excreta collection for greater than 10% of the post–exposure period are summarized in Table 9.

Table 9. ^{239}Pu Oxide Study Dogs Housed in Metabolism Cages for Excreta Collection and Analysis Greater than 10% of Life Span

Exposure-Level Group	*Number of Life–Span Observation Dogs*	*Number of Dogs with Protracted Excreta Collection*	*Dog*	*Percent Time Post–exposure*
0CON	20	0		
1LST	21	1	850F	87
2LOW	22	3	813F	13
			776M	79
			806F	92
3MLO	21	2	802M	79
			884M	80
4MED	24	4	777M	99
			787M	99
			809F	99
			814F	99
5MHI	20	3	889F	11
			840F	15
			880F	98
6HST	8	6	829M	15
			910M	96
			747F	98
			890F	98
			896F	98
			906F	98

Maintenance of "Ideal" Weight

At the start of the study, a veterinarian and the animal care supervisor examined and weighed each dog and assigned an "ideal" weight based on conformation. Throughout life, dogs were weighed at 3 to 4 week intervals. The weight was compared with the previous 2 weights and the ideal weight. If an animal was considered too heavy, a reduction in diet was made to maintain optimum weight. If an animal was below the ideal weight, it was given supplemental food and in some its diet was changed from dry kibbles to prepared wet ration. A change to wet ration also reflected age or palatability (lack of acceptance of dry diet). Animals whose weight deviated significantly from ideal were brought to the attention of the veterinarian. This policy continued throughout the study, although the concept that a dog could be maintained at an ideal weight selected at age 14 months was abandoned. The ideal weight of most of the dogs on this study were eventually changed to reflect the conformation of the dog at a more mature age. Table 10 shows the distribution of final estimates of ideal weight by sex in this study.

Table 10. Distribution of ^{239}Pu Oxide Study Ideal Weights by Sex

Ideal Weight (kg)	♀	♂	*Total*
7.0	3		3
7.5	1		1
8.0	10		10
8.5	4		4
9.0	10		10
9.5	6		6
10.0	10	4	14
10.5	6		6
11.0	10	2	12
11.5	7	7	14
12.0	5	8	13
12.5	1	10	11
13.0	1	16	17
13.5	1	8	9
14.0		5	5
14.5		6	6
15.0		8	8
15.5		1	1
16.0			
16.5		3	3
Total	75	78	153

Change this to a figure

Histogram of ideal weights
with scattergram of exposure weights superimposed

Dosimetry

External thorax counting provided an estimate of the quantity of plutonium deposited in the lung of each dog. Excreta collection and radioanalysis provided an estimate of the quantity of plutonium cleared from the body by biological and physical processes. Tissue radioanalysis provided an estimate of the quantity of ^{239}Pu in the body at death.

External Thorax Monitoring

Plutonium is a difficult isotope to measure *in vivo* since it is a very low intensity alpha–emitter and due to the variations in isotopic composition. (Swinth, *et al*, 1967) A whole body counter had to be developed that could accurately count the progeny isotopes of ^{239}Pu—^{240}Pu, ^{238}Pu, and ^{241}Am—in the experimental animal. "These other isotopes are significant because they have different and generally greater photon emission rates than the ^{239}Pu isotope. For a typical source containing 94% ^{239}Pu it was calculated that less than 50% of the X–rays were from ^{239}Pu." (Swinth, *et al*, 1967)

The external thorax counter developed "consisted of fifty–two detectors each composed of a photomultiplier optically coupled to a 1mm thick NaI(Tl) crystal. The cleaved crystals [were] 1¾in. in diameter, mounted on a quartz base and with a 1 mil aluminum entrance window. These detectors [were] arranged into four arrays of thirteen each. Each array [was] housed in a 6 in. X 12 in. X 7 in. deep stainless shell, light–tight box with a 10 mil mylar window....High voltage [was] supplied from a single power supply through a voltage divider to the individual boxes. The anodes of the phototubes [were] connected in parallel and the signal [was] fed to a pulse height analyzer". (Swinth, *et al*, 1967)

Monitoring Prior to 1970

In the early exposures at 100–F, prior to the ^{239}Pu Oxide study, dogs were counted unanesthetized and placed on their backs in the center of the four plutonium counter units. Counting was done in a cylindrical cave made of pre–world war II steel (a spare battleship amunition elevator which was uncontaminated by radioactive fallout) and lined with lead. A technician remained with each animal in the counter to position the dog. Controlling the horizontal position of an unanesthetized dog was a major obstacle and accounted for a major portion of the

daily variations in whole body counts. (Swinth, *et al* 1968)

After initial exposure, the dogs were bathed to remove external contamination, wrapped in a thin polyethylene sheet, and whole body counted. Initial counting began within one hour after exposure. Counting continued on a daily schedule for two weeks, then once a week for two months, and finally biweekly. (Swinth, *et al* 1968)

Dogs were anesthetized with thiopental sodium to facilitate handling (Swinth, *et al*, 1967) when whole body counting was done. To achieve the best counting geometry, the animals were counted in a supine position with the thorax surrounded by the four counting boxes and with the anterior edge of the counting boxes aligned with the first rib. Thus the volume counted extended from the first to the last rib and included the tissues expected to account for 90% of the plutonium body burden. (Swinth, *et al*, 1967)

Monitoring the ^{239}Pu Oxide Study Dogs

A new thorax monitor was developed which used two of the detector boxes mounted on a lucite box into which a unanesthetized dog could be placed comfortably. The dogs were counted in an upright posture, with plexiglass supporting the sternum. The sodium iodide detector arrays were mounted on the sides of the thorax monitor, with 13 crystal plus photo multiplier tubes on each array.

The new throax monitor was much more suited for routine measurements because the procedure was less stressful on the dogs and more dogs could be counted in a normal working day. Two dogs were brought to the counting room, each was counted for 10 or 20 minutes, they were returned to their kennel or metabolism cage, and another pair was started. The counting room operator also monitored the background and a standard set of ^{239}Pu sources housed in a tissue equivalent phantom.

Phantom Dog

A dog phantom was developed to "investigate experimental variables in these experiments. The phantom was patterned after a typical (10kg) beagle dog and contain[ed] the skeleton of a control beagle from our colony. The lung area [was] filled with a lung equivalent material and the phantom [was] divided in to thirty–three 2–cm thick sections containing a grid of holes in a 2–cm centers. The holes in the phantom normally contain[ed] tissue–equivalent plugs; the plugs [could] be replaced by tissue–equivalent capsules containing a radionuclide....Tissue– and fat–equivalent pads representing the addition of 2 kg of

tissue [were] used to adjust the phantom's weight. The pads enclose[d] the trunk of the phantom; distribution of the pad material [was] similar to the condition in a larger dog and represent[ed] an addition of about 1 cm of tissue....The phantom was not used for final calibration of the counter although results from it agreed with the direct method used." (Swinth, *et al*, 1967) The phantom was used as the reference standard. It was counted each day, and if results deviated from normal, re–calibration of the detectors was indicated.

Lung Lavage

In 1972-73, an investigation of the efficacy of lung lavage was conducted using six dogs (753, 829, 890, 896, 906, and 910) in exposure-level 6. The dogs were anethesized in conjunction with a routine radiographic survey at 8 to 32 months post exposure, and the right side of the lung was irrigated with 200 ml or 400 ml of saline solution. About two-thirds of the lavage solution was recovered for radiographic analysis. This procedure was performed at 13 and 20 months for dog 890.

Excreta Radioanalysis

Another approach to dosimetry for this study was through excreta collection and analysis. The plutonium recovered in tissue at death plus that which was recovered in excreta would equal the quantity of plutonium administered. Excreta was collected daily for 7 days and weekly for 3 weeks in the first month post–exposure while the dogs were held in metabolism cages. Subsequent excreta was pooled into weekly samples. Most dogs were returned to the metabolism cages on a semi–annual or annual basis. A few dogs remained in metabolism cages for life–span excreta analysis as indicated in Table 9. The excreta radioanalysis results are stored in the RIB database.

Tissue Radioanalysis

"At necropsy the tissues from these dogs were digested in nitric acid and ashed in a muffle furnace. The residues were dissolved in 0.3 N HCl and the plutonium extracted with thenoyltrifluoroacetone. Aliquots were then counted in an alpha proportional counter. From these analyses the body burden of the dogs was determined with an accuracy of ± 5% as estimated from counting statistics and experimental recoveries." (Swinth, *et al*, 1967)

THIS IS AN OLD METHOD ADD WORDS FROM POWERS PROTOCOLS HERE DESCRIBE ALPHA ENERGY ANALYSIS TECHNIQUES

Estimation of Initial Lung Deposition

External Thorax Monitoring

The Initial Lung Depsoition (ILD) was estimated by thorax monitoring of the 17 KeV x-rays associated with plutonium in the lung. Counts obtained at 14 and 28 days were averaged. As the dogs died, final body burden (FBB) estimated by radioanalysis of the tissues was compared with the whole body monitoring estimates of ILD. In a few animals, listed in Table 11, the FBB was greater than the ILD. In most of these cases the ILD was very close to the detection limit of the whole body monitoring apparatus. It was decided to substitute the FBB for the thorax estimate of ILD for these dogs for the purposes of dosimetric evaluation.

Table 11. ^{239}Pu Oxide Dogs for which Initial Lung Burden was based on Final Body Burden in lieu of Whole Body Count

Dog	*Exposure-Level Group*	*Survival (days after exposure)*	*Whole Body Count (nCi)*	*Final Body Burden (nCi)*	*Percent change*
697M	3MLO	3478	140	141.35	1.0
702F	5SAC	141	1682	1682.35	0.02
709M	5SAC	141	1726	1726.43	0.03
734M	5SAC	142	914	914.37	0.04
739F	5SAC	142	1511	1511.4	0.03
756M	1LST	4475	0	1.57	100
762M	1SAC	2197	0	1.71	100
766M	0CON	4907	0	0.1	100
792M	0CON	1786	0	0.12	100
801M	0CON	3913	0	0.02	100

813F	2LOW	4669	32	35.66	10.3
815M	3SAC	757	68	73.79	7.8
816M	4SAC	16	398	398.46	0.1
817M	6HST	628	3164	3793.58	16.6
823M	3MLO	4777	65	71.70	9.3
825F	1LST	4180	1.4	1.99	30.0
832F	1LST	5425	2	2.04	2.0
836M	4MED	3569	256	344.47	25.7
847M	1LST	4950	0	0.61	100
853M	1LST	4815	7.7	8.1	4.9
858M	1LST	5566	0	0.42	100
859M	2LOW	4674	35	47.88	26.9
860M	4MED	4034	254	335.4	24.3
865F	1LST	5551	0	0.62	100
872F	0CON	4140	0	0.05	100
879M	1LST	4677	0	0.93	100
885F	0CON	4118	0	0.02	100
886F	1LST	4529	0	0.85	100
899F	1LST	3427	3.6	6.62	45.6
904F	1LST	4422	0.7	1.32	47.0
907F	1LST	5295	0	0.97	100

Materials Balance Approach

An alterternative approach to estimation of initial lung deposition was to estimate the quantity of plutonium excreted in the urine and feces and combine that with the final body burden based on tissue radionalysis. This approach was considered as an

independent confirmation of the ILD estimated by thorax monitoring. This analysis is not complete; the count records are stored in the RIB system and must be evaluated.

Animal Care and Recordkeeping

Philosophy

The animal care philosophy was to care for the dogs in a manner similar to that provided human subjects with similar diseases. Dogs on this study were given the best care available and were not allowed to suffer. When a dog needed treatment, the guiding philosophy was to aggressively treat diseases and syndromes unless it was felt that the appropriate treatment might confound the results of the study (i.e., using cytotoxic or mutagenic drugs or x–ray therapy to treat cancer), but to be more conservative in treatment of radiation induced tumors. For example, we treated hypothyroidism with hormone replacement therapy, but monitored the development of lung tumors without therapy.

Cancer, whether radiation–induced or spontaneous, was surgically treated when possible.

Routine Procedures

Many animal care technicians were involved in care of the beagle colony. Care was provided 24 hours per day. Animal care specialists provided information as well as care. Their protocol called for completion of a "Daily Observation" form if they noted anything out of the ordinary while they were cleaning the pens, feeding the dogs, etc. Each animal was given a physical exam by a technician in conjunction with the weekly (later bi–weekly, then monthly) weighing. Observations about the health of the dog were noted on the weighing form. The reviewing animal care supervisor and veterinarian selected animals from the daily observations and weighing reports for further examination, diagnosis, and/or treatment.

Each dog was scheduled for a semi–annual (or annual) physical examination by the veterinarian. The dog was anesthetized after the examination and thoracic and skeletal radiographs obtained. The physical examination protocol used the "abnormal only" reporting philosophy. Items on the examination which were "normal" were not recorded in the computerized records, nor were they noted on the data collection form. It was, however, standard operating procedure that all items on the form were included in the examination.

Routine immunizations were integral to the development and maintenance of a colony free of infectious diseases. Puppies were immunized as follows: distemper–measles (Nordens Enduracel D–M) at 6 weeks of age;

distemper–hepatitis–leptospirosis (Nordens Enduracel D–H–L) at 10 and again at 16 weeks of age, and rabies (Nordens Endurall–R) at 18 weeks of age. Annual revaccinations were performed for distemper–hepatitis–leptospirosis, and every two years for rabies. Parvovirus vaccines (Norden:DA2PLtCPV) were added when they became available.

Antiparasitic treatments were not routinely performed since the colony was free of endoparasites prior to the start of this study. Although the colony was screened on an irregular basis to assure that it remained free of parasites.

Caging

General Colony Caging

Study animals were matched for caging with respect to exposure level and kept in same–sex pairs in indoor–outdoor runs. The indoor–outdoor dog kennel facility consisted of 18 X 2 foot contiguous runs constructed of concrete and sealed with a chemically inert epoxy. The indoor and outdoor sections of each run were separated by a metal guillotine door that could be manually opened and closed by the technician. The guillotine door contained a flap–door that permitted the animals to freely go in and outdoors. The floor of each run was heated for comfort. The kennels were washed daily. The indoor space was maintained at 65±5 F year around. # of air changes/hr?

Sick animals were removed from the run and put into special care cages in the animal hospital to facilitate medical monitoring and treatment when prescribed by the attending veterinarian.

The regular kennels could be modified for a mother with pups by removing one wall and providing the litter with a double run. A divider was designed and constructed low enough for the mother to walk over, but too high for the pups to climb over, to provide the mother some privacy. A similar divider was constructed to fit in the internal cage door to keep the pups in the kennel when the door was open. The divider fit so the swinging door could be closed with it in place.

Background radiation measurements were made in the animal colony in 1979. ~~(Memo, Murphy to Park, 1979) [WE CANNOT USE THIS AS A REFERENCE WITHOUT ELABORATION]~~ Environmental thermoluminescent dosimeters (TLD) were suspended 2.5 to 3 feet above dog runs. Based on 35 day exposures the dose rates were calculated to have a maximum of 17.0, a minimum of 10.0, and an average of 12.0 μR/hr (express as Coulombs here?); these levels were

considered essentially equivalent to the background levels in the remaining offices and laboratory facilities of the Biology Department. Later studies in 1981 showed gamma exposures to the kennel of 21.8± 1.8 mR and 26.3± 2.6 mR(express as Coulombs here ?), for outdoor and indoor runs, respectively, for one quarter; these levels were also considered within the range of background levels. Radon levels were not measured.

Metabolism Cages

During the immediate post–exposure period it was necessary to maintain them in metabolism units. Dogs remained in the metabolism units until the ^{239}Pu level in their excreta fell below 2X background radiation.

Standard metabolism cages, ?? by ?? meters, with fiberglass (or stainless steel?) collection pans. [GET ED WIERMAN TO SUPPLY DESCRIPTION?]

Feed Specifications and Administration Procedures

Water was available ad libitum with a LixIt® mechanism, and a individualized quantity of kibbled commercial diet was fed once daily. Animals were trained as pups to separate during feeding to prevent fighting and allow analysis of food consumption. One pup was pushed out the guillotine door so one animal was inside and the other animal outside, then both animals were fed. As the dog matured, and after dominance between cage mates was established, the animals voluntarily separated to their feeding area.

The animals were given only a certain amount of time to eat, then any remaining food was removed from the run, unless the dog was sick, recovering, or under special dietary orders. Uneaten food was measured and any amount uneaten recorded in the dog's clinical record as a possible indication of a sick animal.

Feed was provided to adult dogs once daily in stainless steel feeding bowls. Commercially obtained kibbled food (Wayne Certified Lab Dog Diet) was usually fed dry, but occasionally softened with water for dogs temporarily not eating. Canned dog foods were also occasionally offered to sick dogs.

In the initial years of the ^{239}Pu Oxide study, ox tails were fed once a week to provide the dogs with additional nutrition, provide social enrichment, and to control dental tartar build–up. When ox tails were fed, the animals were separated as they were for a normal feeding. Each dog was given an ox tail. After several hours, the kennel separation door was opened. Ox tails were removed from the diet in about 1978 (1981–1982 ???). Some of the animals would hide their ox tail,

wait for the separation door to open, run into the other portion of the kennel, steal it's cage–mate's ox tail, and hide it with it's own. Many animals were injured in the ensuing fights and required medical treatment. Another problem associated with feeding ox tails was broken teeth. The additional cost of treating dogs injured in fights or needing dental prophylaxis led to a decision to termiate the feeding of ox tails.

After ox tails were removed from the diet, chew toys were tried for environmental enrichment, but broken teeth remained a problem and this practice was also abandoned. At this point a more rigorous dental care routine began. During the routine physical examinations, teeth were checked and severe plaque build–up was removed from conscious animals where possible. Dogs with severe plaque build–up, dental caries, broken or abscessed teeth, gingivitis, or bad temperaments were anesthetized for more thorough examination and treatment. Tarter/plaque was removed with an ultrasonic scaler and the teeth routinely polished. Antibiotics were given prophylactically to preclude bacteremia.

Clinical Care

The dogs were provided with care by a team of veterinarians and animal care technicians and specialists. The annual monitoring program for the lifespan animals consisted of physical examinations, including radiographic examinations of the skeleton and thorax, complete blood counts, serum biochemical profiles, fecal analysis for parasites, and routine immunizations. Clinical evaluation of disease was conducted by staff veterinarians, including board–certified specialists in internal medicine/oncology, pathology, radiology, laboratory animal medicine, practitioners, and a clinical pathologist. Specific tests and procedures for disease diagnosis or treatment were ordered at the discretion of the attending veterinarian. Dogs received treatment as needed according to accepted veterinary procedures, although treatments that would potentially alter the neoplasms produced by $^{239}PuO_2$ were not used.

The dogs were observed daily by animal caretakers as the runs/cages were cleaned. At 3 to 4 week intervals the dogs were weighed, and a more detailed inspection was made. If the animal care technicians observed any problems, the animal care supervisor and veterinarian was notified. Protocols were followed for management of disease conditions. Veterinary technicians followed protocols for the following conditions: bite wounds, otitis externa, interdigital cysts, conjunctivitis, dermatitis, dental care, and anal sacculitis. Veterinary care provided by attending veterinarians included history taking, physical examinations, differential diagnosis, diagnostic

procedures, and therapy procedures. Analgesics and glucocorticoids were occasionally used, under the direct supervision of the veterinarian with the agreement of the principal investigator, for herniated intervertebral discs or similar appropriate painful disorders. For after hours care, weekends, and holidays, the home phone numbers of four veterinarians were available to the animal caretakers. GE Dagle, JF Park, SE Rowe, and RE Weller were the attending veterinarians. Rosters were provided for weekends, and the "on–duty" veterinarian carried a beeper device to let him know when he was needed. Due to the experience and quality of the animal care staff, many problems could be resolved via telephone consultation with the attending veterinarian.

Serial Hematology and Clinical Chemistry

Hematologic changes in the $^{239}PuO_2$ exposed beagles were carefully monitored for general health and exposure related effects. "Several hematologic and serum chemistry parameters were evaluated before and after radiographic evidence of lung tumors to identify changes in the tumor–bearing dogs. Blood samples were obtained at 3 to 4 month intervals throughout the life of the [tumor bearing] dogs for complete blood counts and serum chemistry tests, including urea nitrogen, glucose, alkaline phosphatase, the transaminase, protein and protein electrophoretic fractions." (Ragan, *et al*, 1981)

The blood samples were obtained from the juglear vein of fasted dogs and were collected in heparinized and non heparinized 5 or 10 ml tubes. The animals were scheduled for blood collection based on their cohort group. A computer generated calendar was used for scheduling; the goal was to obtain blood samples at 3 month intervals up to 30 months post exposure and at 4 month intervals thereafter. In practice, blood collection was sometimes delayed for a week or more due to conflict with colony management tasks of a more life-threatening nature. Therefore, an artificial "adjusted months post exposure" was assigned to each blood sample so that trends for exposure-level groups could be compared. This field exists in the computer files, but is not necessarily noted on the original data collection forms. Table 12 illustrates the assignment of adjusted months post exposure to cohorts within exposure-level 1 for typical 3 and 4 month intervals.

Table 12. Comparsion of Adjusted Months Post Exposure with Actual for Cohorts of Exposure-Level 1 of ^{239}Pu Oxide Study

Adjusted Months Post Exposure	*Cohort C*	*Cohort D*	*Cohort F*	*Cohort H*	*Cohort J*	*Cohort K*	*Cohort L*
16	5/16/72	6/9/72	8/24/72	10/6/72	11/2/72	2/6/73	3/7/73
	15.87	15.93	15.97	15.97	15.94	16.03	15.89
19	8/16/72	9/8/72	11/22/72	1/5/73	2/7/73	5/8/73	6/5/73
	18.89	18.92	18.92	18.96	19.12	19.02	18.83
22	11/16/72	12/8/72	2/22/73	4/6/73	5/9/73	8/1/73	9/5/73
	21.91	21.91	21.96	21.96	22.11	21.82	21.85
47	12/19/74	1/7/75	3/26/75	5/9/75	6/6/75	9/5/75	10/8/75
	46.98	46.88	46.98	47.01	47.01	46.95	46.92
51	4/30/75	5/2/75	7/18/75	9/10/75	10/7/75	1/8/76	2/12/76
	51.34	50.66	50.73	51.09	51.06	51.06	51.09
55	8/29/75	9/3/75	11/19/75	1/9/76	2/9/76	5/6/76	6/22/76
	55.29	54.74	54.80	55.06	55.16	54.97	55.39

Serum for biochemical determinations was collected from the clotted blood samples and assayed immediately, or frozen at -70°C, within 2 hours after the samples were taken, for subsequent analysis. The serum samples were analyzed using a variety of instruments during the course of the study. Methods and reagents used were those recommended by the manufacturer. Samples were routinely assayed for serum urea nitrogen (SUN), creatinine, total protein, albumin, glucose, protein electrophoretic fractions, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase. Serum samples with ≥ 200 IU/L ALP were heated to 56°C and re-analyzed to determine residual ALP activity. This step was necessary because the bone-derived fraction of ALP is destroyed by heating, leaving only hepatic-derived ALP, which is heat stable. Several dogs had bone lesions or bone cancer, which also could increase ALP. In order to further identify the source of elevated ALP, gamma glutamyl transferase activity, which is associated only with liver function, was determined in 10 dogs with markedly elevated ALP activity. These same dogs also exhibited significantly elevated ALT activity, so sulfobromophthalein sodium retention tests were

performed to assess whether this increased enzyme activity reflected functional impairment (Hoffmann et al. 1989). **{WELLER - Is this TRUE for Expt 140 as well as 158???}**

Serial Radiographic Survey

Skeletal and thoracic radiographs were taken at intervals ranging from 1–3 years prior to a tentative tumor diagnosis, and at 6 month or shorter intervals after a suspected lung or bone tumor was diagnosed radiographically. The radiographs were interpreted and a diagnosis was entered in the SNOVET record storage system.

A retrospective study of serial thoracic radiographs of dogs which developed lung tumors was conducted by RM Perry. The object was to determin the first date the tumor was detected and the last date the lung appeared normal. These data are summarized in a computer file.

Necropsy Protocol

Euthanasia was performed on life span observation dogs that were moribund or failed to respond to routine nursing or veterinary care. This included dogs with intractable pain, such as those with herniated intervertebral discs. Euthanasia was performed by inducing deep anesthesia with intravenously injected pentobarbital and exsanguination via a catheter placed in the carotid artery following intravenously injected heparin. Additional dogs scheduled to be sacrificed were killed in the same manner.

The necropsy protocol following euthanasia or spontaneous death included a complete gross pathological evaluation, with emphasis on those tissues or organs which had been clinically dysfunctional or which had demonstrated lesions when examined radiographically.

Gross necropsy was done to accomplish two primary goals. The first goal was to find, accurately describe, and save appropriate specimens for characterizing disease processes. The second goal was to collect specimens for radioanalysis for dosimetric purposes. Generally paired organs were randomly separated for

radioanalysis or saved in 10% neutral–buffered formalin for histopathologic examination; if a unilateral lesion was observed in a paired organ, the abnormal organ was generally saved in formalin and the normal organ was submitted for radioanalysis. Weights were also obtained for those organs specified by protocol.

A complete necropsy examination was made of each dog that died, was euthanized, or was sacrificed. The complete necropsy included examining all viscera in the thorax, abdominal, neck, and pelvic regions, and all peripheral lymph nodes. Following removal of the brain, the head was split in a mid–sagittal plan to examine the nasal cavity and sinuses. Muscle was dissected from all bones and the bones individually examined. Photographs were made of unusual lesions. Occasional tissue specimens, were submitted to ME Frazier, FC Leung, or GL Stiegler for, respectively, oncogene, growth factor receptor, or gene mutation evaluation. The necropsies were generally performed by senior scientists including a pathologist, assisted by either one or two technicians. Gross observations were dictated, transcribed, and reviewed.

Each lobe of lung was palpated and gross abnormalities noted. Following palpation and weighing, the lungs with attached trachea were instilled with 10% neutral buffered formalin at a hydrostatic pressure equal to the length of the trachea. Occasional specimens of lung tumors were fixed by immersion in 2% glutaraldehyde in preparation for electron microscopy. Following fixation and hardening in formalin, the individual lobes of lungs were removed and multiple transverse slices approximately 0.5 cm thick were made. The slices were numbered beginning from the distal (apex) and extending towards the proximal areas (hilus). Each slice was then individually palpated and visually examined for nodules or any other abnormalities. Three sections from each lung lobe (from distal, middle, and proximal areas) were selected for histologic examination to represent the most severe lesions found in those lobes.

Radiographs of individual bones (humeri, radii, femora, tubuli, ulnae, scapula, sacrum, pelvis, and, in males, os penis) were prepared. The feet, ribs, vertebrae (cervical, thoracic, lumbar, and coccygeal) and head bones were left attached and radiographed as groups. Following radiography, samples of bones were saved in formalin and the remainder were frozen for radioanalysis. Bone marrow smears were routinely saved from a rib and air dried for later staining (Wright Stain). Occasional specimens were saved in acetone or alcohol for submission to Dr. Web Jee at the University of Utah. Radiographs were examined by a veterinarian, or consultant veterinarian radiologist, to see if occult bony lesions were present that were not evident upon gross examination.

The whole brain was examined at necropsy and then, following hardening in 10% neutral buffered formalin, transversely sliced into approximately 0.5 cm sections for further evaluation. Following examination of each specimen, there were 4 routine sections saved for histopathology: 1) Cerebral cortex at level of basal ganglia; 2) cerebral cortex at level of hypothalamus and hippocampus; 3) cerebellum; 4) medulla oblongata at level of decussation of the pyramids. The remainder of the brain was submitted for radioanalysis.

Histopathologic examinations were made of hematoxylin and eosin stained specimens of formalin fixed and paraffin embedded specimens. This routinely included 3 sections of each lung lobe, (from apical, mid–lobe, and hilar areas), additional lung tumors or nodules, trachea, tracheal bifurcation, a cross section of nasal turbinate, tracheobronchial lymph nodes (right, left, and middle), mediastinal lymph nodes, mandibular lymph node, hepatic and splenic lymph nodes, peripheral lymph nodes (Prescapular, cervical, axillary, popliteal, and parasternal), tonsil, liver, pancreas, salivary gland, spleen, kidney, urinary bladder, adrenal, thyroid (including thyroid), pituitary gland, testis and prostate (or ovary and uterus), heart (left and right ventricles and ventricular septum), diaphragm, brain (4 sections as described above), eye, esophagus, stomach, small intestine (jejunum), colon, skin, grossly observed lesions of an uncertain nature, and all suspected tumors. Decalcified bone sections examined included cross sections through proximal and distal metaphyses and the mid–shaft of the femur, tibia, and humerus, and cross sections of a rib and at least one vertebra. Tissues were assigned codes based on the SNOP (Systematized Nomenclature of Pathology, College of American Pathology Committee on Nomenclature and Classification of Diseases, 1965, Chicago) topography codes. Special stains were applied to selected specimens; these included PAS–Alcian blue, Congo red, Mallory trichrome, and Prussian blue. Selected specimens of lung, liver, and lymph nodes were coated with Ilford K-4 emulsion for autoradiography.

Narrative descriptions of histopathologic lesions were dictated, transcribed, reviewed, and inserted into bound Laboratory Books. Diagnoses were based on World Health Organization International Histologic Classifications of Tumors of Domestic Animals and generally accepted diagnostic terms in contemporary literature of Veterinary Pathology. Lesions of an uncertain nature were frequently shown to other pathologists for additional opinions. Suitable diagnostic codes were selected from the SNOP codes and entered into our Datatrieve database.

Data Management

Several data management approaches were used to store the information about the beagle colony over the course of the experiments. The earliest data storage method was the clinical file record. A unique clinical file record was kept each animal. The file cabinets were labeled with the experiment number and clinical records were filed in dog number order. This storage method worked well for recording and retrieving materials associated for unique dogs, but cross comparisons among groups of animals was difficult. Update was slow and the possibility existed that records could be lost or inadvertently destroyed, but retrieval of a medical history on a single dog was efficient. However, this manual retrieval and summary was inefficient for data from groups of dogs.

SNOOPY System

In the late 1960's and early 1970's the manual records were converted to a punched–card batch–updated, magnetic–taped–based computer system. This system was a collection of in–house FORTRAN programs known as SNOOPY; it was implemented on a CDC CYBER mainframe computer and converted to a UNIVAC in 1972. The idea was to put all the information about the dogs in one location. Updating the records was still slow, but retrievals about single animals was efficient. It was still difficult to compare information about groups of animals unless specific computer programs were written for each retrieval request. The integrity and legibility of the records was greatly improved over the clinical file system. The storage of some types of information, i.e., pathologist's observations, were incompatible for storage in this system, and had to be stored separately. (Watson, *et al*, 1980) Information stored in SNOOPY was recorded on data collection forms in the laboratory, reviewed by the PI, transcribed to punched cards in the Mathematics Department, transferred to tape on a weekly basis. Weekly transaction reports and routine summary reports were provided for PI review. Corrections were by the "drop and add" method, with the earlier (erroneous) record flagged and saved on the tape.

DATATRIEVE System

In 1977, a PDP 11/70 minicomputer was obtained for biometrics applications and in 1978 DATATRIEVE, a commercially developed interactive query language, was purchased. All the biomedical information about the beagle colony was transferred to this system. The data was stored on random access disk files, data was clustered by record type rather than by grouping data by each dog. The information was accessible from any computer terminal in the laboratory. The types of information available about each animal through DATATRIEVE were:

Vital Statistics, Weights, General Observations, Hematology, Chemistry, Respiration rates and volumes, Radioanalysis, Pathology and Whole Body Counts. (Watson, *et al*, 1980)

Data entry was moved to the laboratory, and in some cases, data entry forms were not used. The PI and technicians were given responsibility for reporting and editing of records; corrections were made interactively via hard wired computer terminals.

The DATATRIEVE storage/retrieval system remained stable throughout the remainder of the study, although computer hardware was upgraded several times. The final configuration was a VAX cluster operated through a local area network, with access via terminal emulator software on desk top personal computers.

RIB System

The Radioanalysis Inventory for Biology (RIB) system was developed in 19??. Initially, it focused on the inventory aspects of the excreta and tissue specimens. There was often a backlog of several months or years between specimen collection and radioanalysis. The RIB system was based on three relational tables: definitions, counts, and remarks. There was one definition per specimen. The specimens were identified by unique accession numbers, whicwhich were affixed to the containers and recorded on data collection forms. The remarks table was used to track progress of the sample through the analysis process, from collection to ashing to interim storage to chemical preperation, to the count room, and finally to long term storage. Remarks also described lost or discarded samples. The count table contains one or more record per accession number (many samples were re-analyzed). The counts and remarks are identified by sequence numbers; the count with the highest sequence number is the "final" count for that specimen.

The RIB system evolved into an analylitical tool. A suite of computer programs was developed which produced reports summarizing the excreta and/or tissues of each dog. Linear extrapolation was used to fill in missing days in excreta.

SNOP System

A system was developed for interactive storage and retrieval of pathologic observations in 1979 using DATATRIEVE and SNOP (Systematized Nomenclature of Pathology) codes. Observations of each tissue were entered in computer files by an interactive program which performed elementary error checking (i.e. wrong sex, illogical date, etc.). Numerical SNOP topography and morphology codes were also entered. The written translations were immediately

displayed to ensure proper code selection and entry into the system. The SNOP coding scheme was also enhanced to allow for severity grading of lesions on a scale of 1 to 5 where 1 is mild, and 5 is severe. A proof reading report could be generated at any time. This system also was used for retrievals and queries about combinations of tissues or lesions. The system was eventually expanded to include the radioanalysis data for correlation of radiation dose with the biological effect. (Watson, *et al* 1980)

The SNOP system also included a suite of computer programs which tabulated the observations for individual dogs or exposure-level groups or entire studies.

SNOVET Coding

Describe SNOVET here -
NEED TO POINT OUT THAT IN 1981–82? OR SO, PNL DEVELOPED OWN SNOVET CODING SYSTEM AND CLINICAL/SURGICAL/BIOPSY RECORDS WERE RETRO AND PROSPECTIVELY ENCODED TO FACILITATE RETRIEVAL AND DATABASE MANAGEMENT (WELLER, ET.AL.)
include reference to Weller et al. 1986.

Transfer of Information to the National Radiobiology Archives

In 1994, the records stored on the VAX computer were transferred to the PC environment and converted to PARADOX tables. This was part of the process of donating the information to the National Radiobiology Archives operated by PNL. These records are now available for secondary analysis through:

Dr. Charles R. Watson
Director
National Radiobiology Archives
Pacific Northwest Laboratory
Richalnd, WA 99352

Telephone: (509) 376 3483
Facsimile: (509) 376 4533
eMail: CR_Watson@PNL.GOV

Table 13 summarizes the frequency of usage for each data collection form and tabulates the number of records transferred to the Archives:

Table 13. Number of Computer Records Related to Dogs in the ^{239}Pu Oxide Study, by Type of Observation

Observation Form Code	*Observation Type*	*Number of Animals with at Least 1 Form*	*Total Number of Electronic Forms Transferred to the Archives*
1	Birth Record	137	137
2	Experimental Assignment	160	492
3	Death Record	44	44
4	Routine Procedures, i.e., TB Testing, Vaccinations	160	3960
8	Physical Examination	102	105
9	Radiograph Interpretation	160	829
10	Radioanalysis Sample Definition	153	22,663
11	Radioanalysis Results in Counts per Minute	147	17,103
12	Radioanalysis Remarks	147	18,019
13	Radioanalysis Percent (post processed)	129	57,028
14	Radioanalysis Nanocuries (post processed)	153	15,645

Table 13. Number of Computer Records Related to Dogs in the ^{239}Pu Oxide Study, by Type of Observation

Observation Form Code	*Observation Type*	*Number of Animals with at Least 1 Form*	*Total Number of Electronic Forms Transferred to the Archives*
15	Radioanalysis Summary, Activity in Major Organs	162	162
18	Aerosol Summary	141	141
21	Weight Remarks (old form)	3	23
22	Aerosol Characterization	2	2
25	Daily Observations and Food Consumption	146	7881
28	Seizures	6	20
29	Clinical Treatment	146	733
30	Weight Data	162	32,015
31	Hematology	158	5775
32	Hematology Remarks	144	1453
33	Chemistry Data	159	5724
34	Chemistry Remarks	133	861
35	Veterinary Observations	153	3810
36	Veterinary Observations Remarks	94	377
37	Pathology (SNOP)	160	13,864

Table 13. Number of Computer Records Related to Dogs in the ^{239}Pu Oxide Study, by Type of Observation

Observation Form Code	*Observation Type*	*Number of Animals with at Least 1 Form*	*Total Number of Electronic Forms Transferred to the Archives*
38	SNOP Remarks	33	57
39	Medical Summary	115	254
	All Forms		209,177

Summary of Individual Dogs

The report which follows summarizes each animal in PNL Biology Department Experiment 140, "Life–Span Effects of Inhaled $^{239}PuO_2$ in Beagle Dogs". The report is organized by exposure-level group. Dogs are sorted within a group in ascending order of initial lung deposition as estimated by whole body count or final body burden (see Table 11). There are 6 lines of information for each dog; up to six dogs appear on a page. The report columns and rows are defined in Table 14.

Table 14. Definition of ^{239}Pu Oxide Study Individual Animal Summary Report Fields

Column Head	***Row***	***Title***	***Description***
Dog	1		Dog number and sex, printed on row 1. The dog number is a 4 digit number. Dogs numbers in this experiment ran between 435 and 920. Sex is either F (female) or M (male).
	2		Blank.
	3	Wgt:	Body weight at time of exposure.
	4	Cohort	Exposure cohort as defined in Table 5 and 6.
	5	Litter	Litter number.
	6	Line	Genetic Line Code. Codes are: D = Davis, P = Pullman, H = Hanford.
Dates	1		
	2	Birth:	Birth date (mm/dd/yy).
	3	Expo:	Date (mm/dd/yy) of exposure. (Controls were not sham exposed; date of cohort exposure is shown.)
	4	Last norm:	Date (mm/dd/yy) of the last routine thorax radiograph which was "normal;" blank if dog did not have lung tumor.
	5	First pos:	Date (mm/dd/yy) of the first radiographic evidence of the tumor; blank if dog did not have lung tumor.
	6	Death:	Date (mm/dd/yy) of death.
Ages (day)	1		Blank.
	2	Birth:	Blank.
	3	Expo:	Age (days) when exposed to $^{239}PuO_2$ aerosol.
	4	Last norm:	Age (days) of last normal thorax radiograph; blank if dog did not have lung tumor.

Table 14. Definition of ^{239}Pu Oxide Study Individual Animal Summary Report Fields

Column Head	***Row***	***Title***	***Description***
	5	First pos:	Age (days) of first radiographic evidence of lung tumor; blank if dog did not have lung tumor.
	6	Death:	Age (days) at death.

Table 14. Definition of ^{239}Pu Oxide Study Individual Animal Summary Report Fields

Column Head	***Row***	***Title***	***Description***
Burdens (nCi)	1		Blank.
	2	WB count:	Quantity of ^{239}Pu (nCi) estimated by external thorax counting of 17Kev X-rays 14 days after exposure.
	3	Material	Blank.
	4	Balance:	Quantity of ^{239}Pu (nCi) estimated by radioanalysis of tissues at necropsy plus the sum of daily excreta measurements (with linear extrapolation between samples to estimate daily excreta when samples were not collected or analyzed).
	5	Final BB:	Quantity of ^{239}Pu (nCi) recovered in tissues at necropsy.
	6		Blank.
Plutonium Radioanalysis Summary (nCi)	1		Blank.
	2	Lung:	Quantity of ^{239}Pu (nCi) in Lung at necropsy.
	3	Bone:	Quantity of ^{239}Pu (nCi) in Bone at necropsy.
	4	Liver:	Quantity of ^{239}Pu (nCi) in Liver at necropsy.
	5	Thor LN:	Quantity of ^{239}Pu (nCi) in Thoracic Lymph Nodes at necropsy.
	6	Abdo LN:	Quantity of ^{239}Pu (nCi) in Abdominal Lymph Nodes at necropsy.
Plutonium Radioanalysis Summary (nCi)	1		Blank.
	2	Muscle:	Quantity of ^{239}Pu (nCi) in Muscle at necropsy.
	3	Kidney:	Quantity of ^{239}Pu (nCi) in Kidney at necropsy.
	4	Spleen:	Quantity of ^{239}Pu (nCi) in Spleen at necropsy.
	5	Skin:	Quantity of ^{239}Pu (nCi) in Skin at necropsy.

Table 14. Definition of ^{239}Pu Oxide Study Individual Animal Summary Report Fields

Column Head	***Row***	***Title***	***Description***
	6	Gonad:	Quantity of ^{239}Pu (nCi) in Gonad at necropsy.

Table 14. Definition of ^{239}Pu Oxide Study Individual Animal Summary Report Fields

Column Head	***Row***	***Title***	***Description***
Plutonium Radioanalysis Summary (nCi)	1		Blank.
	2	Feces:	Estimated quantity of ^{239}Pu (nCi) excreted in Feces or Urine between 4 days post exposure and death. (Samples were collected daily for the first few weeks, less frequently thereafter. Linear extrapolation between samples was used to compute daily excretion values when samples were not collected or analyzed. This field is the sum of those daily estimates).
	3	Urine:	
	4		Blank.
	5	Other:	Quantity of ^{239}Pu (nCi) in miscellaneous tissues not classified in other fields at necropsy.
	6		Blank.
There are 2 columns which display numbrs of fatal or incidental primary tumors. Tumors are considered primary if the SNODOG code has a 3 in the 5th position as defined in the SNOMED glossary. Our convention for recording the number of tumors is to enter a 1 if there is clearly one primary site within the tissue, 2 if there are 2 sites, 3 if 3 sites, and 9 if there are many sites. The values reported here summarize many tissues. If the sum exceeded 9, it was truncated to 9 in keeping with our convention.			
Tumors F	1		
	2	Lung:	Number (1-9) of primary FATAL lung tumors; blank if none.
	3	Bone:	Number (1-9) of primary FATAL bone tumors; blank if none.
	4	Liver:	Number (1-9) of primary FATAL liver tumors; blank if none.
	5	Other:	Number (1-9) of primary FATAL tumors in other tissues; blank if none.
	6		Blank.

Table 14. Definition of ^{239}Pu Oxide Study Individual Animal Summary Report Fields

Column Head	***Row***	***Title***	***Description***
Tumors I	1		Blank.
	2	Lung:	Number (1-9) of primary INCIDENTAL (non–fatal) lung tumors; blank if none.
	3	Bone:	Number (1-9) of primary INCIDENTAL (non–fatal) bone tumors; blank if none.
	4	Liver:	Number (1-9) of primary INCIDENTAL (non–fatal) liver tumors; blank if none.
	5	Other:	Number (1-9) of primary INCIDENTAL (non–fatal) tumors in other tissues; blank if none.
	6		Blank.
Cause of Death	1		Blank.
	2		Cause of death presented as translation of 2 SNODOG codes—diagnosis; topography. The cause of death may print on 1 to 3 lines.
	3		
	4		
	5	SNOP Cause of Death:	SNOP codes (5 characters) for diagnosis and topography, separated by a semi-colon.
	6	SNODOG Cause of Death:	SNODOG codes (7 characters) for diagnosis and topography, separated by a semi-colon.

Appendices