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November 1972

FISSION PRODUCT INHALATION PROGRAM ANNUAL REPORT

1971-1972

by the
Staff of the
Fission Product Inhalation Program

LOVELACE FOUNDATION

for Medical Education and Research

5200 Gibson Boulevard, SE
Albuquerque, New Mexico 87108

Prepared for the Division of Biomedical and Environmental Research
U. S. Atomic Energy Commission under Contract No. AT(29-2)-1013

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ANNUAL REPORT OF THE
FISSION PRODUCT INHALATION PROGRAM
LOVELACE FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH
ALBUQUERQUE, NEW MEXICO 87108

October 1, 1971 through September 30, 1972

by the
Staff of the
Fission Product Inhalation Program

Edited by
R. O. McClellan, Program Director
and
F. C. Rupprecht, Technical Editor

November 1972

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Prepared for the Division of Biomedical and Environmental Research
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INTRODUCTION

The prime objective of the inhalation toxicology research program conducted by the Lovelace Foundation for the U. S. Atomic Energy Commission is to develop knowledge that will contribute to an improved understanding of the biological consequences of inhaling radionuclides, such as might be released in the event of nuclear accidents. The ultimate goal is to establish the relationship between the radiation dose pattern resulting from various levels and types of exposures and the resulting biological response.

The major stimulus for development and continuation of the research program stems from concern for nuclear industry operations that may result in potential accident situations with release of radioactive aerosols. In the past, major attention has been directed toward investigations with fission product radionuclides that are of interest relative to nuclear power reactor and associated fuel reprocessing operations. More recently, increased attention has been directed toward research with plutonium and transplutonium radionuclides recognizing their importance in the future, especially with development and broad use of breeder reactors.

Studies are being conducted to evaluate the importance of the many factors influencing the toxicity of inhaled radionuclides. These include: (1) the radiological characteristics of radionuclides such as half-life, emissions (alpha, beta and gamma), and energies; differences that result in a spectrum of radiation dose patterns and dictate concern for (a) relative effectiveness of alpha, beta and gamma radiation, (b) radiation dose rate, (c) total radiation dose, and (d) the degree of non-homogeneity of irradiation of the tissue, especially lung; (2) the size distribution of the aerosol particles; (3) physical and chemical characteristics of particles, such as density and solubility; and (4) the physiological characteristics of the animals which influence retention of inhaled materials. All of these factors are important in determining the ultimate toxicity of an inhaled radioactive aerosol.

The ultimate aim of the program is to develop information that will be relevant to man. In the absence of information on man himself, it is necessary to conduct animal experiments with a view toward making extrapolations to man. To this end, several experimental animal species are being routinely used including mice, Chinese hamsters, Syrian hamsters, rats, and Beagle dogs, attempting to capitalize on unique characteristics of each species as appropriate. Extensive use is made of the Beagle dog for several reasons including; (1) its moderately long lifespan, which may be of importance in extrapolating findings to man; (2) its size, which allows each animal to be studied intensively as a clinical subject much as a human patient; and (3) the desire to make use of the wealth of information being obtained in other long-term radiation toxicity programs using the Beagle dog, and to develop information that will complement the findings of these programs.

Papers within the report are grouped in six sections which correspond to programmatic areas that have been delineated for budget purposes. Section I reports research directed toward developing an improved understanding of the nature of radioactive aerosols, including their production and characterization for use in animal exposures. During the past year, major attention has been given to developing improved procedures for generating experimental aerosols and characterizing them as to their in vitro solubility. One paper in the section reports research characterizing the operating conditions of two generators commonly used in our program. Three papers describe approaches

to production of submicron or monodisperse aerosols. The last paper presents early results from studies to assess the solubility of aerosols in vitro for use in predicting their in vivo behavior.

Section II includes 15 papers describing research directed toward obtaining an improved understanding of the radiation dose pattern resulting from inhalation of radioactive aerosols of diverse character. The first four papers report research with monodisperse aerosols, research that will ultimately provide improved insight into the basic mechanisms involved in the body's handling of inhaled particles. Of special interest is information that will aid in assessing the manner in which particle size influences the toxicity of inhaled alpha-emitting radionuclides. The fifth paper in this section reports research that emphasizes the importance of species differences in the metabolism of ^{252}Cf . Other papers report research that emphasizes the importance of particle size, chemical form, and heat-treatment of aerosols on their behavior when inhaled.

Section III, which represents the major portion of the total program, reports a series of studies directed toward establishing the relationship between exposure to various levels and types of aerosols and the resulting biological effects. This effort is centered in a series of sequential inter-related studies in which Beagle dogs, exposed to a few carefully selected radioactive aerosols producing significantly different radiation dose patterns, are followed for their total lifespan so as to provide an assessment of the various parameters of radiation dose that influence dose-response relationships for inhaled radionuclides. Such longevity studies are currently under way with dogs exposed at 12-14 months of age (young adults) to $^{90}\text{SrCl}_2$, $^{144}\text{CeCl}_3$, $^{91}\text{YCl}_3$, $^{137}\text{CsCl}$, and ^{90}Y , ^{91}Y , ^{144}Ce and ^{90}Sr in fused clay aerosols. During the past year, two additional studies were initiated with ^{144}Ce fused clay with Beagle dogs exposed at 3 months or 8 to 10.5 years of age with a view toward examining potential age-related differences in their biological behavior and effects of inhaled radionuclides. The title of each of the 8 reports in Beagle dogs has a Roman numeral suffix which represents the number of progress reports for that particular study. Other reports in this section detail a complementary study being performed in rodent species as well as supporting research on survival of control dogs in this colony and age differences in pulmonary function and biochemical parameters.

Section IV relates research under way to develop an improved understanding of the pathogenesis of radiation-induced disease, especially as produced by internally deposited radionuclides. Studies reported in this section extend from the biochemical level, i. e., connective tissue and lipids in the lung to the system level, i. e., studies of pulmonary function.

Section V reports continuing research directed toward developing effective therapeutic procedures for treating individuals who may be accidentally exposed to radioactive aerosols. The first paper is a report of the first use of bronchopulmonary lavage for removing inhaled radioactivity, ^{239}Pu , from an individual who was accidentally exposed. It represents an important milestone in our therapy research that was originally initiated as a serendipitous outgrowth of efforts to use lavage to recover material from the lung for biochemical studies. The second paper reports a study designed to provide an "acid-test" of the usefulness of bronchopulmonary lavage, in that dogs have been lavaged following exposures that resulted in lung burdens of radioactivity that in the absence of therapy would be fatal in a matter of months.

The sixth section reports a new effort in the laboratory, Respiratory Tract Deposition Models, which is being conducted for and supported by the National Institute of Environmental Health Sciences (NIEHS) under an inter-agency agreement between the NIEHS and the U. S. Atomic Energy Commission. The two reports, on an improved technique for casting the lung in situ and a heat transfer analogy for mathematical modeling of deposition in the respiratory tract, are included recognizing that this new effort both gains from and contributes to the other research activities of the laboratory irrespective of the source of support.

At this juncture, it is worthwhile to point out certain educational features of the program which, although not spelled out in detail in the report, do complement and enhance the research activities of the program. During the past year, 19 individuals participated in the program as Faculty Participants, Graduate Fellows or Student Participants under the auspices of the Associated Western Universities, Inc. and the Division of Nuclear Education and Training. An additional 13 students participated in the research program as short-term employees. Students in the basic biological and physical sciences as well as individuals enrolled in dental, medical or veterinary medical schools from a total of 16 different institutions participated. In the majority of cases, an attempt was made to have the student work closely with a doctoral-level staff member so that the student could gain a first-hand impression of science and assess his or her interest in pursuing a career in science. As in the past, the presence of students served as a stimulus to the permanent staff.

Because of the multidisciplinary and long-term nature of the effort, the research reported here is the product of the entire staff. Nonetheless, it is desirable to provide the outside reader with an indication of the principal investigators associated with particular aspects of the research program in the event the reader should desire to contact an investigator for additional information. This has been done by listing the principal investigators associated with each effort. It should be emphasized that these listings are rarely comprehensive in acknowledging all the individuals who have made important contributions to the research. In the unnamed category are the many highly skilled technicians, animal caretakers, maintenance, shop, administration and secretarial personnel whose efforts are essential to the continuation of a productive and meaningful research program. A roster of the staff is shown in Appendix E.

An aerial view (above) of the Inhalation Toxicology Research Facility located 10 miles south of Albuquerque, New Mexico on Kirtland Air Force Base East. The facility, operated by the Lovelace Foundation for Medical Education and Research for the Division of Biomedical and Environmental Research, U. S. Atomic Energy Commission, was constructed in several increments starting in June 1962. The facility now consists of (1) a central laboratory and office building which includes a specially designed and equipped inhalation exposure complex for research with beta-gamma emitting radionuclides, (2) a veterinary hospital with facilities for detailed clinical observations on experimental dogs, (3) a canine metabolism building, (4) a small animal barrier-type building, and (5) ten kennel buildings, nine capable of housing 100 dogs each and one housing 120 dogs. Exposure facilities (6) for research with alpha-emitting radionuclides are nearing completion. Other facilities located at the Lovelace Foundation headquarters site in southeast Albuquerque are also used in the research effort.

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SECTION I

NATURE OF RADIOACTIVE AEROSOLS

OPERATING CHARACTERISTICS OF TWO COMPRESSED AIR NEBULIZERS USED IN INHALATION EXPERIMENTS

ABSTRACT

Nebulizers operated with compressed air are conveniently used for generating radioactive aerosols of both relatively soluble and relatively insoluble forms from aqueous solutions and suspensions for inhalation experiments. The Lovelace nebulizer and the Retec nebulizer are two which have been in use in this program. Selected operating characteristics of representatives of each of these nebulizers have been studied to determine (a) output of usable aerosol, (b) volumetric rate of air required, (c) droplet size distribution of usable aerosol, and (d) rate of evaporation of water which is not associated with the aerosol. The Lovelace nebulizer has a basic aerosol output of about 50 μ l/min of aerosol in an airflow of about 1.3 l/min at an operating pressure of 20 psig. The Retec nebulizer has a basic aerosol output of about 200 μ l/min in about 6 l/min at an operating pressure of 20 psig. The droplet distributions for these two nebulizers were similar and in the respirable size range; the largest droplets had a volume median diameter of 6.9 μ m and geometric standard deviation of 1.7 from the Lovelace nebulizer operating at 20 psig.

PRINCIPAL INVESTIGATOR

O.G. Raabe

INTRODUCTION

A nebulizer is an atomizer which produces a high concentration of droplets in the respirable size range. The use of compressed air operated nebulizers for producing aerosols of radioactive materials for inhalation experiments has a number of advantages over other methods of producing aerosols (such as dry dust generation). Aqueous suspensions of small particles or pure solutions can be aerosolized with a nebulizer in precisely prepared concentrations and, after dilution with dry air and evaporation of the water in the droplets, the resulting aerosols of dry particles can be controlled to provide the desired concentrations of particles if the operating characteristics of the nebulizers are known. Both aerosols of relatively soluble forms and relatively insoluble forms can be prepared. With subsequent heat treatment of an aerosol, the chemical form of the particle can be conveniently altered as desired. Typically, the particles formed are spherical or near spherical depending upon the crystalline properties of the aerosol material.

In this program, aerosols of relatively soluble forms have been generated using nebulizers from aqueous solutions of electrolytes or complexes of radioactive cations of such elements as Y, Cr, Co, Sr, Zr, Nb, Mo, Ru, Cs, Ba, La, Ce, Eu, Tm, Pu, Am, and Cm. Relatively insoluble forms of many of these have been conveniently prepared by ion exchange into clay in aqueous suspensions which are nebulized and heated to 1150°C to form spherical aluminosilicate particles with the radionuclide firmly entrapped. Oxides and other forms have been similarly prepared by nebulization of solution and suspensions and subsequent heating of the aerosols to effect the desired chemical changes.

Basic to the use of nebulizers in producing suitable radioactive aerosols for inhalation experiments is the determination of the operating characteristics of the nebulizer to be used. Important

factors in describing the operation of nebulizers include (a) the output rate of usable aerosol, (b) the volumetric rate of air, (c) the evaporation losses of liquid which are independent of usable aerosol, (d) the droplet size distribution, (e) the volume of liquid required for proper operation, and (f) the maximum unattended operating time. Cognizance of these factors in relation to a particular application determines the choice of a nebulizer.

The output of usable aerosols is usually described as volume of liquid at initial formation associated with droplets that leave the generator and may be expressed in $\mu\text{liter}/\text{minute}$. The number of droplets from most nebulizers is between 10^6 and 10^7 per cc of air, but since the volumetric rate of air often is a number of liters per minute, the droplet output rate is usually in excess of $10^9/\text{min}$.

Evaporation losses increase the concentration of the solute in solution or particles suspended in the aerosolized liquid. This concentration change causes a concomitant increase in the sizes of the particles that are formed when the droplets dry. This evaporation occurs both from the surface of the liquid and from the droplets which evaporate slightly and then hit the wall of the nebulizer to be returned to the reservoir. Evaporation is most important in nebulizers with small reservoirs but large volumetric airflows.

Nebulizers produce droplets of many sizes and resultant aerosol particles after evaporation are concomitantly polydisperse. The droplet distributions described for nebulizers are the initial distributions at the instant of formation; droplet evaporation begins immediately even at saturation humidity since the vapor pressure on a curved surface is elevated. The rate of evaporation depends upon many factors - including surface tension, energy availability, degree of saturation of the air, the solute concentration, the hygroscopicity of the solute, the presence of immiscible liquids or evaporation inhibitors, and the size of the droplets (smaller droplets have higher vapor pressures and dry faster).

The distribution of droplets produced by nebulizers has been described satisfactorily in many ways. Most useful is the assumption that the logarithms of droplet size are normally distributed. This log-normal distribution of sizes allows for simple mathematical transformations and, usually describes droplet volume distributions satisfactorily. The characteristic parameters of a log-normal distribution are the median (or geometric mean) and the geometric standard deviation (σ_g). The median of a distribution of droplet diameters is called the droplet count median diameter (CMD); the median of the mass or volume distribution of the droplets is called either the mass median diameter (MMD) or the volume median diameter (VMD).

Two nebulizers used in inhalation experiments, the Lovelace nebulizer¹ and the Retec X-70 nebulizer (Retec Development Laboratory, Portland, Oregon) (Fig. 1) have been studied and the results are summarized in this report.

EXPERIMENTAL METHODS

General:

Aerosols of solutions containing 0.99 mg of uranine (disodium fluorescein, a water soluble fluorescent dye)/ml, were generated with the Lovelace (reservoir volume ~ 5 ml) and Retec (reservoir volume ~ 10 ml) nebulizers at various operating pressures of compressed dry air up to 50 psig, pressure above normal atmospheric 12.6 psia. The fluorescent properties of uranine were used to quantitatively measure and trace the aerosols produced with these nebulizers. Measurements of fluorescence were made using a Turner Fluorometer. The volumetric flow rate of the nebulizers at various pressures was determined by passing the nebulizer output through a dry gas meter for a measured period of time. Two nebulizer units of each type were studied to indicate variability between nebulizers.

Figure 1. Retec and Lovelace nebulizers used in this study.

Output Rate of Aerosol:

When droplets are formed in the nebulizers, the larger droplets are normally not expelled into the airflow. However, some larger droplets which leave the general body of the nebulizer deplete the reservoir of liquid but do not provide usable aerosol. Two methods were therefore used to determine output rate of aerosol generation. The usable aerosol was measured by collecting the aerosol onto a clean high-efficiency filter after dilution with sufficient dry air to effect drying of the droplets to prevent wetting of the filter. The uranine collected on the filter and in the adjacent aerosol line was dissolved in water and assayed fluorometrically to determine the usable aerosol output rate. Losses very near and in the nebulizer body were not measured.

A second method involving solution concentration changes was used to determine the total nebulizer fluid consumption and the relative effect of evaporation not associated with droplet production. This procedure has been described by Mercer et al.¹ The total aerosol output from the nebulizer is determined by volume changes:

$$V = V_0 - (A + W)Ft \quad (1)$$

with V the volume (μl) of liquid in the nebulizer reservoir after an operating time t (min), V_0 the starting volume of liquid in the nebulizer, A the total aerosol output per liter of airflow ($\mu\text{l/l}$), W the evaporated water per liter of airflow ($\mu\text{l/l}$) and F the airflow rate through the nebulizer (l/min). This yields a volume change rate given by

$$\frac{dV}{dt} = - (A + W)F \quad (2)$$

The mass of solute (uranine), M (grams), in a given volume of aqueous solution is given by

$$M = CV$$

with C the concentration (gm/ μl). The reduction in mass is given by

$$\frac{dM}{dt} = C \frac{dV}{dt} + V \frac{dC}{dt} = -ACF \quad (3)$$

Hence:

$$\frac{dC}{dt} = \frac{CWF}{V} = \frac{CWF}{V_0 - (A + W)Ft} \quad (4)$$

and

$$\int_{C_0}^C \frac{dC}{C} = \int_0^t \frac{WF dt}{V_0 - (A + W)Ft} \quad (5)$$

which yields:

$$\ln (C/C_0) = \frac{W}{A + W} \cdot \ln (V_0/V) \quad (6)$$

from which

$$A = \frac{(V_0 - V) (1 - R)}{Ft} \quad (7)$$

and

$$W = \frac{(V_0 - V) R}{Ft} \quad (8)$$

with

$$R = \ln (C/C_0) / \ln (V_0/V) \quad (9)$$

In order to determine V , the residual volume of ungenerated solution after each run, a concentration technique was used in which a sample volume (normally 1 ml) was taken for fluorometrically determining the concentration C . After adding an equal volume of water (pH 10), another measurement of concentration (C') was made and:

$$V = \frac{C}{C - C'} \quad (10)$$

Although most tests were conducted with the nebulizers in normal room surroundings ($\sim 22^\circ\text{C}$), some were conducted with the nebulizer reservoir submerged in an ice water bath to determine the effect of temperature upon output and evaporation. In all tests, the temperature of the residual liquid in the nebulizer at the end of each run was measured with a thermometer.

Droplet Distributions:

The droplet size distributions of the usable portion of the aerosol generated by the nebulizers was determined by bringing the aerosols to complete dryness using gentle heating (37°C) and diluting into a 20-liter holding chamber with at least 32 l/min of dry air. Samples of the dry uranine particles were collected with a Mercer seven-stage impactor operated at 1200 cc/min for periods from 1 to 4 minutes.² Determination of the relative amount of uranine on each stage and the back-up filter was made fluorometrically. These values were used to fit a log-normal distribution function of the aerodynamic size distribution of the uranine particles by weighted least squares. The resulting mass median aerodynamic diameters and geometric standard deviations were used to calculate the droplet distribution with the known concentration of uranine in the nebulizer solutions ($0.99 \mu\text{g/ml}$) and the density (1.48 gm/cc) of dry uranine particles. These calculations provided the volume median geometric diameter of the droplets and the corrected geometric standard deviation.

RESULTS

The results of these studies for the Lovelace nebulizer are summarized in Table 1. Since the Lovelace nebulizers used in this study were optimized for 20 psig operation, the maximum output rates of usable aerosol were obtained with this pressure and lower outputs were obtained at higher pressures except at 50 psig where the effect of reducing droplet distribution sizes was offset by the high flow rate of air and other factors. A useful means of reducing evaporation without affecting output of aerosol is keeping the reservoir at near 0°C by submerging the nebulizer in an ice water bath.

Table 1

Some Operating Characteristics of Lovelace Nebulizers

Operating pressure (psig)	20	20	20	30	40	50
Unit used	1	1	2	2	2	2
Run time (min)	7.5-11	12	15	15	15	10
Number of runs	3	1	3	2	2	2
Volumetric flow rate (l/min)	1.34	1.34	1.34	1.81	2.28	2.64
Usable aerosol output rate ($\mu\text{l}/\text{min}$)	52 ± 7	55	[45]*	[40]*	[35]*	[50]*
and per air liter ($\mu\text{l}/\text{l}$)	39 ± 5	41	[34]*	[22]*	[15]*	[19]*
Total aerosol output rate ($\mu\text{l}/\text{min}$)	68 ± 2	70	58 ± 3	51 ± 4	45 ± 5	64 ± 0
and per air liter ($\mu\text{l}/\text{l}$)	51 ± 2	52	43 ± 2	28 ± 2	20 ± 2	24 ± 0
Evaporated water rate ($\mu\text{l}/\text{min}$)	15 ± 1	~ 2	18 ± 3	20 ± 1	21 ± 5	30 ± 3
and per air liter ($\mu\text{l}/\text{l}$)	11 ± 1	~ 1	13 ± 2	11 ± 1	9 ± 2	11 ± 1
Reservoir temperature ($^{\circ}\text{C}$) end of run	~ 10	2	~ 10	~ 10	~ 10	~ 10
Surroundings temperature ($^{\circ}\text{C}$)	~ 22	0	~ 22	~ 22	~ 22	~ 22
Nebulizer liquid consumption rate ($\mu\text{l}/\text{min}$)	83 ± 2	72	75 ± 4	71 ± 3	67 ± 0	94 ± 3
and per air liter ($\mu\text{l}/\text{l}$)	62 ± 1	54	56 ± 3	39 ± 2	29 ± 0	36 ± 1
Droplet distribution of usable aerosol VMD (μm)			6.9	4.7	3.1	2.6
σ_g			1.7	1.9	2.2	2.3

* Calculated value

The results of these studies for the Retec nebulizer are summarized in Table 2. Although the outputs of usable aerosol per liter of air are about the same at 20 psig as for the Lovelace nebulizer, the Retec had a remarkably steady output of usable aerosol per liter of air ($36.7 \pm 3.3 \mu\text{l}/\text{l}$) for all tests. The flow rates of total aerosol are about 4.5 times higher at all pressures than for the Lovelace nebulizer at corresponding pressures. The nebulizer consumption was found to be quite high in all cases compared to actual usable aerosol outputs. The effect of submerging the nebulizer in an ice bath was to reduce to almost nil the evaporation rate but reduce only slightly the output of aerosol.

Both the Lovelace nebulizer and the Retec nebulizer had decreasing volume median diameters for droplets produced at higher pressures and correspondingly had increasing geometric standard deviations for the droplet distributions at higher pressures.

Table 2

Some Operating Characteristics of Retec Nebulizers

Operating pressure (psig)	20	20	20	30	30	40	40	50	50
Unit used	1	2	2	1	2	1	2	1	2
Run time (min)	10	11	10	10	10	10	10	10	8
Number of runs	1	1	1	1	1	1	1	1	1
Volumetric flow rate (l/min)	5.9	6.4	6.4	7.9	9.1	9.8	10.1	11.8	12.0
Usable aerosol output rate (μ l/min)	208	257	224	284	378	324	413	376	427
and per air liter (μ l/l)	36	40	35	36	41	33	41	32	36
Total aerosol output rate (μ l/min)		364	349		532		598		667
and per air liter (μ l/l)		57	55		58		61		57
Evaporated water rate (μ l/min)		70	6		98		65		108
and per air liter (μ l/l)		12	1		11		7		9
Reservoir temperature ($^{\circ}$ C) end of run		12	2		11		9.8		9
Surrounds temperature ($^{\circ}$ C)	~22	~22	0	~22	~22	~22	~22	~22	~22
Nebulizer liquid consumption rate (μ l/min)	450	444	355	550	630	575	663	600	775
and per air liter (μ l/l)	77	69	55	69	69		67	51	66
Droplet distribution of usable aerosol VMD (μ m)	5.7 $\pm$ 0.2			3.6 $\pm$ 0.3		3.7 $\pm$ 0.02		3.22 $\pm$ 0.3	
σ_g	1.8			2.0		2.1		2.2	

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APPLICATION OF A HIGH TEMPERATURE GRAPHITE FURNACE FOR THE PRODUCTION OF SUBMICRON AEROSOL PARTICLES BY VAPORIZATION OF CERIC OXIDE

ABSTRACT

A high temperature graphite furnace has been evaluated as a thermal source to produce submicron aerosol particles from oxides of metals. The effect of temperature, diluting air, and collection chamber size upon formation of chains and agglomerates formed by vaporization of CeO_2 was studied. The formation of agglomerates and chains could be reduced and in some cases kept from forming by introducing diluting air and storing the aerosol immediately downstream from the furnace in a large chamber. The particles produced ranged in size from $0.001\ \mu\text{m}$ to $0.3\ \mu\text{m}$ physical diameter.

PRINCIPAL INVESTIGATOR

M.E. Light

INTRODUCTION

In the experimental production of aerosols which might be encountered from a high temperature source, one of the main difficulties encountered is obtaining a device to produce the necessary high temperatures. High temperature or condensation aerosols are of interest since they have a potential for formation in certain types of nuclear industry accidents.

Horn and Castleman, Jr.¹ investigated aerosols produced by vaporization of fast reactor core materials. PuO_2 , UO_2 , and Na aerosols were produced by placing the sample material on a tantalum carbide filament and passing 500 amperes through the filament to produce the temperature required to vaporize the sample material in atmospheres of argon, nitrogen and air. They report a maximum temperature attained of 3000°C with their apparatus.

Chatfield² used an explosive vaporization technique to obtain aerosols consisting of plutonium-sodium particles. Chatfield's apparatus consisted of a chamber of about 4 liters capacity, fitted with two heavy current electrodes which were bridged by a thin wire about 7 mm long. The wire vaporized explosively with 1 kJ at a potential difference of 1.0 kV and the aerosol formed by the reaction of metal vapor with air followed by condensation was collected for examination.

Problems such as equipment interface, temperature control requirements for specialized samples, and limitations on the quantity of aerosol which could be produced, make the experimental devices just described less than optimum for high temperature radioactive aerosol production for inhalation studies. The purpose of this study was to investigate the use of the Perkin-Elmer HGA-70 graphite furnace as a high temperature source to produce aerosols from oxides of metals and to compare the results with other high temperature or condensation aerosol studies.

FURNACE DESCRIPTION

Although the Perkin-Elmer HGA-70 graphite furnace was designed for use as a sampling device in Perkin-Elmer atomic absorption units, its size, shape, operating characteristics and suitability for using larger samples of a variety of material make it an ideal high temperature source for melting and vaporizing material up to a maximum temperature of 2600°C . The heated portion of the furnace consists of a graphite tube 50 mm long and 10 mm outside diameter and about 0.9 mm wall thickness. The tube is contained in a water-cooled aluminum jacket (Fig. 1).

Figure 1. Simplified schematic of graphite furnace.

Heating is produced by an electric current passed through the graphite tube. Low, intermediate, or high current (500 amps) can be applied to the tube. When high current is applied, the tube becomes incandescent within two to three seconds.

The power supply for the furnace is a portable 220 V unit which is programmable for temperatures from 300°C to 2600°C for various time periods. Three power levels may be selected and automatically sequentially applied to provide appropriate low, intermediate, and high temperatures. Automatically-resetting individual timers are provided for adjusting the periods of low, intermediate, and high temperatures. Built-in inert gas controls provide proper purge gas flow to the furnace to prevent combustion of the graphite tube, and built-in safety circuits turn off the control unit if the purge gas flow to the furnace drops below one liter per minute. The safety circuits will also turn off the control unit if the outer surface of the furnace exceeds 60°C due to improper cooling water flow. Figure 2 shows power supply and furnace assembly. The unit operates with 220-240 V, 30 amp power, 3 liters of cooling water per minute, and argon or nitrogen purge gas at 45 psig.

EXPERIMENTAL SETUP

Adaptation of Furnace for Aerosol Production:

The arrangement of experimental apparatus used for this study is shown in Figure 2. The furnace and aerosol collecting chamber were placed in a fume hood with the power supply in front of the hood. The direction of aerosol flow is from the furnace to the collection chamber and out the back end of the chamber through a filtered vacuum line to the house vacuum system. Partially hidden in Figure 2 is a branched section of copper tubing which exits at the rear of the chamber. The vertical branch contains a filter through which the major portion of exhaust aerosol flows and the horizontal branch, which is shown, has provision for insertion of a point-to-plane electrostatic precipitator for particle sampling or for insertion of a quick disconnect fitting to allow particle concentration measurements.

Special venting attachments were made for the ends of the furnace to allow the required piping to be attached to the furnace. The venting attachments were machined from aluminum with a 1/2-inch wide flange at the base. The flange contained an O-ring for an airtight seal between the furnace and venting attachment and the O.D. of the attachment contained two O-rings for an airtight seal between the venting attachment and a 1-1/4 inch O.D. copper transition fitting. The copper transition fitting fits over the outside of the venting attachment and reduces to accept 5/8-inch O.D. copper tubing. Figure 2 shows the copper transition fittings in place over the venting attachments and on the right side of the furnace, immediately downstream from the transition fitting is an in-line air mixer for introduction of diluting air. Immediately downstream from the in-line air mixer is a splicing sleeve which connects the collecting chamber piping to the in-line air mixer.

Figure 2. Apparatus for production of submicron aerosol particles showing power supply, furnace and collection chamber.

Procedures for Aerosol Production:

The procedure followed to produce experimental aerosols was to remove the transition fitting from the right venting attachment, place a sample to be heated or vaporized in a pellet form (mg size pellets used for this study) into the graphite tube through the opening on the right end of the furnace, set the temperature and time controls and set the proper nitrogen and water flow rates, reconnect the piping to the furnace and energize the furnace. Immediately after energizing the furnace, the proper diluting air flow rate into the collecting chamber and exhaust flow rate out of the chamber were set by use of rotameters. At the completion of the heating cycle, the furnace was automatically de-energized, the diluting air line to the chamber and the vacuum exhaust line from the chamber were closed. At this time, particle sampling or concentration counting was performed.

Particle Size Analysis:

Aerosol particles were collected on carbon-coated electron microscope grids using a point-to-plane electrostatic precipitator (PTP). All grids were scanned in an electron microscope. Particle sizes were determined from electron micrographs and, in some cases, a Zeiss analyzer and a computer program were utilized.

RESULTS AND DISCUSSION

Factors Effecting Particle Size, Shape, and Agglomeration:

a. Temperature

Temperature is one of the most important factors in effecting the vapor concentration and determining the nature of aerosol particles formed. With the temperature of the furnace around 1700°C to 2000°C, more individual aerosol particles formed with less chains and agglomerates. The temperature region of 2300°C to 2600°C produced very small particles (0.01 μm and below) and the tendency for chain agglomerate formation was very great. Figure 3 shows some of the largest particles produced in this study. These particles were produced by heating a ceric oxide pellet in the graphite furnace at 1700°C for two minutes. The carrier gas was nitrogen at a flow rate of 2.5 lpm and diluting air flow rate was 30 lpm. The particles were collected in the large 120-liter chamber. Particle concentration was measured with a small particle counter. The average concentration immediately after collection was 8×10^6 particles/cc, and the concentration one hour later was 1×10^5 . All particles above 1.0 μm were detected by the counter.

Figure 4 shows the resultant aerosol particles produced by heating a ceric oxide pellet to a melting temperature of approximately 2600°C. This electron micrograph shows the agglomeration and long twisting chains which are characteristic of high temperature condensation aerosols and similar to those produced by Horn and Castleman¹ in comparisons of UO₂ and PuO₂ agglomerates. The chains and agglomerates of aerosol particles shown in Figure 4 are similar to aerosols produced by Chatfield² using an explosive vaporization technique to produce Pu, Pu-Al, Al, Pu-Mg, Mg, Pu-Zn, and Zn aerosols.

The general trend observed from temperature versus particle size studies was that somewhat larger, individual particles were formed around 1700°C and long twisting chains and agglomerates were formed around 2600°C.

b. Carrier Gas

The carrier gas used in this study was nitrogen. The minimum flow rate used was 1 liter per minute since the furnace would automatically shut off if the flow dropped below this rate. Various flows up to about 6 liters per minute were tried but no observable effect upon particle size, shape, or agglomeration was noticed. In this study, the carrier gas flow rate seemed to have no significant effect if any upon size, shape, and agglomeration.

To prevent rapid deterioration of the graphite tube, the atmosphere in and around the tube must be kept relatively inert by use of a gas such as nitrogen or argon. The average rate of graphite loss for a new tube heated to 2600°C in a nitrogen atmosphere (1.5 l/min nitrogen flow rate) was 3.66 mg per minute averaged over a three-minute time span.

If air is introduced into the graphite tube during heating, the tube deteriorates rapidly and relatively large amounts of graphite are removed from the tube as either gaseous or particulate matter at temperatures beyond 2000°C. Collection of particulate matter produced when an empty graphite tube was heated at 2600°C in a nitrogen air (1.5 l nitrogen/min, 10 l air/min) atmosphere indicates that very little carbon particulate matter is formed and most of the graphite loss from the tube is probably converted to the gaseous state. The average rate of material loss from the tube was 30 mg/min average over a three-minute heating period.

Figure 3. Electron micrograph of aerosol particles produced from ceric oxide. Furnace temperature, 1700°C; nitrogen flow rate, 2.5 LPM; diluting air flow rate, 30 LPM.

Figure 4. Electron micrograph of aerosol particles produced from ceric oxide. Furnace temperature, 2600°C; nitrogen flow rate, 2.5 LPM; diluting air flow rate, 30 LPM.

A typical aerosol produced by heating ceric oxide in a nitrogen atmosphere at 2000°C could possibly contain as much as 8% by weight carbon particulate matter if all the graphite loss were converted to carbon particles.

c. Diluting Air

Diluting air produced a marked effect upon the formation of chains and agglomerates at temperatures around 2000°C to 2300°C. The introduction of diluting air flow rates of 20 to 30 l/min reduced chain size as shown in Figure 5. Figure 5 shows two general types of aerosol particles; the particles which have formed chains and agglomerates and very small individual particles. It could not be determined what condition or conditions prevailed to produce these very small particles. At temperatures around 2600°C, diluting air seemed to have very little effect upon formation of chains.

d. Size of Collection Chamber

High concentrations of small aerosol particles yield rapid coagulation. Most experimental evidence indicates that coagulation results in nearly every collision. Therefore, the effect of a large collection chamber upon formation of chains and agglomerates was studied.

When the aerosol particles were allowed to form in the 5/8-inch O.D. copper tubing, long twisting chains were formed even at 2000°C as shown in Figure 6. When the 120 liter chamber was placed immediately downstream from the furnace, there was a tendency for smaller chains and more individual particles to form. Undoubtedly, the combined effect of rapid dilution and size of collection chamber have a greater effect upon particle size and shape for a given temperature than either parameter acting alone.

Effect of Reheating Aerosol Particles:

Tests were conducted to determine the effect of reheating previously collected aerosol particles on particle size and shape. The aerosol was generated at a temperature of 2000°C using ceric

Figure 5. Electron micrograph of aerosol particles produced from ceric oxide showing smaller chain and agglomerate formations. Furnace temperature, 2000°C; nitrogen flow rate, 2.5 LPM; diluting air flow rate, 20 LPM.

Figure 6. Electron micrograph of aerosol particles produced from ceric oxide showing formation of twisting chain. Furnace temperature, 2000°C; nitrogen flow rate, 1.5 LPM; diluting air flow rate, 2 LPM.

oxide and collected in the chamber as previously described. The chamber was closed at both ends and the necessary piping changes were made to the inlet and outlet of the furnace and the collecting chamber was reattached to the furnace. The aerosol was drawn back through the furnace, reheated at a temperature of 2400°C, and particles were collected on an electron microscope grid by electrostatic precipitation. Figure 7 shows typical results of this procedure. The particles tended to be spherical in shape and ranged in size from possibly as small as 0.001 μm diameter to about 0.1 μm diameter real size and many of the smaller particles were hollow spheres. Clearly, long chains had melted and consolidated into spherical particles. The results of the reheat test suggest that the graphite furnace could be used to heat treat previously prepared aerosol particles.

Figure 7. Electron micrograph of aerosol particles produced from ceric oxide showing effect of reheating particles. Initial furnace temperature, 2000°C; nitrogen flow rate, 2.5 LPM; diluting air flow rate, 20 LPM; reheat temperature, 2400°C.

Characterization of Aerosol Particles by Electron Microscopy:

A meaningful size distribution analysis on particles produced by high temperatures and subsequent condensation during accident or simulated accident conditions is at best very difficult. A rigorous size distribution analysis on aerosol particles such as those in Figures 4-6 is not possible due to the chains and agglomerates. A size distribution analysis on the larger, dense particles of Figures 3 and 7 was made using a Zeiss particle analyzer. The results of this analysis for Figure 3 were; CMD = 0.064 μm , MMD = 0.22 μm , and σ_g = 1.9 and for Figure 7 it was; CMD = 0.03 μm , MMD = 0.079 μm , and σ_g = 1.63.

CONCLUSIONS

The Perkin-Elmer HGA-70 graphite furnace is an excellent device for quickly generating high temperatures in the range of from 1000°C to 2600°C and maintaining the preselected temperature within this range for a given amount of time. The furnace is readily adaptable to the production of experimental aerosols from a variety of material vaporized at the desired temperature up to 2600°C.

This arrangement could be used for production of submicron aerosols from a variety of materials by vaporization of the materials at the desired temperature up to 2600°C. This method therefore is useful in studying the nature and behavior of submicron aerosols produced in high temperature accidents, including their biological fate following inhalation.

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PRODUCTION OF SUBMICRON METAL OXIDE AEROSOL PARTICLES
FROM THEIR THD COMPOUNDS BY VAPORIZATION-DEGRADATION METHODS

ABSTRACT

An improved procedure has been developed for producing submicron aerosol particles of rare earth elements such as may be accidentally released in nuclear operations. The Y and La compounds of 2,2, 6,6 tetramethyl 3,5 heptane dione [Y(THD)₃ and La(THD)₃] were used to produce submicron aerosol particles of mass median diameters < 0.3 μm. Two methods were used in these studies. The first consisted of vaporization of the Y(THD)₃ or La(THD)₃ and direct heat degradation of the vapor. This procedure yields chains of extremely small particles. The second method consisted of vaporization, condensation, revaporization, subsequent condensation and heat degradation. This method provided distinct submicron size particles, ranging from 0.3 to < 0.1 μm. Several factors which affect size distribution were investigated.

PRINCIPAL INVESTIGATORS

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INTRODUCTION

Improved procedures for the production of submicron aerosol particles are of interest for research directed toward an improved understanding of aerosols that may be accidentally released in nuclear operations.

Controlled condensation of vaporized materials for the production of submicron size inorganic aerosol particles of narrow size distribution have been used.^{1,2} This method, however, may be used only when the material exhibits sufficient vaporization and chemical stability at the operating temperatures. The oxides of rare earth and actinide elements, which are of interest because of the abundance of these elements in nuclear industry, have insignificant vapor pressure below 1500°C and are relatively unstable at temperatures where significant vaporization exists. Use of other compounds of these elements, which are volatile at relatively low temperatures and could be readily converted to their oxides, is therefore desirable. Some β-diketone chelates of most metallic elements are volatile and chemically stable below 300°C.³ Aerosols containing organometallic particles may be readily converted into metallic oxide particles by passing the aerosol through a heating column at 1100°C.⁴ Therefore, β-diketone chelates of rare earth and actinide elements may be used for the production of their oxide aerosol particles in the submicron range by vaporization, condensation, and thermal degradation.

Although the simplest β-diketone, acetylacetone, forms volatile compounds with many metals, the rare earth acetyl acetonates are not volatile.⁵ The β-diketone 2,2, 6,6 tetramethyl 3,5 heptane dione (THD) forms volatile chelates with all metals, except that of alkali metals.⁵⁻⁷ These volatile compounds are therefore suitable for the production of submicron size aerosol particles of most metal oxides by vaporization, condensation, and subsequent thermal degradation technique.

When solutions of volatile compounds such as acetyl acetonates of Co and Zr and chlorides of Al and Zr are nebulized and the resulting aerosols are heated, there is no significant vaporization. However, Y₂O₃ and La₂O₃ aerosols can be prepared by direct vaporization of solid THD chelates of Y and La with subsequent thermal degradation. The two methods which were studied were the method of direct thermal degradation of the vapor without prior aerosol formation and the method of condensation of the vapor to form an aerosol with subsequent revaporization of the particles and recondensation before thermal degradation. The effects of several factors were studied including

(a) the rate of vaporization of the solid material, (b) the presence of water vapor in the degradation system, and (c) the flow rates used in the degradation system. Aerosol particles ranging from 0.06 to 0.3 μm and small chains of primary particles of less than 0.01 μ were prepared.

EXPERIMENTAL METHODS

Reagents:

The β -diketone, THD, was obtained from Eastman Organic Chemicals and used without further purification. The ^{90}Y used as a radiolabel was separated from ^{90}Sr using a procedure described elsewhere.⁸ All other reagents used were of analytical grade.

Preparation of $\text{La}(\text{THD})_3$ and $\text{Y}(\text{THD})_3$:

The general method described by Hammond et al.⁶ was used for the preparation of $\text{Y}(\text{THD})_3$ and $\text{La}(\text{THD})_3$. Ten mmoles of Y or La nitrate were dissolved in 100 ml of 90% ethyl alcohol containing 1 mmole of HNO_3 and used after filtration through a Whatman No. 42 filter. Thirty-eight (38) mmoles of THD was mixed with 38 ml of 1 M NaOH in 90% ethyl alcohol so that the THD was in a readily chelating form. The La or Y solution was mixed thoroughly with the THD solution and then diluted with 200 ml of distilled water. The pH of this solution was then adjusted to 7.4 with the addition of 1 M (aqueous) NaOH. $\text{Y}(\text{THD})_3$ or $\text{La}(\text{THD})_3$ precipitation was complete at this stage and was separated by vacuum filtration through a Whatman No. 42 filter in a Buchner funnel. Purification of $\text{Y}(\text{THD})_3$ or $\text{La}(\text{THD})_3$ was done by reprecipitation from alcohol and air drying after filtration. The ^{90}Y containing $\text{Y}(\text{THD})_3$ was prepared with the same procedure. The ^{90}Y was mixed with the yttrium nitrate solution in ethyl alcohol and then combined with the THD solution. The melting points of $\text{Y}(\text{THD})_3$ and $\text{La}(\text{THD})_3$ were observed to be between 151°C to 154°C and 235°C to 240°C, respectively, in good agreement with Berg et al.⁵

Experimental Arrangements for the Production of Aerosols:

Two different experimental arrangements were used in this study for the production of aerosols. The first arrangement (Fig. 1) consisted of a vaporizer, a degrader and a sampling chamber. Figure 2 shows the second arrangement with a vaporizer, condenser, revaporizer, a second condenser, degrader and a sampling chamber. Electrical heating columns, consisted of 50 cm long and 3 cm diameter quartz tubes wound with nichrome wire, were used as vaporizers and degraders. Aluminum tubing, 10 cm diameter, was used for condensers and sampling chambers. The first condenser, 60 cm long, was heated with electrical heating tape. The first half of this condenser was normally maintained at 100°C and the second half at about 50°C. Both the sampling chamber

Figure 1. Experimental arrangement used for the preparation and sampling of Y_2O_3 aerosol particles produced by the direct degradation of $\text{Y}(\text{THD})_3$ vapor.

Figure 2. Experimental arrangement for preparation and sampling of Y_2O_3 and La_2O_3 aerosol particles produced by controlled condensation-revaporization and degradation.

and the second condenser were about 30 cm long. Copper tubing, 1.5 cm diameter and approximately 10 cm long, was used to connect between the various units.

The samples for vaporization were placed in a boat made of 1 cm diameter pyrex tubing with a 0.8 cm diameter circular opening. The sample boat with about 100 mg sample was placed in the middle of the vaporizer column and heated for 10 to 15 minutes to obtain temperature equilibrium. The temperature of the vaporizer column was monitored with a thermocouple. The temperature of other units reported in this study are estimated temperatures as determined from calibration curves. Filtered dry air was used throughout these studies except when water vapor was intentionally introduced. Air saturated with water vapor at 60°C was prepared by passing air over a water reservoir kept at 60°C and then introduced into the first condenser. Both experimental arrangements were operated with vacuum at about 15 inches of water.

Particle Size Analysis:

Aerosol particles were collected on carbon-coated electron microscope grids using a point-to-plane electrostatic precipitator (PTP). Grids were scanned in electron microscopes and particle sizes determined from electron micrographs using a Zeiss analyzer and a computer program.

Chemical Characteristics:

Infrared spectrum of aerosol particles collected at different stages were examined by a KBr macropellet technique using a Perkin-Elmer Model 621 I.R. spectrometer.

RESULTS AND DISCUSSION

Rate of Vaporization of $Y(THD)_3$ at Different Temperatures:

Particle sizes of the aerosol may be affected by the vapor concentration and hence by the rate of vaporization of $Y(THD)_3$. The rates of vaporization of $Y(THD)_3$ at different vaporization temperatures were determined by using ^{90}Y labeled $Y(THD)_3$. The experimental arrangements shown in Figure 1 was used in these studies. The $Y(THD)_3$ vapor carried by flowing air, 1.5 liters per minute, was degraded at 1100°C, diluted with 10 liters per minute air and the aerosol particles collected on 47 mm (diameter) 0.3 μm pore size Millipore membrane filters. Several samples at the same temperature were collected in intervals ranging from 3 to 20 minutes. The results are shown in Figure 3 as μg of $Y(THD)_3$ vaporized per minute versus the temperature of the vaporizer column. The μg of $Y(THD)_3$ vaporized were calculated from the ^{90}Y count rate on the aerosol collection filters and from the count rate per μg of the original $^{90}Y(THD)_3$ sample. Quantitative retention of ^{90}Y by the 0.3 μm membrane filter was indicated by lack of ^{90}Y activity on back-up filters.

Figure 3. Rate of vaporization of $Y(THD)_3$ with respect to the vaporization temperature as determined by ^{90}Y count rate of aerosol particles collected on filters after degradation.

With the flow rate of air at 1.0 liter per minute or above, the flow rate had no effect on the ^{90}Y count rate collected on the filter. With the flow rate of air at 300 cc per minute, the ^{90}Y count rate on the filters decreased by 10% compared to the count rates at higher flow rates. ^{90}Y collected on filters in similar tests conducted with the experimental arrangements shown in Figure 2 was not significantly different from that of earlier experiments provided the flow rates were ≥ 1.5 liter per minute. At lower flow rates, ^{90}Y loss mainly in the first condenser, was indicated. At an air flow rate of 500 cc per minute, this loss was about 50%.

Y_2O_3 Aerosol Particles by Direct Degradation of the $\text{Y}(\text{THD})_3$ Vapor:

Aerosol samples were prepared by vaporization of $\text{Y}(\text{THD})_3$ and directly degrading the vapor at 1100°C using the experimental arrangement shown in Figure 1. The total flow rates of aerosol and the diluting air were about 20 liters per minute. The aerosol particles were all chains when the vaporizer temperature was at 180°C and the flow rate of air through the vaporizer were 5, 2, 1 and 0.5 liter per minute. An example is shown in Figure 4. The primary particles of these chains were smaller than $0.01\text{ }\mu\text{m}$. Slight increase of primary particle sizes with lower air flow rate through the vaporizer is indicated. The aerosol particles produced with the vaporizer temperature at 200°C were chains with a number of larger individual particles with diameters at $\sim 0.1\text{ }\mu\text{m}$.

Aerosol prepared with the vaporizer temperature at 150°C consisted of small particles ($< 0.01\text{ }\mu\text{m}$) of low density and probably are aggregates of even smaller particles.

Effects of Condensation and Revaporization of $\text{Y}(\text{THD})_3$ Particles:

The physical and chemical nature of $\text{Y}(\text{THD})_3$ aerosol particles formed from $\text{Y}(\text{THD})_3$ vapor after controlled condensation, revaporization and recondensation were studied using the experimental arrangements shown in Figure 2. All experiments were conducted with vaporization at 180°C , no heating of the degrader column, an air flow rate of 1 liter per minute through the vaporizer and another 500 cc per minute of dry air or air saturated with water vapor at 60°C introduced in the first condenser.

$\text{Y}(\text{THD})_3$ particles themselves were not stable under the electron beam in the electron microscope possibly due to melting. Aerosol particles prepared from $\text{Y}(\text{THD})_3$ vapor, with dry air flow, first condenser temperature at 100°C and revaporizer at 300°C were stable under the electron beam in the electron microscope. However, very fine particles were also present in these samples. Introduction of water vapor in the carrier gas at the first condenser yielded particulates without the very fine particles (Fig. 5). Water vapor at high temperatures enhances the degradation of THD compounds.⁷ The degradation process may have prevented excessive vaporization of the particles in the revaporizer.

Infrared absorption spectra of aerosol particles produced by vaporization of $\text{Y}(\text{THD})_3$ at 180°C and revaporized at 250°C were identical with that of the original $\text{Y}(\text{THD})_3$. Strong and sharp absorptions at 18 different wave numbers ranging from 2940 to 350 cm^{-1} are seen for $\text{Y}(\text{THD})_3$. Aerosol particles produced by vaporization of $\text{Y}(\text{THD})_3$ at 180°C , condensation at 100°C in the presence of water vapor, and revaporization at 300°C shows only three broad absorptions at wave numbers 1510 , 1400 and 860 cm^{-1} . Chemical change of the yttrium compound to yttrium carbonate was indicated.

Y_2O_3 Aerosol Particles Produced with Controlled Condensation and Revaporization:

The formation of Y_2O_3 aerosol particles produced from $\text{Y}(\text{THD})_3$ vapor by controlled condensation and thermal degradation with the experimental arrangement shown in Figure 2 is assumed to involve the following steps: (1) the $\text{Y}(\text{THD})_3$ vapor gradually condenses in the first condenser to

Figure 4. Electron micrograph of Y_2O_3 aerosol particles produced by the direct degradation of $Y(THD)_3$ with the experimental arrangement shown in Figure 1. Vaporizer column and degradation column temperatures were 180 and 1100°C. The flow rate of air through the vaporizer column was 1 liter per minute.

Figure 5. Electron micrograph of aerosol particles produced from $Y(THD)_3$ vapor. Using experimental arrangement in Figure 2: vaporization temperature at 180°C, first condenser at 100°C, revaporizer at 300°C, no heating of the degrader, flow rate through the vaporizer at 1.0 liter per minute and an additional 500 cc of air saturated with water vapor at 60°C introduced in first condenser.

form solid particles. (2) Partial or complete vaporization of the $Y(THD)_3$ particles in the revaporizer occurs, depending on the temperature and whether chemical degradation takes place in the revaporizer. (3) Non-volatile degradation products of $Y(THD)_3$ recondense in the revaporizer and $Y(THD)_3$ vapor recondenses in the second condenser. (4) Complete degradation of Y compounds to Y_2O_3 occur in the degrader. If $Y(THD)_3$ exists in the aerosol particles, revaporization may occur in the degrader and fine particles may be produced. Otherwise, individual particles of narrow size distribution are obtained.

Many experiments were performed in attempts to produce distinct Y_2O_3 aerosol particles of submicron size and narrow size distribution. With revaporizer temperature between 260 to 300°C and in the presence of water vapor, relatively dense, larger particles and free of very fine particulates were obtained (Figs. 6, 7). When the temperature of the revaporizer went below 260°C or above 325°C, extremely fine particles were also present, along with relatively larger particles (Fig. 8). The tendency to form very fine particles was increased when the temperature of the vaporization was above 200°C.

Particle sizes and size distributions of Y_2O_3 aerosol particles produced under different conditions are shown in Tables 1, 2 and 3. The effect of vaporization temperature on the size distribution is given in Table 1. Count median diameters (CMD) and mass median diameters (MMD) increased with increasing temperature of the vaporizer from 170°C to 220°C. The particle size distributions were narrower at the lower temperatures as indicated by the lower geometric standard deviations (σ_g).

The effects of revaporization temperature on the size distribution may be observed from the data in Table 2. The MMDs were only slightly affected by the revaporization temperature. The CMDs increased with revaporization temperatures between 260°C to 310°C and a decrease occurred with a further increase to 325°C. The size distribution, as indicated by the σ_g s, was narrower with revaporization at about 300°C.

Figure 6. Electron micrograph of Y_2O_3 aerosol particles produced from $Y(THD)_3$ vapor using experimental arrangements shown in Figure 2: vaporizer column, first condenser, re-vaporizer column and degrader column at 200, 100, 310 and 1100°C, respectively. The flow rate of air through the vaporizer column was 1.0 liter per minute and an additional 500 cc of air saturated with water vapor at 60°C introduced in the first column. MMD = 0.123 μm and $\sigma_g = 1.45$.

Figure 7. Electron micrograph of Y_2O_3 aerosol particles produced from $Y(THD)_3$ vapor using experimental arrangements shown in Figure 2: vaporizer column, first condenser, re-vaporizer column and degradation column at 180, 100, 280 and 1100°C, respectively. The flow rate of air through the vaporizer column was 1.0 liter per minute and 500 cc of air saturated with water vapor at 60°C was introduced in the first condenser. The electron micrograph grid was shadowed with Cr at 30° angle. MMD = 0.093 μm and $\sigma_g = 1.26$.

Figure 8. Electron micrograph of Y_2O_3 aerosol particles produced from $Y(THD)_3$ vapor using experimental arrangements shown in Figure 2: vaporizer column, first condenser, re-vaporizer column and degrader column at 200, 100, 340 and 1100°C, respectively. The flow rate of air through the vaporizer was 1.0 liter per minute and 500 cc of air saturated with water vapor at 60°C was introduced in the first condenser.

Table 1

Effect of Vaporizer Temperature on the Size Distribution of Y_2O_3 Aerosol Particles*

Vaporizer Temp. °C	Revaporizer Temp. °C	CMD μm	MMD μm	σ_g
170	280	0.057	0.071	1.31
180	280	0.079	0.093	1.26
200	280	0.084	0.16	1.60
220	280	0.084	0.30	1.86
180	325	0.051	0.07	1.42
200	325	0.082	0.20	1.73

* Flow rate air, through the vaporizer, was 1000 cc per min. An additional 400 cc of air saturated with water vapor at 60°C was introduced at the first condenser which was heated to 100°C.

Both CMDs and MMDs increased with lower air flow rates through the vaporizer. However, at flow rates of 500 and 100 cc per minute fewer particles were observed. Very fine particles, which could not be counted with the Zeiss analyzer, were also present with flow rates of 2500 cc per minute.

These studies show that submicron Y_2O_3 aerosol particles of narrow size distribution may be produced by the above described method. Particle sizes may be varied by varying vaporization temperature and flow rate of air. The revaporization temperature at about 300°C seems to be optimum.

Production La_2O_3 Aerosol from $La(THD)_3$:

$La(THD)_3$ does not have significant vapor pressure below 220°C. Several experiments suggested that between 220°C and 270°C, $La(THD)_3$ vaporization was sufficient to produce aerosol particles. Prolonged heating above 270°C degrades the $La(THD)_3$ and therefore the vaporization temperature for this material is limited to between 220°C and 270°C. The revaporization temperature, slightly higher than that used for $Y(THD)_3$ was found to be satisfactory. No other difference between $La(THD)_3$ and $Y(THD)_3$ during the production of their respective oxide aerosol particles could be observed.

CONCLUSIONS

The use of THD compounds of Y and La is a convenient approach to continuous production of aerosol of spherical submicron aerosol particles of different sizes. Aerosols of small chains of extremely small particles may be conveniently produced by this method. These aerosols will be useful for inhalation deposition studies and for *in vivo* and *in vitro* dissolution studies. Further, the method used for the production of Y_2O_3 aerosol from $Y(THD)_3$ may be easily adapted for the production of other metal oxide aerosols. Though attempts to make monodisperse particles by

Table 2

Effect of Revaporizer Temperature on the Size Distribution of Y_2O_3 Aerosol Particles*

Revaporizer Temp. °C	CMD μm	MMD μm	σ_g
240	0.063	0.17	1.76
280	0.090	0.16	1.54
295	0.13	0.18	1.40
310	0.15	0.18	1.28
325	0.082	0.20	1.73

* Flow rates through the vaporizer were 1000 cc. An additional 400 cc of air, saturated with water vapor at 60°C was introduced in the first condenser. All experiments were conducted with the vaporizer temperature at 200°C.

Table 3

Effect of Air Flow Rate Through the Vaporizer on the Size Distribution of Y_2O_3 Aerosol Particles*

Air Flow Rate In The Vaporizer cc/min	CMD μm	MMD μm	σ_g
100	0.13	0.28	1.64
500	0.12	0.19	1.45
1000	0.090	0.16	1.54
1500	0.081	0.12	1.45
2000	0.078	0.12	1.46
2500	0.071	0.093	1.35

* 400 cc of air saturated with water vapor at 60°C was introduced in the first condenser. All experiments were conducted with vaporizer temperature at 200°C and revaporizer at 280°C.

this method were unsuccessful, refinements of experimental conditions described in this paper may provide a means of producing monodisperse particles of MMD < 0.3 μ m.

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PRODUCTION OF MONODISPERSE AEROSOLS OF $^{239}\text{PuO}_2$ LABELED WITH ^{51}Cr

ABSTRACT

Monodisperse aerosols of $^{239}\text{PuO}_2$ labeled with ^{51}Cr have been prepared by (1) nebulizing an aqueous preparation containing both ^{239}Pu and a small mass of ^{51}Cr , (2) heat treating the resulting aerosol to form a polydisperse aerosol of particles of $^{239}\text{PuO}_2$ labeled with ^{51}Cr , (3) separation and collection of the particles into monodisperse size groups using the Lovelace Aerosol Particle Separator, and (4) resuspending the monodisperse particles on small segments of the collection foil from the particle separator to provide aqueous suspensions for nebulization to form monodisperse aerosols or for other studies. During an 11½ hour continuous separation, monodisperse particles of $^{239}\text{PuO}_2$ labeled with ^{51}Cr were prepared for injection into Chinese hamsters for cytogenetic studies, test inhalation exposures with Syrian hamsters, in vivo solubility studies in rats and hamsters for in vitro solubility studies. The in vitro solubility studies showed that the monodisperse aerosols had a readily soluble fraction possibly associated with small contaminating particles of ^{51}Cr in that over 11% of the ^{51}Cr was readily dissolved in the first two days of the dissolution study. Near the end of the 24-day study, dissolution stabilized somewhat and the PuO_2 dissolution based on the ^{51}Cr label had a rate constant of specific solubility of about $6.8 \times 10^{-8} \text{ gm/cm}^2\text{-day}$ as compared to a value of about $1.9 \times 10^{-10} \text{ gm/cm}^2\text{-day}$ based on dissolved plutonium. Clearly, the ^{51}Cr was leaving the plutonium particles much faster than they were dissolving with a half-time of about 2 years as compared to about 700 years for the plutonium particles themselves.

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INTRODUCTION

Since the low-energy photon emissions of most of the important actinides are at best difficult to count under in vivo conditions, these studies were undertaken to develop an effective method for incorporating ^{51}Cr as a tracer in $^{239}\text{PuO}_2$ particles. Some of the monodisperse particles were prepared for cytogenetic studies in Chinese hamsters (this report, pp. 61 - 64). Other monodisperse particles were subsequently used for an inhalation experiment with Syrian hamsters (this report, pp. 50 - 54) and for both in vivo (this report, pp. 55 - 60) and in vitro solubility studies. This report describes the basic methodology used in the production of ^{51}Cr -labeled monodisperse $^{239}\text{PuO}_2$ particle. All ^{51}Cr counts were corrected to the day of the test inhalation exposure (Sept. 6, 1972) for ease of comparison.

CHEMICAL PROCEDURES

$^{239}\text{PuO}_2$ obtained from Oak Ridge National Laboratory was dissolved in a mixture of HNO_3 -HF. The resultant solution was evaporated to dryness several times with the dry residue, each time redissolved in 6 M HNO_3 . After the final evaporation, the residue was dissolved in 2 M HCl and treated with NaNO_2 - HNO_3 . The precipitate formed by subsequent addition of NH_4OH was filtered, washed with water and dissolved in HCl, to provide the basic stock solution containing 5.28 mg ^{239}Pu /ml for the experiment. The aerosol nebulizer solution was prepared as summarized in Figure 1. A 41.4 ml aliquot of the ^{239}Pu stock solution was treated with HNO_3 and NaNO_2 and stirred to insure the presence of Pu^{4+} . After stirring for 15 minutes, 1.15 ml (containing about 250 mCi) of the $^{51}\text{Cr}^{3+}$ in 0.5 M HCl stock solution was added. About 15 ml of 5 N NH_4OH was

Figure 1. Flow chart showing chemical procedure for ^{51}Cr - ^{239}Pu preparation used in nebulizer to produce ^{51}Cr - $^{239}\text{PuO}_2$ particles after heat treatment at 300 C and 1100 C.

added to precipitate the hydroxides and the precipitate was filtered and washed with 100 ml H_2O . The precipitate was then resuspended in 87.5 ml of 0.6 N HCl. An assay of the final solution with aliquots and liquid scintillation counting yielded concentrations 2.65 mg ^{239}Pu /ml (164 $\mu\text{Ci}/\text{ml}$) and 0.88 mCi ^{51}Cr /ml. Based upon the assay provided by the ^{51}Cr supplier (ICN), the chromium should have represented only about 6 $\mu\text{g}/\text{ml}$ in the nebulizer solution.

AEROSOL GENERATION AND PARTICLE SEPARATION

Polydisperse aerosols labeled with ^{51}Cr were generated using a Lovelace Nebulizer (which yielded 45 $\mu\text{l}/\text{min}$ of aerosol and 1.3 l/min of air at an operating pressure of 20 psig), and passed through two heating columns in succession, the first at 300 C and the second at 1150 C. The resulting polydisperse aerosol of $^{239}\text{PuO}_2$ labeled with ^{51}Cr was mixed into a holding chamber with 4.7 l/min of clean dry air from which a sample at 0.35 l/min was collected into a Lovelace Aerosol Particle Separator (LAPS), a device which separates particles into monodisperse size groups onto a collection foil.¹ The separator was operated at 4500 rpm with a total channel flow of 5 l/min. The aerosol generation procedure and particle collection were basically as described by Kotrappa, Wilkinson and Boyd.²

Prior to the production run, a 10-minute calibration run was performed in which a suspension of montmorillonite clay was nebulized rather than the plutonium solution. The density of the fused clay particles which form in the 1150 C heating column have been previously measured in the range 2.0 to 2.3 gm/cm^3 . The LAPS foil was replaced with a Whatman filter strip on which electron microscope grids had been placed at 5.4 cm, 7.2 cm, 11.0 cm, 11.5 cm, 12.0 cm, 12.5 cm, 15.5 cm and 30.0 cm down the channel. After the clay generation, the plutonium nebulizer solution was generated for 10 minutes to collect a sample of the ^{51}Cr -labeled $^{239}\text{PuO}_2$ particles onto

the same electron microscope grids which had the clay samples. A calibration was prepared by sizing electron micrographs of the particles using a Zeiss particle size analyzer. The calibration is shown in Figure 2 for the $^{239}\text{PuO}_2$, the clay, and the Lovelace aerodynamic equivalent diameter values calculated for clay with $\rho = 2.3$ and for $\rho = 2.0$. Since a recent measurement of the clay in this experimental system gave values closer to 2.0, this density was assumed in the following discussion.

An 11-1/2 hour continuous production run to produce monodisperse particles was conducted with a thin stainless steel collection foil in the LAPS. The aerosol nebulizer was loaded with a fresh volume of solution (about 7 ml) twelve times during this period. After the run, the foil was removed from the separator and cut into segments as shown along the abscissa in Figure 2. The segments were counted using a double crystal low-energy photon detector to count 17 KeV x-rays to determine the ^{239}Pu on the segments and the 30 to 40 KeV range to determine the ^{51}Cr . The results and ratios of activity are given in Table 1. Also given in Table 1 are the count mean aerodynamic diameter and $^{239}\text{PuO}_2$ mean geometric diameter corresponding to the middle of each seg-

Figure 2. Lovelace aerosol particle calibration for $^{239}\text{PuO}_2$ labeled with ^{51}Cr including mean geometric diameter of $^{239}\text{PuO}_2$ particles versus foil position, mean geometric diameter of reference clay particles versus foil position, and calculated Lovelace count mean aerodynamic equivalent diameters assuming $\rho = 2.0$ and $\rho = 2.3$ for the reference clay particles. Also shown are the segment numbers and sections used in various studies.

Table 1
⁵¹Cr Labeled ²³⁹PuO₂ Foil Segment Summary

Segment Number	Distance (cm - cm)	Mid-Range Aero. Diam.	Mid-Range Mean. Diam.	Calculated Density (gm/cm ³)	μ Ci ²³⁹ Pu	μ Ci ⁵¹ Cr (8-7-72)	⁵¹ Cr/ ²³⁹ Pu
0	0- 3.92	-	-	-	0.14	0.53	3.79
1	3.92- 4.15	4.6	1.50	8.3	0.22	2.57	11.68
2	4.15- 4.35	4.35	1.40	8.4	0.38	4.84	12.74
3	4.35- 4.60	4.05	1.28	8.7	1.11	11.85	10.67
4	4.60- 4.87	3.75	1.18	8.6	2.01	24.53	12.20
5	4.87- 5.10	3.50	1.08	8.9	4.20	47.96	11.42
6	5.10- 5.37	3.30	1.00	9.1	5.66	62.81	11.1
7	5.37- 5.67	3.10	0.92	9.3	8.05	94.54	11.74
8	5.67- 6.00	2.90	0.85	9.4	9.30	106.49	11.45
9	6.00- 6.33	2.70	0.78	9.5	11.16	127.04	11.38
10	6.33- 6.65	2.53	0.72	9.7	12.55	139.13	11.09
11	6.65- 7.0	2.35	0.66	9.7	10.98	128.29	11.68
12	7.0 - 7.6	2.18	0.59	10.2	18.84	205.84	10.93
13	7.6 - 8.2	1.97	0.53	10.0	17.82	195.14	10.85
14	8.2 - 9.0	1.75	0.46	10.1	18.12	205.37	11.33
15	9.0 - 9.7	1.58	0.40	10.4	14.26	150.08	10.52
16	9.7 -10.8	1.41	0.355	10.1	17.45	156.56	8.97
17	10.8 -12.2	1.25	0.31	9.9	12.00	127.16	10.6
18	12.2 -13.4	1.15	0.275	10.1	8.00	85.71	10.71
19	13.4 -14.7	1.09	0.254	10.3	5.03	57.62	11.44
20	14.7 -17.2	1.00	0.23	10.1	8.27	73.24	8.86
21	17.2 -19.7	0.92	0.212	9.7	5.94	49.33	8.30
22	19.7 -23.0	0.84	0.195	9.2	4.63	48.75	10.53
23	23.0 -26.5	0.765	0.179	8.6	3.83	37.10	9.69
24	26.5 -32.0	0.685	0.161	8.1	4.08	32.05	7.86
25	32.0 -39.0	0.61	0.144	7.5	4.02	28.02	6.97
26	39.0 -46.2	0.55	0.13	7.1	3.54	19.44	5.49

ment and the calculated density for the ²³⁹PuO₂ based upon these numbers. The density values near the tails are probably least reliable because of the extrapolations on the calibration curve. The density value for the middle segments is about 10 gm/cm³. If the calibration clay were assumed to have a density greater than 2 gm/cm³, the calculated density for the plutonium would be correspondingly larger.

The original generator solution had a ⁵¹Cr/²³⁹Pu activity ratio of 5.4. The separated aerosol had ⁵¹Cr/²³⁹Pu ratios generally lower than this value for most segments with much lower values associated with the smaller particle sizes suggesting some vaporization of chromium during the heat treatment. When the chromium activities and the plutonium activities on the segments of the LAPS foil were independently used to fit a log-normal distribution to the aerodynamic diameter calibration, the results were nearly identical with an AMAD about 1.5 μ m and a σ_g about 1.5 which indicate that the ⁵¹Cr label was initially satisfactorily uniform with respect to total aerosol activity and particle distribution.

MONODISPERSE AEROSOL GENERATION

Monodisperse particles from segments 13 and 14 (1.65-2.05 μm count mean aerodynamic diameter based on clay $\rho = 2.0$) were combined to provide an aqueous suspension for nebulization. The particles were ultrasonically suspended in 15 ml water with 0.02% Dowfax 2A1 (a surfactant) and concentrated by centrifugation and removal of 9 ml of supernatant to reach the final volume of 6 ml and the particles were again stirred by ultrasonic agitation.

Aliquots of 0.025 ml were taken from the 15 ml resuspension volume prior to centrifugation and from the final 6 ml generator suspension and analyzed separately for ^{239}Pu and ^{51}Cr . The ^{239}Pu activity was determined by liquid scintillation counting and ^{51}Cr with 4" x 8" NaI crystal detectors and gamma spectrum analysis. These data are summarized in Table 2. The results show a loss of about 21.9% of the ^{51}Cr and 23.0% of the ^{239}Pu in achieving concentration increases of 1.95 and 1.92, respectively. Also, they show that about three-fourths of the activity on the foil segments were recovered from the foil in the resuspension procedure.

Table 2
 ^{51}Cr and ^{239}Pu Activity in Resuspension and Aerosol Generation Solutions

	^{51}Cr		^{239}Pu		$\frac{^{51}\text{Cr}}{^{239}\text{Pu}}$
	Conc. ($\mu\text{Ci/ml}$)	Total Activity (μCi)	Conc. ($\mu\text{Ci/ml}$)	Total Activity (μCi)	
Resuspension Volume	7.57	113.6	1.79	26.9	4.22
Generator Volume	14.8	88.7	3.45	20.7	4.29
Activity Loss in %	21.9		23.0		
Concentrating Factor	1.95		1.92		

A Lovelace nebulizer measured to consume about 60 $\mu\text{l/min}$ of solution at an operating pressure of 20 psig was used to aerosolize the monodisperse particles. The nebulizer was immersed in ice water to reduce evaporation. The aerosol droplets generated by the nebulizer at an air volumetric rate 1.3 l/min were passed through a heating column at 300 C to dry to the droplets and then through another heating column at 1100 C to degrade the Dowfax. The resulting aerosol containing monodisperse particles of $^{239}\text{PuO}_2$ labeled with ^{51}Cr and smaller residue particles of droplets which had no plutonium was passed to the inhalation exposure equipment and mixed with diluting air to a final volumetric rate of 23 l/min. The test exposure lasted 133 minutes due to failure of the nebulizer to operate properly during the entire run; 5.9 ml of the generator solution was nebulized for an average consumption of only 44 $\mu\text{l/min}$.

A variety of samples were taken to characterize the aerosol including (a) 3 filter samples at flow rates about 1 l/min each, (b) 3 cascade impactor samples at flow rates about 0.5 l/min, and (c) 2 electrostatic precipitator samples for electron microscopic study. The results are summarized in Table 3. An electron micrograph of the aerosol is shown in Figure 3. A density of 10.3 gm/cm^3 for the $^{239}\text{PuO}_2$ particles was calculated based on the cascade impactor data (average activity median aerodynamic diameter 2.04 μm) and the average mass median diameter (MMD = 0.525 μm based upon sizing of only particles near 0.5 μm geometric diameter) from the micrographs.

When the gross ^{51}Cr on the cascade impactor stage was used to fit a size distribution the values were very much lower than for the plutonium. Study of the data showed that about one-fourth of the ^{51}Cr activity was collected on the back-up filter of the impactors as compared to only about one-hundredth of the plutonium activity. Clearly from these impactor results the separation of at least one-fourth of the ^{51}Cr from the particles with subsequent association with the smaller particles was indicated. Dissolution studies described below indicated that this separation of chro-

Table 3

Summary of Sampler Data for 133 Minute Hamster Exposures to Monodisperse ^{51}Cr - $^{239}\text{PuO}_2$

Sampler Type	Location In Apparatus	Operating Time During Run (Minutes)	Concentration ($\mu\text{Ci/l}$)**	Median Diameter (μm)*	σ_g *	% Singlets	Cr/Pu
Filter	Sampler Port	0 - 133	0.0026 (Pu) 0.0091 (Cr)	--	--	--	3.5
Filter	Exposure Port 2C6	0 - 133	0.0029 (Pu) 0.0010 (Cr)	--	--	--	3.4
Filter	Exposure Port 1C9	0 - 133	0.0021 (Pu) 0.0075 (Cr)	--	--	--	3.6
Impactor JJ	Sampler Port	10 - 15 52 - 57	0.0018 (Pu) 0.0060 (Cr)	AMAD (Pu) $2.02 \pm 0.03 \mu\text{m}$	1.08 ± 0.01	--	3.3
Impactor FF	Exposure Port 2B3	10 - 15 52 - 57	0.0016 (Pu) 0.0058 (Cr)	AMAD (Pu) $2.08 \pm 0.02 \mu\text{m}$	1.08 ± 0.01	--	3.6
Impactor GG	Exposure Port 1B6	20 - 25 35 - 40	0.0014 (Pu) 0.0014 (Pu)	AMAD (Pu) $2.03 \pm 0.04 \mu\text{m}$	1.09 ± 0.02	--	2.3
Electrostatic	Sampler Port	16 - 26 50 - 60	--	Count Mean Geometric $0.50 \mu\text{m}$	1.09	86.5	--
Electrostatic	Exposure Port 1A1	4 - 14 45 - 55	--	Count Mean Geometric $0.53 \mu\text{m}$	1.09	86.1	--

mium from the plutonium oxide particles occurred as a result of the aerosolization procedure, possibly under the influence of the 1100 C heat treatment. Also, the ratios of $^{51}\text{Cr}/^{239}\text{Pu}$ on the samples were lower than in the generator suspension possibly due to loss of small chromium particles by diffusion to the walls of the inhalation apparatus.

When the ^{51}Cr on the back-up filters was ignored and the ^{51}Cr activity on the remaining stages of the impactors used to fit size distributions, the results yielded AMAD value of $1.96 \mu\text{m}$, $2.11 \mu\text{m}$ and $2.02 \mu\text{m}$, and σ_g values of 1.11, 1.07 and 1.16 for impactors JJ, FF and GG, respectively. These values are not significantly different from the distributions obtained for the ^{239}Pu alone, indicating that the ^{51}Cr consists of two distributions, one with $^{239}\text{PuO}_2$ and one of very small particles ($\ll 0.5 \mu\text{m}$ aerodynamic diameter).

IN VITRO DISSOLUTION STUDIES

One of the back-up filters from the inhalation exposure apparatus and a swiped sample from segment 15 (the next smaller particle size) of the LAPS foil were entered into a larger plutonium in vitro dissolution experiment in progress (this report, pp. 29 - 36). Each of these samples was placed in separate special holders for dissolution studies after being sandwiched between $0.1 \mu\text{m}$

Figure 3. Electron micrograph of monodisperse aerosol of ^{51}Cr - $^{239}\text{PuO}_2$ as generated for the test inhalation experiment. Sample has been shadowed with chromium vapor at an angle of about 20° .

pore size membrane filters. In these holders a serum simulant can be run by and around the sample to carry off the dissolved material, but the particles themselves cannot leave the holder. The system is started with ten minute rapid flow flush samples after which the eluant from each sample was collected on a daily basis with a serum simulant flow rate of 1 ml/min. Plutonium in the eluant was determined by ion-exchange, electroplating and alpha spectroscopy with a Pu-236 tracer. As part of the ion-exchange procedure, the fractions of ^{51}Cr -containing material leaving the system was collected, concentrated to 10 ml counting volumes and gamma counted.

The back-up filter from the inhalation exposure (sample 1) was determined to have 2.92 μCi ^{239}Pu and 9.03 μCi ^{51}Cr as of the exposure day; the $^{51}\text{Cr}/^{239}\text{Pu}$ ratio was 3.1. The MMD for these particles was about 0.525 μm with a σ_g of 1.09. Basically, the dissolutions had three parts: (a) nine days with serum simulant flowing at room temperature (23 C), (b) eight days with serum simulant at 37 C, and (c) eight days with serum simulant with 0.2 mmoles/liter of disodium EDTA at 37 C. The fractions per day dissolved for both ^{51}Cr and ^{239}Pu are shown in Figure 4.

The swipe of segment 15 (sample 2) represented particles with an MMD of 0.41 μm and σ_g of 1.07. Dissolution for these particles was conducted: (a) eight days with serum simulant at 37 C and (b) eight days with serum simulant with 0.2 mmoles/liter of disodium EDTA at 37 C. This sample had 3.9 μCi of ^{239}Pu and 19.5 μCi of ^{51}Cr . The results for ^{51}Cr are also shown in Figure 4.

The results show a significant difference (over two orders of magnitude) between the slow dissolution of $^{239}\text{PuO}_2$ and the rapid elution of ^{51}Cr . Clearly, the ^{51}Cr being eluted is either on the

Figure 4. Summary of *in vitro* dissolution data for two ^{51}Cr - $^{239}\text{PuO}_2$ samples showing fraction of activity dissolved per day of study for the ^{51}Cr in both samples and for the ^{239}Pu in sample 1 plotted versus elapsed days from start of each dissolution study.

surface of the particles, not associated with the particles, or diffusing out of the particles. In addition, the aerosolized sample shows a marked elution of ^{51}Cr in the first few days with 3.4% dissolving in the starting flush and an additional 6% in the remainder of the first day. Most of this rapidly eluted fraction of ^{51}Cr was probably not associated with the $^{239}\text{PuO}_2$ particles, but rather the very small particles with ^{51}Cr identified on the cascade impactors. Thereafter the elution rate dropped off exponentially with a half-time of about 4 days. The swipe sample only had about 0.5% of the ^{51}Cr eluted in the starting flush, and only an additional 0.5% in the following day. If an exponential is fit to the elution rates, they tended to fall with a half-time of 25 days. Hence, the un-aerosolized sample 2 had a fairly regular elution throughout the 16 days it was studied while the sample 1 ^{51}Cr elution proceeded downward 2 orders of magnitude. This supports the hypothesis that the heating columns may have effected a release of ^{51}Cr from the particles in the inhalation experiment.

Near the end of the dissolution study the fractions per day for ^{239}Pu and ^{51}Cr became fairly regular although the ^{51}Cr count rates in the eluants were near the limit of statistical significance and the dissolution rates may still have been proceeding downward in an exponential fashion. The rate constant of specific solubility of the PuO_2 calculated from the dissolved ^{239}Pu during the last 12 days of the study was $1.9 \pm 1.2 \times 10^{-10} \text{ gm/cm}^2\text{-day}$. If the rate constant for the PuO_2 had been calculated from the ^{51}Cr data, it would have been only $6.8 \pm 1.2 \times 10^{-8} \text{ gm/cm}^2\text{-day}$ based on the last seven days of elution of sample number 1 and $5.1 \pm 0.6 \times 10^{-8} \text{ gm/cm}^2\text{-day}$ based upon the last six days of elution of sample number 2. Based upon these values the long-term half-life of dissolution of ^{51}Cr in sample 1 was only about 2 years compared to a value of 700 years for the plutonium particles themselves.

SUMMARY

The use of ^{51}Cr as a gamma-emitting label for monodisperse particles of $^{239}\text{PuO}_2$ was tested yielding the following general conclusions. The ^{51}Cr incorporated into the original ^{239}Pu preparation may have been partially vaporized during the heat treatment required to form the $^{239}\text{PuO}_2$ particles. However, the original particles were fairly uniformly labeled with the ^{51}Cr . Although the ^{51}Cr dissolved out of these particles under in vitro solubility conditions, the level of loss would not prevent satisfactory use of the tracer in some applications with only about 2% being lost from the particles during the first 16 days.

A different result was obtained when some ^{51}Cr -labeled monodisperse particles of $^{239}\text{PuO}_2$ were resuspended in water, nebulized, and passed through two heating columns, one at 300 C and one at 1100 C. At least one-fourth of the ^{51}Cr was separated from the $^{239}\text{PuO}_2$ particles and was collected associated with very small submicronic particles of the back-up filter of cascade impactors while the remaining ^{51}Cr appeared to be with the $^{239}\text{PuO}_2$ particles. In vitro solubility studies of samples of collected particles showed that there was a significant fraction of readily dissolved ^{51}Cr with about 11% being eluted out of the sample in the first two days of elution.

In order to use ^{51}Cr as a gamma-emitting label for aerosols of actinides to be used in inhalation experiments, the mechanism of the separation of ^{51}Cr from the $^{239}\text{PuO}_2$ must be unequivocally determined and avoided or the separated ^{51}Cr must be preferentially removed from the aerosols before it is used. Future studies will be directed towards developing a system for producing aerosols of $^{239}\text{PuO}_2$ more coherently labeled with ^{51}Cr .

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A STUDY OF THE IN VITRO SOLUBILITY OF $^{239}\text{PuO}_2$

ABSTRACT

The solubility rates of submicrometer particles of $^{239}\text{PuO}_2$ under non-equilibrium conditions are being studied using an in vitro system. A continuously flowing stream of serum simulant (at 37 C) passes over and around a sample of particles which is sealed between membrane filters. Dissolved plutonium is carried off in the stream and measured to determine the rate constant of specific solubility, which for $^{239}\text{PuO}_2$ prepared at 1100 C was usually found to be of the order of 10^{-11} gm/cm²-day. A study of an accidentally produced soluble plutonium aerosol of unknown chemical form or particle size distribution demonstrated the potential usefulness of the in vitro solubility system for rapid range finding of the possible in vivo solubility of aerosol samples collected in accidental releases.

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INTRODUCTION

The main objectives of this study were (a) to determine the in vitro dissolution rates of samples of monodisperse aerosol particles of plutonium oxide with a flowing solvent of a lung fluid simulant at 37 C and (b) to test the usefulness of the in vitro dissolution system in predicting in vivo dissolution of inhaled particles.

This report describes the experimental details of the study with emphasis upon the criteria used to plan the experiment. Also described are preliminary data on the dissolution of laboratory prepared monodisperse spherical particles of $^{239}\text{PuO}_2$. Although these data show great fluctuations, they provide valuable insight into the very insoluble nature of even small particles of $^{239}\text{PuO}_2$.

In order to test the usefulness of the in vitro solubility system for rapid range-finding and study of plutonium aerosols of unknown chemical form or particle size distribution such as might be produced in an accidental release, a sample of such an aerosol has been studied.

METHODS

Samples of monodisperse particles of $^{239}\text{PuO}_2$ were prepared by the methods described by Kotrappa et al.¹ Basically, this involved generation of polydisperse aerosols of spherical particles of $^{239}\text{PuO}_2$ by heat treatment at 1000 C of aerosols produced by nebulization of a plutonium preparation in 0.6 M HCl and separation of the polydisperse particles in monodisperse size groups using the Lovelace Aerosol Particle Separator.² These particles can either be studied directly or they can first be resuspended in water, aerosolized for inhalation exposure of animals, and then filter samples of these monodisperse aerosols collected from the exposure chamber can be studied. This latter procedure is preferred to provide data which complement the animal data and allow the in vitro data to be compared to the observed behavior of the inhaled particles in animals.

After collection of a sample onto a filter it was assayed either by alpha counting or by spectral analyses of the 17 KeV x-rays using a composite crystal detector. It is then sandwiched between two 0.1 μm pore size membrane filters and sealed into a specially designed and constructed flowing solvent sample holder made of lucite (Fig. 1). This sample holder has a small chamber

Figure 1. Cross-section view of dissolution sample holder.

(~ 0.6 ml volume) on one side of the sample through which the solvent passes and another small space (~ 0.2 ml volume) on the other side of the filter sandwich in the space around the O-ring seal of the chamber. In this arrangement the solvent flows into the larger cavity at a predetermined rate and elutes any dissolved material entering the cavity. The particles are in contact with the solvent only indirectly through the membrane filter which prevents the particles themselves from leaving the sample holder. The sample residue, after studies are ended, was dissolved in an HNO_3 -HF mixture and assayed by liquid scintillation counting of an aliquot to verify the original assay.

In order to prepare a solvent to simulate lung fluids with their natural chelating agents, a serum simulant with pH 7.3 was prepared using the composition described by Kanapilly et al.³ (Table 1). In some cases the effect of adding a strong chelating agent to the lung fluid simulant was studied with the addition of 0.2 mmoles disodium EDTA/liter. The pH of the lung fluid simulant was relatively stable at 7.3, checked throughout the experiments, and readjusted to 7.3 with small additions of HCl or NaOH as required.

Both gravity feed from a 20-liter reservoir and pressurized air feed have been used to provide the head pressure for the solvent flow. The gravity feed was easier to operate. The reservoir was placed about five feet above the sample holders. All connections, valves, and other components of the flow system were of non-metallic forms, usually plastic or teflon, to prevent changes in solvent character. A small valve was used to adjust the flow rate of the lung fluid simulant for each sample holder. The flowing solvent passed out of the sample holder through a small bore plastic tube and was dripped into the plastic collection containers. Flow rates were regularly checked by first calibrating the drop rate to volumetric rate and maintaining the required drop rate.

When solubility was studied at 37 C, the filter holder and associated lead tubes were submerged in a thermostatically-controlled, electrically-heated water bath. Temperature was measured with a thermometer with control within ± 1 C. Small caliber spaghetti tubing approximately 1 meter long, coiled and kept in the warm water bath was used to achieve thermal equilibrium of the solvent before it reached the sample holder. A bubble chamber in the line just prior to the filter holder was used to remove from the flow lines gas formed when the solvents were heated.

Before starting the dissolution system for normal operation, each sample was given a rapid (about 10 min) flush, consisting of 100 to 200 ml of solvent to clear out air from the sample chamber and bubbles from the flow lines. The flow rate was then adjusted and eluants were collected daily into separate bottles for analysis. The first three samples were run at about 0.3 ml/minute in anticipation that the plutonium in the eluant would be of high enough concentration to allow ana-

Table 1
Composition of Blood Plasma and Lung Fluid Simulant

Chemical Species	Blood Plasma m Moles/L	Lung Fluid Simulant m Moles/L
Sodium	142	145
Potassium	5	--
Calcium	2.5	0.2
Magnesium	1.5	--
Ammonium	--	10
Protein	6.6	--
Chloride	103	126
Bicarbonate	27	27
Phosphate	1.2	1.2
Citrate	0.2	0.2
Organic Acid	6	6
Sulfate	0.5	0.5
Chemical Compound	NaCl NH ₄ Cl NaHCO ₃ NaH ₂ PO ₄ Na ₃ Cit Glycine H ₂ SO ₄ CaCl ₂	
m Moles dissolved per liter	116 10 27 1.2 0.2 6 0.5 0.2	

lysis by liquid scintillation counting. Because this was not possible in most cases, other samples were run at about 1 ml/min, a flow rate which is more easily adjusted and which provides greater assurance of efficient clearance of the dissolved plutonium. Most samples were analyzed for ²³⁹Pu by ion exchange, electrodeposition and alpha spectroscopy adding 50 to 150 dpm of ²³⁶Pu to each sample as a reference tracer in the radioanalytical techniques.

Data were analyzed in terms of the fraction of the ²³⁹Pu which was eluted per day of the study. The rate constant of non-equilibrium dissolution was determined using the mathematical dissolution model described by Mercer.⁵ A direct relationship of mass rate of dissolution to the surface area of particles was assumed by Mercer for describing the dissolution of sparingly soluble materials such as ²³⁹PuO₂. This relationship is given by:

$$\frac{dM}{dt} = -kS \quad (1)$$

where M is the particle mass, S is the particle surface area, t is time, and k is the dissolution rate constant of dissolution with units of mass dissolved per unit time for each unit of surface area of the particles. The units normally used are gm/cm²-day. Equation (1) is equivalent to:

$$\frac{dF}{dt} = -\frac{k'}{D} \quad (2)$$

where F is the mass fraction, D the particle diameter, k' a constant equal $k\alpha_s/\rho\alpha_v$ where α_s is the particle surface shape factor, α_v the particle volume shape factor, and ρ the particle density. The ratio $\alpha_s/\alpha_v = 6$ for spheres. The half-life of a single size particle is described by Mercer⁵ as

$$t_2 - t_1 = 0.206 \left(\frac{3\alpha_v\rho D_0}{\alpha_s k} - t_1 \right) \quad (3)$$

with D₀ the particle size at time t₁ and t₂ - t₁ the time period required for the particle to be reduced to 0.5 its original mass. At a time

$$t = \frac{3a_v \rho D_o}{a_s k} \quad (4)$$

the particle will be completely dissolved.

RESULTS

Laboratory Generated Aerosols:

Dissolution rates of four monodisperse samples of spherical particles of $^{239}\text{PuO}_2$ have been studied. The first three were collected from inhalation chamber filters during dog inhalation experiments. These samples were the first studied and numerous experimental difficulties in adjusting the flow rate of solvent led to irregular collection times and pooling of samples from a number of days on some occasions. These three samples were (a) 1.5 μCi of 0.227 μm geometric diameter, (b) 3.8 μCi of 0.41 μm geometric diameter, and (c) 3.2 μCi of 0.6 μm geometric diameter with σ_g in all cases less than 1.12. The results in fraction per day dissolved are shown in Figure 2. The numerical data are summarized in Table 2.

Another monodisperse sample (6.8 μCi) of 0.66 μm geometric diameter was taken directly from the Lovelace Aerosol Particle Separator without being used to generate an aerosol. This sample dissolution was used to compare to the three aerosolized samples and to test the effects of adding 0.2 mmoles/ml of disodium EDTA and the effect of room temperature on the observed dissolution. The results are summarized in Figure 3 and in Table 2. The EDTA appeared to stabilize the observed dissolution rate without affecting the average rate. The effect of lowering the temperature to 22 C was to increase the apparent dissolution rate by about twofold. After returning to 37 C the sample dissolution dropped back to the original level in about one week.

Figure 2. Dissolution data histograms giving fraction dissolved per day versus elapsed days in experiment for the three monodisperse $^{239}\text{PuO}_2$ aerosol particle sample (a) 0.27 μm , (b) 0.41 μm and (c) 0.60 μm . Missing data is indicated by the gaps in the histograms. All data were collected at 37 C lung fluid simulant.

Table 2
Summary of $^{239}\text{PuO}_2$ Dissolution at 37C

Geometric Diameter (μm)	ρ (gm/cm^3)	Sample Activity (μCi)	Dissolution Rate (DPM/day)	Dissolution Rate (fraction/day)	k ($\text{gm}/\text{cm}^2/\text{day}$)	Dissolution Half-Time (years)
0.227	9.1	1.5	4.5 ± 3.1	$(1.3 \pm 0.9) \times 10^{-6}$	$(4.6 \pm 3.2) \times 10^{-11}$	1300
0.41	8.5	3.8	7.2 ± 6.1	$(8.6 \pm 7.2) \times 10^{-7}$	$(5.0 \pm 4.2) \times 10^{-11}$	2100
0.60	>10	3.2	0.78 ± 1.27	$(1.1 \pm 1.8) \times 10^{-7}$	$(1.2 \pm 1.8) \times 10^{-11}$	14000
0.66	>10	6.8	12.4 ± 9.2	$(8.2 \pm 6.1) \times 10^{-7}$	$(9.0 \pm 6.7) \times 10^{-11}$	2100

Figure 3. Dissolution data histogram giving fraction dissolved per day versus elapsed days in experiment for the monodisperse $^{239}\text{PuO}_2$ particle sample of $0.66 \mu\text{m}$. Missing data are indicated by the gaps in the histogram. The experiment began with lung simulant at 37 C; subsequent single parameter changes are indicated.

The overall average fractions of ^{239}Pu dissolved per day for the four samples were used in Figure 4 to calculate the unweighted least-squares line of slope k' as given in equation (2). The k value for this slope is equivalent to $5.2 \pm 0.9 \times 10^{-11} \text{ gm}/\text{cm}^2\text{-day}$ assuming a particle density 10 for all samples. Correlation was demonstrated at the 5% significance level by the correlation coefficient of 0.79.

Also given in Table 2 are the half-times for dissolution predicted by equation (3). Clearly, the $^{239}\text{PuO}_2$ particles which were prepared and studied were of very low solubility.

Accident Aerosol:

A sample of a filter containing a plutonium aerosol accidentally released at another laboratory was studied with the aim of evaluating the usefulness of the *in vitro* solubility system for rapid solubility range finding over a short study period. The filter was assayed with the composite crystal counting system and found to have $6.2 \mu\text{Ci}$ of ^{239}Pu and $0.2 \mu\text{Ci}$ of ^{241}Am for a $^{239}\text{Pu}/^{241}\text{Am}$ ratio of about 31. Daily collections were analyzed for ^{239}Pu by liquid scintillation counting and for ^{241}Am by NaI crystal spectroscopy for the 59 KeV photon emissions. A summary of these data is shown in Figure 5. Two of the collections were selected for ^{241}Am analysis by ion exchange separation, electrodeposition and alpha pulse height analysis using ^{243}Am as a tracer. The analyses gave 920 dpm of ^{241}Am eluted on day 4 and 360 dpm on day 18 with respective $^{239}\text{Pu}/^{241}\text{Am}$ ratios of 67 and 44 for these two days.

The data indicate a higher solubility rate for Pu than Am. While the statistics of the counting data for ^{241}Am are poor, the general trend of higher solubility rate is confirmed by the two more

Figure 4. Dissolution data for the four monodisperse samples plotted as the average fraction per day dissolved for the four samples versus the reciprocal of particle diameter yielding a least-squares line of slope representing a rate constant of dissolution of $(5.2 \pm 0.9) \times 10^{-11}$ gm/cm²/day.

Figure 5. Dissolution data histograms of dissolved ²³⁹Pu and ²⁴¹Am in accident sample at 37 C giving fraction dissolved per day versus elapsed days in experiment. A missing datum is indicated by a gap in the histogram.

accurate ²⁴¹Am determination. The addition of EDTA to the solvent results in an increase in the solubility rate for both Pu and Am. The Pu data suggest that after the initial increase with EDTA the decrease of the rate of dissolution proceeds at the same rate as before the EDTA. However, because of the poor statistics for Am it is difficult to make any similar predictions. During the twenty days the sample was studied, 9.5% and 3.4% of the initial Pu and Am activity dissolved, respectively. Using the data from the initial parts of the graph (Fig. 5) the solubility half-times for Pu and Am in the original sample are estimated to be 90 and 300 days, respectively.

SUMMARY

In vitro studies of four monodisperse sizes of spherical particles of ²³⁹PuO₂ demonstrated a very low solubility under non-equilibrium condition. Although the data suffered from considerable variability, the rate constant of specific solubility for these particles was found to be of the order of 10^{-11} gm/cm²-day in each case. These rate constants imply dissolution half-times of the order of thousands of years.

On the other hand, a short study of samples of an aerosol of unknown chemical or physical form demonstrated a marked solubility for the plutonium and americium in the sample with about 10% of the total alpha activity of the sample being removed by dissolution in the 20 days of the study. Even the first days eluant quickly demonstrated that the sample contained a relatively soluble form of plutonium, very useful information which could be obtained immediately after an accidental inhalation to help in planning the course of therapy.

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SECTION II

RADIATION DOSE PATTERN FROM INHALED RADIONUCLIDES

INTERPRETATION OF RESULTS FROM STUDIES WITH MONODISPERSE FUSED CLAY AEROSOL PARTICLES

ABSTRACT

The availability of monodisperse, radionuclide-containing particles of respirable size has opened avenues to many new phases of research in the inhalation field. To assess better the most important routes to pursue and to establish priorities, several studies have been conducted with monodisperse, ^{137}Cs -labeled, fused clay aerosol particles, using both in vivo and in vitro systems. Dogs have been exposed by inhalation to monodisperse ($\sigma_g \sim 1.1$) particles having aerodynamic diameters of ~ 0.4 , 1.7 and $3.0 \mu\text{m}$. Syrian hamsters have inhaled aerosols with aerodynamic diameters of ~ 0.6 , 0.7 , 0.8 , 1.5 and $2.6 \mu\text{m}$ plus a polydisperse aerosol with aerodynamic median size of $1.9 \mu\text{m}$ and $\sigma_g = 1.5$. In vivo solubility characteristics of some of these same particle sizes have been determined following intramuscular injection of particle suspensions into Syrian hamsters. In vitro solubility studies have also been performed on similar aerosol particle suspensions. These data are interpreted in several ways including the following: 1) initial pulmonary deposition versus particle size; 2) absorption from the lung to internal organs as a function of particle size; 3) interlobar patterns of initially deposited particles; 4) in vitro solubility characteristics compared to in vivo results; 5) interpretation of in vivo solubility results in terms of lung clearance mechanisms; 6) gross whole-body and organ retention patterns as related to particle diameter; 7) comparison of initial deposition patterns of monodisperse and polydisperse particles following inhalation. Future plans with monodisperse particles are discussed in light of the relationships established.

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INTRODUCTION

The variables involved in assessing hazards associated with the inhalation of toxic substances are many and are often complex. Many of these concern the nature of the particles comprising the potentially damaging aerosol. In real life the accidentally released pollutant is generally of an extremely heterogeneous nature regarding particle size distribution and chemical form. To evaluate the practical situation in the laboratory, it has been necessary to select carefully only a few variables from the multitude of those that might occur and to study these thoroughly. To the present time, the only extensive inhalation toxicological studies with particles have used log-normally distributed or "polydisperse" aerosols. A device, the Lovelace Aerosol Particle Separator (LAPS), has been perfected in this laboratory that will enable the production of "monodisperse" aerosols, namely those having geometric standard deviations (σ_g) about the mean size of approximately 1.1.¹

The studies to be discussed are from several different experiments in which the aerosol particles used were of either monodisperse or polydisperse fused montmorillonite clay labeled with ^{137}Cs . The studies involved both in vivo and in vitro systems and centered around the effect of particle size upon biological behavior with regard to initial deposition and subsequent retention patterns.

METHODS

In raw ground-up form, montmorillonite clay, an aluminosilicate, will exchange some of its binding sites with cations. In our laboratory the raw clay is first "packed" with sodium, an easily exchangeable cation. The radionuclide cation of interest, in these studies ^{137}Cs , is then exchanged with the sodium until the available sites are essentially saturated. A suspension of the washed particles may then be nebulized and the droplets passed through a high temperature heating column operated at about 1100°C in these studies. The fused spherical particles containing the ^{137}Cs may be directed in this polydisperse form to animals for inhalation studies, or collected on filters for either in vitro solubility studies or for resuspension and subsequent use for in vivo studies. These polydisperse particles may also be passed on to the Lovelace Aerosol Particle Separator (LAPS) where they can be deposited in a continuum by size on a collecting stainless steel foil. This foil may be cut into strips of desired size, the particles from a given strip now being essentially monodisperse. Resuspension of these particles yields a starting material of essentially one size of ^{137}Cs -labeled fused clay particles which may be regenerated as an aerosol and used in the same manner as with the polydisperse particles. The basic methodology for this procedure has previously been described.^{2, 3}

The in vivo studies to be presented used Beagle dogs or Syrian hamsters as the subjects. Generally, animals inhaled the aerosol, some were sacrificed soon after exposure for determining initial deposition and some were sacrificed at subsequent times for determining tissue distribution patterns. As part of some studies, particles from filters that were operated during the inhalation exposures were resuspended in distilled water and injected intramuscularly into hamsters to obtain measurements of in vivo solubility. Parts of such filters were also used to study the in vitro solubility characteristics with time, using a system in which serum simulants were brought into contact with the collected particles in a parallel-flow system. Detailed procedures for each type of study have been elaborated in previous Annual Reports. The experimental studies to be discussed are described briefly in Table 1.

RESULTS AND DISCUSSION

Initial Whole-Lung Deposition:

Data from Studies I to IV were treated in the same manner to obtain initial whole-lung deposition. For the dogs, actual breathing patterns were obtained for the exposure period. For Syrian hamsters a minute volume of 100 cc was assumed, although this is probably high. A representative value for the quantity of radioactivity that was aerosolized was calculated from exposure time and the air concentration ($\mu\text{Ci}/\text{minutes}/\text{liter}$). Multiplying by the volume of air breathed gave the total amount of radioactivity breathed. The fraction deposited in the whole lung was obtained by dividing the radioactivity in the lung plus absorbed activity by the total breathed. In some cases ^{137}Cs entered blood and internal organs during or shortly post-exposure and this material has been assumed part of the initial lung deposit. All of the data, as mean values, are shown in Figure 1. Plotted also is a curve calculated for human deposition by the ICRP Task Group on Lung Dynamics.⁴ The particular tidal volume of 2150 cm^3 was chosen because it gave a curve that more closely fit the dog data. From Figure 1 it is obvious that the animal data follow the general pattern of the calculated curve. With only one dog at a point it is not possible to actually estimate the deposition pattern but the general shape is implied to be similar to the drawn curve. The hamster data are more extensive and do enable drawing the dashed line, but only in a manner to give a general shape as the human curve. Also of interest are the two points for the monodisperse and polydisperse aerosols at 1.5 and $1.9\text{ }\mu\text{m}$, respectively. Because of the large number of animals involved, these two points are statistically highly different from each other. The fact that they seem to fit the pattern, however, would imply that the aerodynamic median diameter of a polydisperse aerosol may be perfectly valid for predicting initial lung deposition. This is further borne out when the

Table 1
Experimental Design for In Vivo and In Vitro Studies with ^{137}Cs Labeled Fused Clay Particles

Study Designation	Nature of Aerosol	Particle* Size (μm)	Standard* Deviation σ_g	Type of Study	Subject	Number of Animals	Duration of Exposure (minutes)	Sacrifice Times (days)	Purposes of Study
I	Monodisperse	0.6	1.4	Inhalation & Injection	Hamster	80	120	0.25, 1, 2, 4, 8, 16, 24, 32, 40, 48, 56, 64	Initial deposition, <u>in vivo</u> solubility, whole-body and tissue retention.
		2.6	1.1			80			
II	Monodisperse	1.5	1.15	Inhalation	Hamster	80	20	0.1	Initial whole lung and inter-lobar distribution
	Polydisperse	1.9	1.46		Hamster	80	20		
III	Monodisperse	0.74	1.08	Inhalation & Injection	Hamster	40	30	0.1, 1, 2, 4, 8, 16, 24, 32, 40, 48, 56, 64, 72	Initial deposition, <u>in vivo</u> solubility, whole-body and tissue retention, effect of multiplets.
		0.74	1.15			40	30		
		0.80	1.12			40	30		
IV	Monodisperse	0.4	1.66	Inhalation	Dog	2	60	0.1, 4	Initial deposition, whole-body tissue retention.
		1.65	1.18			2	30		
		3.0	1.03			2	30		
V	Monodisperse	0.4	1.66	<u>In Vitro</u>	Serum	-	-	Measurements over 28 days	<u>In vitro</u> solubility characteristics
		1.65	1.18		Simulant				
		3.0	1.03						

* Activity Median Aerodynamic Diameter (AMAD) and geometric standard deviations (σ_g) measured with cascade impactors. These values are not often accurate for the smallest particles because the effective cutoff diameter of the last collection stage is very nearly the size of the particle.

present monodisperse dog data of Figure 1 are compared to similar polydisperse sizes (LF-41, p. 84) and when a similar comparison is made between the hamster data and other small animal deposition data collected in this laboratory.⁵

The data presented in Figure 1 are sparse but the trends are encouraging. With the monodisperse aerosols it now becomes imperative to carry out studies with additional sizes and to continue the comparison with results from polydisperse aerosols. This comparison is important for the practical field situation where heterogeneous aerosol distributions exist. If the mass median diameter of an industrial aerosol is a representative parameter for assessing initial deposition, then our experimental results are much easier to interpret for this first step in hazards evaluation.

Initial Interlobar Deposition:

In estimating radiation dose to the lung from inhaled particles the total amount of radioactivity is generally assumed to be evenly spread throughout the total weight of the lung. One approach to making more sophisticated estimations of dose is to determine if there are significant differences in deposition patterns between lung lobes. Heretofore, such determinations have been made using polydisperse aerosols and the question always arose as to whether the differences in interlobar deposition were due to different portions of the inhaled aerosol distribution being selectively deposited in regions of the respiratory tract as a result of differences in airway branching and size. In this study of interlobar distribution, monodisperse and polydisperse particles were inhaled by hamsters (Study II), the animals sacrificed shortly post-exposure and each lobe separated carefully for ^{137}Cs analysis. Results of the study are shown in Tables 2 and 3 for the total lobe content and the lobar concentration, respectively.

Table 2 shows conclusively that there is no real difference in total lobar content between males and females or between monodisperse and polydisperse aerosols. There is a definite difference between lobes however, with the total right lung receiving 66% of the total deposited. Among the right lobes the right diaphragmatic is seen to contain at least twice as much as any of the other lobes. More meaningful from the radiation dosage viewpoint are the data expressed on a weight basis in Table 3. There is a systematic difference between the males and females for each lobe and for total lung as seen in Part A. This was a curiosity that led to an analysis of the lung weights between males and females. These are shown in Table 4 for all animals in each study. It is obvious that the total lungs of female hamsters are a considerably larger fraction of the body weight. It was thought that these differences may be due to the difference in sex organ weights and it was found that the testes averaged about 6.5% of the body weight and the female organs about 0.8%. This was not enough difference to compensate for those seen in Table 4, however it did bring the relative weights closer together. Apparently there is a real sex difference in hamster lung weights.

Figure 1. Deposition of inhaled ^{137}Cs -labeled fused clay particles in the lung of Beagle dogs and Syrian hamsters.

Table 2
Lung Lobe Content of ^{137}Cs as Mean Total Lung Content $\pm$ 1 Standard Error*
Lobar Content as Percent Total Lung

	<u>Right Apical</u>	<u>Right Cardiac</u>	<u>Right Diaphragmatic</u>	<u>Right Intermediate</u>	<u>Left</u>
Monodisperse					
Males	13.9 $\pm$ 0.5	12.5 $\pm$ 0.6	27.4 $\pm$ 0.9	12.8 $\pm$ 0.6	33.5 $\pm$ 1.3
Females	13.4 $\pm$ 0.6	12.3 $\pm$ 0.5	27.5 $\pm$ 1.2	11.9 $\pm$ 0.4	35.0 $\pm$ 1.6
Total	13.7 $\pm$ 0.4	12.4 $\pm$ 0.4	27.4 $\pm$ 0.7	12.4 $\pm$ 0.4	34.0 $\pm$ 1.0
Polydisperse					
Males	13.6 $\pm$ 0.7	11.9 $\pm$ 0.6	29.0 $\pm$ 1.5	11.5 $\pm$ 0.7	34.1 $\pm$ 1.6
Females	13.7 $\pm$ 0.7	12.1 $\pm$ 1.0	28.2 $\pm$ 1.8	11.7 $\pm$ 1.0	34.4 $\pm$ 1.6
Total	13.6 $\pm$ 0.5	11.9 $\pm$ 0.5	28.6 $\pm$ 1.1	11.5 $\pm$ 0.6	34.2 $\pm$ 1.1

* Standard Error of the Mean (SD/ $\sqrt{n}$)

Table 3
Absolute and Relative Lobar Concentrations of ^{137}Cs as Mean $\pm$ 1 Standard Error*

	<u>Right Apical</u>	<u>Right Cardiac</u>	<u>Right Diaphragmatic</u>	<u>Right Intermediate</u>	<u>Left</u>	<u>Total Lung</u>
<u>A. Absolute Lobar Concentration as Percent Total Lung Burden per Gram</u>						
Monodisperse						
Males	95.4 $\pm$ 3.9	74.0 $\pm$ 4.2	68.4 $\pm$ 2.8	86.5 $\pm$ 4.5	80.8 $\pm$ 4.1	77.6 $\pm$ 3.3
Females	87.4 $\pm$ 5.3	69.2 $\pm$ 4.6	61.8 $\pm$ 3.9	76.3 $\pm$ 4.3	70.7 $\pm$ 4.4	69.9 $\pm$ 4.0
Polydisperse						
Males	100.1 $\pm$ 5.4	70.7 $\pm$ 3.9	75.4 $\pm$ 3.9	86.5 $\pm$ 5.2	80.5 $\pm$ 4.0	78.8 $\pm$ 3.9
Females	77.4 $\pm$ 5.9	62.2 $\pm$ 5.7	62.6 $\pm$ 5.0	66.5 $\pm$ 5.0	64.5 $\pm$ 3.5	64.1 $\pm$ 4.3
<u>B. Lobar Concentration as Percent Total Lung Concentration</u>						
Monodisperse						
Males	1.23	0.95	0.88	1.11	1.04	1.00
Females	1.24	0.99	0.88	1.09	1.01	1.00
Polydisperse						
Males	1.27	0.90	0.96	1.10	1.02	1.00
Females	1.21	0.97	0.98	1.04	1.01	1.00

* Standard error of the mean (SD/ $\sqrt{n}$)

Table 4
Mean Wet Weights of Individual Syrian Hamster Lung Lobes and Total Lung
in Grams. Total Lung also Expressed as Percent Body Weight

	<u>Right Apical</u>	<u>Right Cardiac</u>	<u>Right Diaphragmatic</u>	<u>Right Intermediate</u>	<u>Left</u>	<u>Total Lung</u>	<u>Percent Body Weight</u>
Monodisperse							
Males	0.15	0.18	0.41	0.15	0.43	1.32	1.02
Females	0.16	0.19	0.47	0.16	0.53	1.50	1.25
Polydisperse							
Males	0.14	0.17	0.39	0.14	0.43	1.27	0.99
Females	0.18	0.21	0.50	0.17	0.55	1.61	1.38

The data in Table 3A were converted to fractions of the total lung concentration for each lobe, as shown in Part B. This emphasizes the greater concentration in the right apical lobe and the lowest in the right diaphragmatic. This method of presentation also emphasizes the similarities between the deposition of polydisperse and monodisperse aerosols. At least for the Syrian hamster, the difference in lobar distribution of inhaled particles does not appear to be a function of action of anatomical structure on a given segment of the inhaled aerosol particle distribution. The finding of a greater relative concentration of activity in the apical lobe is of special interest because this lobe frequently has more severe lesions than other lobes in the dog. Studies similar to that reported here for the Syrian hamster should be conducted in the Beagle dog to verify the applicability of the hamster findings to other species.

Whole-Body Retention:

A comparison of whole-body retention data for two different size monodisperse aerosol is shown in Figure 2 for Syrian hamsters. The data, expressed as percent of initial body burden, show a difference in initial clearance between the two sizes. The larger particles are cleared to a greater extent during the first day with the relative difference maintained thereafter. On day one the absolute difference is ~ 25% (65% vs 40%) and at 99 days it is ~ 5% (11.5% vs 6.5%). The relative fractions of the smaller particle size body burdens, however, are 0.38 (25% ÷ 65%) and 0.43 (5% ÷ 11.5%) at these times, indicating a similar clearance pattern. Whole-body data very similar to those in Figure 2 were observed for the hamsters described in Study III (Table 1). The "long-term" effective half-lives in Figure 2 were estimated to be about 65 days; the half-life estimated in Study III was about 45 days but the experiment has only been followed for 60 days. Study III is being done primarily to see if differences in biological behavior will be apparent as the number of multiplet particles increases. At this point no differences have been seen in whole-body retention characteristics but it may be too early in the study.

Figure 2. Retention of inhaled monodisperse ^{137}Cs fused clay particles in Syrian hamsters. Data expressed as percent of initial body burden.

Whole-body retention in the Beagle dog over the four days of Study IV are shown in Figure 3 in disintegrations per minute. As with the hamsters in Figure 2, the larger particle size left the body to a greater extent than with the 0.4 or 1.65 μm particles. The 1.65 μm particles were cleared somewhat faster than the smallest ones, but the difference is subtle. By four days the body burdens had reached ~ 23 , 72 and 78% of the initial values in going from larger to smaller sizes. The differences observed in Figures 2 and 3 were no doubt reflections of the upper respiratory tract deposition of the larger particles, with subsequent greater clearance via the feces during the early times post-exposure.

Tissue Distribution:

In Study IV with Beagle dogs, the tissues taken for analysis at necropsy were separated into four major groups. These corresponded to those comprising (a) the upper respiratory tract, (b) the lower respiratory tract, (c) those containing ^{137}Cs that was released from the fused clay matrix and absorbed, and (d) those representing external contamination. The decision as to which tissue belongs in each group was in part arbitrary but it enables a comparison between animals on a standard basis. The tissues and groupings are listed below:

(a) Upper Respiratory Tract	(b) Lower Respiratory Tract	(c) Absorbed Activity	(d) Possible External Contamination
Skull	Total Lung	Salivary Glands	Head Skin
Turbinates	Tracheobronchial	Testes or Ovaries	Nares
Trachea & Larynx	Lymph Nodes	Spleen	Paws and Tail
GI Tract	Mediastinal Tissue	Adrenal Glands	Pelt
		Kidneys	Miscellaneous (gauze sponges, plastic gloves, etc.)
		Liver	
		Heart	
		Bone	
		Soft Tissues (not specifically named)	
		Blood	

Table 5 lists the tissue levels of ^{137}Cs at 4 days post-exposure as percentages of initial body burden. The relative tissue distribution for all six dogs for the two groups of three dogs sacrificed at 0.1 and 4 days post-exposure is given in Table 6 as percent sacrifice body burden. Several differences were noted among the dogs sacrificed at 0.1 hour after exposure. First, the amount of external contamination associated with the dog exposed to the 3.0 μm aerosol is approximately

Figure 3. Whole-body retention in Beagle dogs of ^{137}Cs inhaled in monodisperse particles.

Table 5
 ^{137}Cs in Tissue and Excreta Samples for
Dogs Sacrificed at 4 Days after Inhalation
of Monodisperse Fused Clay Particles

Containing ^{137}Cs			
	Percent of Initial Body Burden		
Dog No.	<u>2</u>	<u>4</u>	<u>6</u>
AD μm	<u>0.4</u>	<u>1.65</u>	<u>3.0</u>
Upper Respiratory Tract	4.6	.76	1.2
Lower Respiratory Tract	19	65	14
Absorbed Activity	44	5.1	5.0
Possible External Activity	3.0	5.2	.88
Urine	11	1.6	1.6
Feces	<u>8.5</u>	<u>18</u>	<u>71</u>
% Recovery	90	96	94

Table 6
Relative Tissue Distribution of ^{137}Cs
Inhaled in Monodisperse Fused Clay Particles

	Percent Sacrifice Body Burden					
Sacrifice	0.1 Day			4 Days		
AMAD(μm)	<u>0.4</u>	<u>1.65</u>	<u>3.0</u>	<u>0.4</u>	<u>1.65</u>	<u>3.0</u>
Organ Groupings						
Upper Respiratory Tract	18	11	11	6.6	1.1	5.8
Lower Respiratory Tract	32	65	21	27	91	66
Absorbed Activity	38	12	11	63	7	24
Possible External Activity	12	12	57	4	0.7	4

five times higher than that seen for the other two dogs. This was primarily the result of high activity levels for head skin, nares and miscellaneous samples. Second, if only the internally deposited activity is considered, i.e., the initial body burden minus the external contamination, the lowest percentage of this activity was in tissues other than the respiratory tract in the dog exposed to the 1.65 μm aerosol. The percentage "absorbed" was twice as high in the dog exposed to the 3.0 μm aerosol and three times higher in the dog exposed to the 0.4 μm aerosol. These results may be influenced somewhat by the length of exposure time and the consequent delay in sacrifice for the 0.4 μm dog whose exposure lasted 60 minutes instead of the 30-minute periods used for the other two sizes. Regardless, it is notable that such a large amount of translocation of ^{137}Cs occurred in such a short period of time.

By 4 days post-exposure, the tissues potentially attributable to external contamination were much lower with most of the residual activity associated with the head skin and pelt samples. In the dog exposed to the 1.65 μm aerosol, most of the sacrifice body burden was found in the lung (92%) with approximately 7% in other tissues. This is within the same order of magnitude as seen with the polydisperse ^{137}Cs clay dogs at this time period (LF-41, pp. 105-110). In agreement with the early sacrifices, the two dogs exposed to the largest and smallest particle sizes, respectively, had larger relative amounts of ^{137}Cs and absorption into the systemic circulation seen in dogs exposed to the 1.65 μm particles. This may be the result of greater solubilization due to the increased surface area present for the 0.4 μm particles. Although the opposite might be expected in the case of the 3.0 μm particles, such was not the case. In these dogs, the increased absorption of ^{137}Cs from the respiratory tract may have resulted from a lesser degree of fusing than occurred for the 1.65 μm particles. Future expansions of this experiment should enhance our understanding of these particle-size-related effects and provide data for comparison with results from our study of the behavior in the Beagle dog of inhaled polydisperse clay particles containing ^{137}Cs which has now been in progress for over five years. Of special interest will be studies directed at determining the influence of differences in particle fusing and related differences in particle surface characteristics which may be reflected in altered biological behavior.

Date on the tissue distribution in the hamsters of Study I are shown in Table 7 for the two particle sizes. Initially there is evidence for considerable absorption of ^{137}Cs from the smaller particle size with a substantial portion of the ^{137}Cs in remainder (primarily the carcass including

Table 7
Distribution of Inhaled ^{137}Cs Fused Clay in Tissues of the Syrian Hamster
Data Presented as % Sacrifice Body Burden Per Organ

Day *	AMAD 0.6 μm				AMAD 2.6 μm			
	Lung	GI Tract	Pelt **	Remainder	Lung	GI Tract	Pelt **	Remainder
0	31	19	28	22	44	45	7.7	3.3
1					62	21	9.0	8.0
2	51	14	13	22				
4					82	9.8	4.3	3.9
8	75	2.3	8.7	14	90	4.4	3.4	2.2
16	85	1.0	11	3.0	93	2.3	1.4	3.3
24	84	1.7	8.3	6.0	95	1.7	.9	2.4
32	85	1.2	12	1.8	97	1.1	.9	1.0
40	81	.9	14	5.1	97	.9	.6	1.5
48	85	1.2	10	3.8	98	.7	.4	.9
56	84	1.2	12	2.8	98	.4	.5	1.1

* Sacrifice Day Post-Exposure

** Includes headskin

muscle which accumulates ^{137}Cs). As a function of the sacrifice body burden, this decreases fairly rapidly and has reached rather low levels by 16 days. Because the absorbed ^{137}Cs is retained with a shorter effective half-life than that in lung, the lung ^{137}Cs becomes relatively larger with increasing time post-exposure. It is interesting that this absorption occurs as readily as with the dogs. The pelt appears to gather a larger fraction of the sacrifice body burden at the smaller particle size but this is only a relative observance in that the GI tract contains so much more at the early times. At later times, however, there is more absorbed ^{137}Cs (remainder) with the smaller particles. This is attributed to the larger surface-to-mass ratio, as will be discussed in more detail later. The important particle size differences noted in the tissues from this study are the seemingly greater retention of the larger particles in the lung, due to relatively less dissolution.

These tissue distribution differences as a function of particle size may be extremely important in assessing long-term radiation effects from inhaled radionuclides. With cations other than cesium that may be held tenaciously by internal organs following absorption, the fraction absorbed could be very important in the selection of a critical organ for long-term dosage accumulation. Straightforwardly this says that the degree of radiobiological effect on an organ from a given radionuclide may very well be a function of particle size.

In Vivo Solubility and Lung Clearance:

The intramuscular injection of aerosol particles in suspension has proven a successful tool in establishing in vivo solubility characteristics. One of the unknowns in lung clearance of particles is the fraction that may leave by macrophage movement up the ciliary escalator or, in another expression, the fraction that leaves by dissolution and entrance to blood. There are ways of estimating these fractions by using the data from intramuscular injections. Such data were obtained in Studies I and III and those from Study I are shown in Figure 4. The injection data plotted near the top of the graph are expressed as percent of initial body (muscle) burden and the lung data from hamsters in the same study after inhalation are plotted as percent of initial lung burden. The lung burdens were obtained by applying sacrifice lung data to the whole-body curves of Figure 2. The

two sets of curves are shown as a comparison of lung clearance primarily by the two methods mentioned compared to that by solubilization alone following the intramuscular route. The interplay between these two may be described mathematically.

The exponential loss from the muscle may be described as:

$$-\frac{dM}{dt} = \lambda_m M \quad (1)$$

where M is the amount in the injection site at any time and λ_m is the rate constant of loss. The loss from lung by dissolution is therefore a function of λ_m . The clearance by ciliary action may be assumed to be exponential also and loss from the lung in this manner may be similarly expressed as

$$-\frac{dL}{dt} = \lambda_c L \quad (2)$$

where λ_c is the rate constant of loss by ciliary action. The obvious assumption has to be made that rate of clearance by either route is proportional to the entire lung content at any time, ignoring the fact that there are different pools that could be considered. Combining the equation for an expression of overall lung clearance gives

$$-\frac{dL}{dt} = \lambda_m L + \lambda_c L \quad (3)$$

Integration yields an expression for the fraction of initial lung burden remaining at any time:

$$\frac{L}{L_0} = e^{-(\lambda_m + \lambda_c)t} \quad (4)$$

It should be recognized that the clearance referred to and the initial lung burden is that of the long-term component. Early clearance, as observed for the first 20 days in the data shown in Figure 4, would be another matter and essentially described only by λ_c . From the intramuscular data of Figure 4 one can obtain rate constants of loss from each particle size, making the reasonable assumption that kinetics of loss from the whole-body are essentially the same as those from the injection site. With ^{137}Cs this is a good assumption due to the short biological half-life and the small rate of loss from injection site.

To use equation (4) the long-term half-life in lung is obtained, such as shown by the dashed lines in Figure 4. With L/L_0 being 0.5 and t being the half-life, substitution of λ_m from the injection data will yield a value for λ_c . This may be transformed into a half-life of clearance by ciliary activity. This in itself is not of great interest, but it may be used to obtain values for the fractions of lung burden being cleared by each mechanism.

Let F = fraction being cleared by solubility and $1 - F$ = fraction cleared by ciliary action. Then

$$F \lambda_m + (1 - F) \lambda_c = \lambda_t \quad (5)$$

where λ_t is the rate constant for total clearance. The value for λ_t may be obtained from the overall lung clearance components in Figure 4, and substituted in equation (5). Solution for F leads to the fractions being cleared by each mechanism.

Figure 4. Whole-body retention of intramuscularly injected ^{137}Ce fused clay and pulmonary retention of inhaled ^{137}Cs fused clay in Syrian hamsters.

Data obtained from Figure 4 may be used to illustrate the use of these equations and to indicate the kinds of values that are generated. The half-lives and rate constants obtained from the lines drawn by eye are as follows:

$$\begin{aligned}\lambda_m (0.6 \mu\text{m}) &= 0.0015 \text{ day}^{-1} \\ \lambda_m (2.6 \mu\text{m}) &= 0.00043 \text{ day}^{-1} \\ T_{1/2} (0.6 \mu\text{m}) &= 63 \text{ days} \\ T_{1/2} (2.6 \mu\text{m}) &= 64 \text{ days}\end{aligned}$$

By substitution into equation (4) the following values for λ_c were obtained:

$$\begin{aligned}\lambda_c (0.6 \mu\text{m}) &= 0.013 \text{ day}^{-1} \\ \lambda_c (2.6 \mu\text{m}) &= 0.011 \text{ day}^{-1}\end{aligned}$$

Substitution in equation (5) yields values for F of 0.14 (0.6 μm) and 0.04 (2.6 μm). Thus, about 14% of the clearance of the small particles is estimated to be by dissolution, and 4% for the larger particles. Conversely, macrophage removal would account for 86% and 96% of the removal for the small and large particles, respectively. These are probably reasonable estimates, inasmuch as the portion of the lung retention curves being observed over the 60 days really does not represent the long-term loss. Thus, it would be expected that macrophage removal would be the greatest mode of loss. It would also be expected that a greater fraction of the larger sized particles would leave via this route.

It is interesting, but not really surprising, how so many of the variables discussed fit together. The real diameters of the two particles used in Study I calculate to be 0.3 and 1.6 μm . Assuming that mass dissolved is proportional to surface area of the particle, one should be able to predict the difference in dissolution rates observed in Figure 3. The surface area to mass ratio is essentially the inverse of the diameter, or, $1/0.3 = 3.3$ and $1/6 = 0.63$. This would imply a greater rate of dissolution from the smaller particle of $3.3 \div 0.63 = 5.2$. From the rate constants used above, this appears to be a factor of 3.5 ($0.0015 \div 0.00043$). This discrepancy is not as great as it may appear when the length of the study (60 days) is considered in calculating half-lives of 450 and 1600 days ($\lambda_m = 0.0015$ and 0.00043).

Another interesting facet arises from the tissue data of Study I (Table 7). As mentioned, after 16 days the remainder values appeared to have reached equilibrium. The early surge of ^{137}Cs into the blood apparently was the result of a soluble fraction on the particles, a relatively larger fraction with smaller particles as would be expected if it were a surface phenomenon. A plot of the ^{137}Cs in the remainder sample showed that it left with a short half-life (5-10 days) as might be expected with cesium.⁶ When ratios of remainder samples to lungs are obtained at the later times in Table 7, say from 32 days on, mean values of 0.042 and 0.011 are calculated for the small and large particle sizes, respectively. This implies that the relative ^{137}Cs burden in the internal organs is $0.042 \div 0.011 = 3.8$ times greater with the smaller size. Bearing in mind the short half-life of retention of ionic cesium, one would expect equilibrium between amount absorbed by dissolution from lung and excretion in the urine to be reached soon and become relatively constant due to the large lung reservoir. On this basis it is therefore safe to assume that the ratio of 3.8 between particle sizes represents the relative rates of solubility. This value is between those obtained above (3.5 and 5.2).

In Vitro Solubility:

Filters from the inhalation exposure in Study IV were obtained for in vitro solubility studies. The studies were performed with the parallel flow system operated at 37°C and used serum simulant as a dissolving medium. Fractions were collected for short periods at early times (20 min)

and for much longer periods (8-16 hrs) at later times. The percent dissolving at various times is presented in Table 8 for each of the three particle sizes (as determined by analysis of cascade impactor sampling). As explained in Table 1, the small particle sizes reported from cascade impactor analyses are often not reliable. The data have been calculated as a fraction dissolved per day, F_D , for various times, and these have been plotted in Figure 5.

Table 8
Dissolution of Monodisperse
 ^{137}Cs -Fused Clay Particle With Time

AMAD (μm)	Percent Total Dissolution		
	1st Hour	1st Day	28 Days
0.4	15.9	60.3	63.7
1.65	1.4	3.6	6.0
3.0	12.1	17.6	25.8

Unusually high dissolution of both 0.4 μm and 3.0 μm particles was noted at the early times. In fact, during the first two days the rates of dissolution of 3.0 μm particles were higher than that of 1.65 μm particles. After the first two days the rates of dissolution decreased only slightly. Both the unusually high early dissolution of samples with AMAD of 3.0 μm and 0.4 μm and the decreasing rate of dissolution were unexpected. It is evident that these two ^{137}Cs fused clay samples were quite different from other fused clay samples. The results here are reminiscent of those obtained in the dogs (Table 6). Much more of the 0.4 and 3.0 μm particle ^{137}Cs was absorbed to the internal organs than was seen with the middle-sized particles. These *invitro* studies correlate quite well with the *in vivo* dog data, a very encouraging finding.

Following the 28 days of dissolution study and after 64% dissolution, the particles with 0.4 μm AMAD should have shown a 30% decrease in diameter. The diameters of these particles determined from electron micrographs showed only about 5% instead of the expected 30%. This would

Figure 5. Rates of dissolution of monodisperse ^{137}Cs fused clay particles shown as the fraction dissolved per day versus elapsed days since the beginning of the elution. The dissolution experiments were conducted in a parallel flow system with serum simulant at pH 7.3 and at 37°C as the solvent. The fraction dissolved per day was calculated from the ^{137}Cs activity eluted per day and the ^{137}Cs activity remaining in the filter sample during the day.

indicate that the high dissolution observed was not representative of the clay matrix but preferential higher elution of ^{137}Cs from the particle surface. It appears that a higher specific activity of ^{137}Cs must be occurring at the surface which would lend itself to a high elution rate. This *in vitro* finding suggest that the unusually high *in vivo* dissolutions of monodisperse ^{137}Cs -fused clay particles of 0.4 μm and 3.0 μm AMAD are due to the nature of the particles rather than biological causes.

In Vitro versus In Vivo Solubility:

There is a striking similarity between the *in vitro* and *in vivo* solubility characteristics of these ^{137}Cs -labeled monodisperse fused clay particles. Some of these similarities have already been stressed above. One other common similarity involves calculation of k , the constant of specific solubility described by Mercer in terms of the particle diameter and the half-life of dissolution.⁷ In the present work, k values were calculated from results of *in vivo* dissolution studies (intramuscular injections) from Studies I and III (Table 1), and the *in vitro* dissolution studies just described (Study V). The results of these calculations are given in Table 9. The values are very similar and indicate a trend toward slightly increasing with particle size. This may be an artifact and will be particularly looked for in future studies of this nature.

Table 9
Comparison of In Vivo and In Vitro Systems for Determining the
Constant of Solubility (k)

Particle* Diameter (AMAD μm)	Constant Of Specific Solubility (Grams/cm ² /Day)	System	Study
0.4	1.6×10^{-8}	In Vitro	V
0.6	1.6×10^{-8}	In Vivo	I
0.8	1.5×10^{-8}	In Vivo	III
1.65	2.1×10^{-8}	In Vitro	V
2.6	2.3×10^{-8}	In Vivo	I
3.0	3.1×10^{-8}	In Vitro	V

* Sized from cascade impactor analysis.

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EXPOSURE OF SYRIAN HAMSTERS TO ^{51}Cr LABELED AEROSOLS
OF $^{239}\text{PuO}_2$

ABSTRACT

Syrian hamsters were exposed to a ^{51}Cr labeled $^{239}\text{PuO}_2$ aerosol to determine the effectiveness of the gamma emitting tag as an aid in the determination of lung burden of $^{239}\text{PuO}_2$ during and soon after experimental exposures. Aerosol sampling during this exposure indicated a bimodal particle size distribution with about 25% of the ^{51}Cr activity associated with particles less than $0.5\text{ }\mu\text{m}$ AMAD and of unknown density. The remaining ^{51}Cr and all ^{239}Pu activity was associated with particles of $2.0\text{ }\mu\text{m}$ AMAD and density of 10.3 gm/cm^3 . Whole-body retention, initial tissue deposition, and lung retention of the labeled aerosol was consistent with the bimodal particle size distribution. In vitro solubility studies on the reclaimed aerosol particles used in the exposure confirmed the aerosol characterization and animal data regarding the nature of the aerosol. Results of this study indicated that a modification of the techniques and methods was necessary to insure complete incorporation of ^{51}Cr into the $^{239}\text{PuO}_2$ aerosol particles.

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INTRODUCTION

The use of a thoracic radioactivity monitoring system has proven of value in this laboratory during the exposure of Beagle dogs to aerosols of gamma-emitting radionuclides as a means of achieving desired lung burdens of radioactivity (see this report, pp. 114-118). Accurate assessment of the total initial lung burden of a radioactive material following such an exposure, and for periods of several days to weeks thereafter, is also of considerable importance in retention, distribution, excretion and dosimetric studies. Such measurements during and after exposure of Beagle dogs to ^{238}Pu , ^{239}Pu , ^{242}Cm and ^{244}Cm aerosols are not possible at present since the specialized counting system employed to detect low energy x-rays emitted by these radionuclides following internal deposition cannot be readily modified for this purpose.

To circumvent these difficulties, experiments were conducted to determine the feasibility of incorporating a gamma-emitting radionuclide into aerosols of $^{239}\text{PuO}_2$. The usefulness of the "tag" is entirely dependent upon its complete incorporation into $^{239}\text{PuO}_2$ particles and subsequent release at the same rate as the solubilization of the PuO_2 aerosol. The present report describes research to evaluate the usefulness of ^{51}Cr as the "tagging" radionuclide for exposure of Syrian hamsters to aerosols of $^{239}\text{PuO}_2$.

METHODS

The techniques employed in the production and collection of monodisperse particle size fractions of ^{51}Cr labeled $^{239}\text{PuO}_2$ aerosols are described elsewhere in this report (see pp. 21 - 28). For this study two segments of the Lovelace Aerosol Particle Separator (LAPS) production foil containing particles in the size range 1.8 to $2.2\text{ }\mu\text{m}$ count mean aerodynamic diameter were resuspended in 15 ml distilled water using ultrasound. The resuspension volume was reduced by centrifugation, discarding the supernatant, and reagitation with ultrasound in 6 ml distilled water. This solution was placed in the reservoir of a Lovelace nebulizer for generation of the aerosol used in the animal exposure.

The methods and apparatus used for exposure of small animals to radioactive aerosols have been described (1970-1971 Annual Report, LF-44, pp. 34-39). In the present study, 60 Syrian hamsters were exposed to a ^{51}Cr labeled $^{239}\text{PuO}_2$ aerosol over a 133-minute period. The duration of animal exposure coincided with complete aerosolization of the generator solution. Animals were removed from the apparatus immediately and placed in pint plastic containers for subsequent whole-body counting. Following counting, animals were placed in individual cages with food and water provided ad libitum. Four animals were sacrificed immediately after initial whole-body counts and at each sacrifice time thereafter; 7, 14, 21, 28, 35 and 42 days post-exposure. The following tissues were obtained by dissection on each animal; head, head skin, trachea, lung, gastrointestinal tract and carcass.

Whole-body counting and tissue counting for ^{51}Cr was accomplished using a pair of opposed 4" x 8" diameter NaI (Tl) crystals coupled to a multi-channel analyzer. Crystal separation was 5.75 inches and counting for ^{51}Cr accomplished by integration over the 320 KeV photopeak area. ^{239}Pu was extracted from the ashed tissue samples using di-2-ethylhexylphosphoric acid and the extracted fraction counted in a liquid scintillation counting system. Data from these counting systems allowed comparison of the ^{51}Cr to ^{239}Pu activity ratio in all tissue samples by separate means for comparison to the ^{51}Cr to ^{239}Pu ratio determined for the initial aerosol generator solution and the resultant aerosol. A ZnS alpha scintillation counting system was employed to determine ^{239}Pu activity in certain samples.

The aerosol produced in the animal exposure was characterized by multiple cascade impactor, small filter, and electrostatic precipitator samples taken at various positions in the exposure apparatus. Further documentation of aerosol characteristics was obtained in two correlary studies. The aerosol particles used in these latter studies were reclaimed from the exposure apparatus back-up filters following the run. Details of the aerosol characterization during the exposure and the in vitro solubility study of the reclaimed particles are given in this report (pp. 21 - 28). The in vivo solubility of the particles was followed as part of a larger study also described in this report (pp. 55 - 60).

RESULTS AND DISCUSSION

Details of aerosol characterization for this animal exposure are presented in this report (pp. 21 - 28). The data regarding the relative concentration of ^{51}Cr and ^{239}Pu in the LAPS foil segments resuspension and generator solution preparations indicated no differential solubilization of the two radionuclides during these procedures. The impactor data showed the presence of a bimodal particle size distribution with about 25% of the total ^{51}Cr activity associated with particles smaller than 0.5 μm AMAD while 75% of the ^{51}Cr and all the ^{239}Pu was associated with particles of 2.0 μm AMAD and a density of 10.3. Electron micrographs of the aerosol confirmed the presence of numerous small particles. Filter samples of the animal exposure aerosol displayed a ^{51}Cr to ^{239}Pu activity ratio lower than was present in the generating solution.

The extended exposure run time (133 minutes) was the apparent result of generator malfunction. Probably due to the extended period spent in the exposure tubes, animal survival through the exposure was unusually low; only 47% (28 of 60) of the animals survived through the period of exposure and initial whole-body counting. Initial body burdens (IBB) of ^{51}Cr were determined on the 33 animals surviving the exposure. The mean IBB for these animals was 46 nCi ^{51}Cr with a range of 26 to 63 nCi. Whole-body retention of ^{51}Cr was followed on the four animals scheduled for sacrifice at 42 days post-injection. The mean retention and range of these data are shown in Figure 1. The mean retention data points for day 14 through day 42 were fitted by least squares analysis to yield an effective long-term retention half-time of 28 days. Considering the data scatter, it must

be concluded that the effective long-term retention of ^{51}Cr following this aerosol exposure was not different from the physical half-life of 27.8 days. By successive subtraction techniques, the short-term rapid elimination of nearly 85% of the initial body burden was found to have a half-time of 2.5 days. The data indicate that for periods post-exposure greater than about 14 days the ^{51}Cr label must be associated with the insoluble $^{239}\text{PuO}_2$ particles.

Tissue distributions of ^{51}Cr and ^{239}Pu expressed as a percent of the total tissue burden for nine animals which were sacrificed or died during initial whole-body counting are shown in Table 1. For ^{51}Cr , the total tissue activity obtained by summation is virtually identical to the total burden measured by whole-body counting. These data are notable in the dissimilar fractions of ^{51}Cr and ^{239}Pu found in the tissues and the resultant variation in the $^{51}\text{Cr}/^{239}\text{Pu}$ ratio

found in the several tissues. This variation represents additional confirmation that two aerosol components were present in the exposure apparatus. The tissue data further confirm that a sizeable fraction of the aerosol was composed of quite small particles containing only ^{51}Cr . For example, in the lung enhanced deposition of small particles would lead to an increase in the $^{51}\text{Cr}/^{239}\text{Pu}$ ratio as seen in Table 1. Similar reasoning regarding the relative effect of impaction, sedimentation and diffusion of two different size fractions of aerosol on initial deposition can account for the variety of $^{51}\text{Cr}/^{239}\text{Pu}$ ratios noted in Table 2.

By the seventh day post-exposure and for the remaining experimental period, lung was the single tissue containing statistically significant quantities of ^{51}Cr and ^{239}Pu . In order to follow the retention of these radionuclides in the lung, initial lung burdens of ^{51}Cr and ^{239}Pu were calculated for each animal in the experiment, based on the initial whole-body burden of ^{51}Cr . Thus, the initial lung burdens of ^{51}Cr and ^{239}Pu as a percent of the initial ^{51}Cr whole-body burden were 23 and

Figure 1. Whole-body retention of ^{51}Cr labeled $^{239}\text{PuO}_2$ aerosol in Syrian hamsters.

Table 1
Tissue Distribution of ^{51}Cr and ^{239}Pu Expressed as a Percent of the Total Tissues
for Nine Animals Which Were Sacrificed or Died During Initial Whole Body Counting
(Mean $\pm$ S. D.)

Tissue	^{51}Cr	^{239}Pu	$\frac{^{51}\text{Cr}}{^{239}\text{Pu}}$
Lung	23.3 $\pm$ 2.9	12.3 $\pm$ 2.7	5.88
GI Tract and Contents	31.1 $\pm$ 15.3	42.5 $\pm$ 15.9	2.27
Head Skin	21.5 $\pm$ 7.9	28.8 $\pm$ 8.3	2.32
Head	6.3 $\pm$ 10.5	4.5 $\pm$ 10.6	4.35
Trachea	2.6 $\pm$ 4.2	2.0 $\pm$ 4.6	1.79
Carcass	15.1 $\pm$ 4.9	12.0 $\pm$ 5.3	3.91
Total Tissue Burden (nCi)	52.7 $\pm$ 9.4	17.0 $\pm$ 3.7	3.10
Burden by Whole Body Count (nCi)	53.1 $\pm$ 11.5		
Aerosol Sampled During Run			3.54

Table 2
Computer Half-Times of Retention of ^{51}Cr and ^{239}Pu in the Syrian Hamster Lung

	λ	T_e	T_e Range ($\lambda + \text{S. E.}$)	T_p	T_b
^{51}Cr	0.0358	19.3 day	18.5 - 20.4	27.8 day	63.1 day
^{51}Cr Decay Corr.	0.0123	56.3 day	48.1 - 67.9	-	56.3
^{239}Pu	0.0137	50.5	34.1 - 97.6	-	50.5

3.9%, respectively. These data are presented in Figure 2. The curves drawn through each set of datum points represents the fitted single exponential equation which best describes the data. The effective and biological half-times calculated from the equations are listed in Table 2. These data indicate that for the period from 7 through 42 days post-exposure the ^{51}Cr and ^{239}Pu are leaving the lung at a statistically indistinguishable rate. In contrast, the percent initial lung burden intercepts predicted for the ^{239}Pu and ^{51}Cr lung retention equations were different. Slightly over 100% of the initial lung burden of ^{239}Pu could be accounted for by the retention equation whereas only 78 to 80% of the initial lung burden of ^{51}Cr could be accounted for by the ^{51}Cr retention equations. These data indicate that a negligible quantity of ^{239}Pu was eliminated from the hamster lung from day 0 to day 7 in contrast to approximately 20% of the initial ^{51}Cr lung burden being eliminated during the same period post-exposure.

The quantity of ^{51}Cr and ^{239}Pu present in the lung at each sacrifice date is shown in Table 3 along with the $^{51}\text{Cr}/^{239}\text{Pu}$ ratio for the lung. If the assumption is made that ^{51}Cr and ^{239}Pu are contained within the same particles at day zero and if the two radionuclides are leaving the lung with the same biological half-time, then the $^{51}\text{Cr}/^{239}\text{Pu}$ ratio obtained at zero day corrected for ^{51}Cr decay to each sacrifice time should agree with the values obtained from the lung counting. As can be seen in Table 3 the tissue ratio observed is lower at every sacrifice date than is the ratio based on zero day sacrifice and corrected for decay to each sacrifice date. In contrast, when the day zero lung burden of ^{51}Cr is reduced by 20%, corresponding to the burden predicted by the retention of ^{51}Cr from day 7 through day 42, the calculated ^{51}Cr to ^{239}Pu ratios compares closely with the observed lung ratios. These data indicate that more ^{51}Cr than ^{239}Pu is leaving the lung at early times post-exposure. This early loss of ^{51}Cr from the lung would be compatible with the presence of numerous small particles of chromium in the exposure aerosol being rapidly solubilized *in vivo*.

Figure 2. Lung burden of ^{51}Cr and ^{239}Pu , measured separately, as a percent of the calculated initial lung burden.
 ^{51}Cr ILB = $0.233 \times \text{IBB of } ^{51}\text{Cr}$
 ^{239}Pu ILB = $0.039 \times \text{IBB of } ^{51}\text{Cr}$

Table 3
Summary of ^{51}Cr and ^{239}Pu Content in the Lung

Days P. E.	^{51}Cr (nCi)	^{239}Pu (nCi)	$\frac{^{51}\text{Cr}}{^{239}\text{Pu}}$	$\frac{^{51}\text{Cr}}{^{239}\text{Pu}}$	$\frac{^{51}\text{Cr}}{^{239}\text{Pu}}$
				Decay Corrected From Day Zero	Day 0 ^{51}Cr Reduced by 20%
0	12.28	2.09	5.88	5.88	4.70
7	6.63	1.74	3.81	4.94	3.95
14	4.74	1.50	3.16	4.15	3.32
21	3.90	1.19	3.28	3.48	2.78
28	3.61	1.91	1.89	2.92	2.34
35	1.97	0.96	2.05	2.46	1.96
42	1.86	1.02	1.82	2.06	1.65

The in vivo solubility study conducted on reclaimed aerosol particles from the animal exposure run supported the whole body and lung retention results of this experiment. The injected particles had a lower ^{51}Cr to ^{239}Pu ratio than that of the aerosol due to rapid ^{51}Cr solubilization during the resuspension and centrifugation operations. After injection of these particles the ^{51}Cr to ^{239}Pu ratio did not change indicating no differential solubility in the animal.

The in vitro solubility of the reclaimed aerosol also showed a rapid initial solubilization of ^{51}Cr , about 10.3% during the first 3 days, followed by a declining solubilization from day 4 through day 20, after which the ^{51}Cr activity in the daily samples was at the lead of detection and therefore not measurable. During this same period only a minute fraction, 0.02%, of the ^{239}Pu activity was found in the daily samples.

The cause of this differential solubility of ^{51}Cr relative to ^{239}Pu for short periods may be related directly to the above noted nature of the aerosol, i. e., a sizeable portion of the total aerosol was of very small particle size containing only ^{51}Cr whereas the ^{239}Pu and the remaining ^{51}Cr were contained in larger particles. The small ^{51}Cr particles were solubilized with a half-time of about 5 days versus the ^{51}Cr - ^{239}Pu particles being solubilized at a very slow rate.

CONCLUSIONS

The generation of ^{51}Cr labeled $^{239}\text{PuO}_2$ particles using the techniques described in this report (pp. 21 - 28) resulted in the formation of an aerosol which had a bimodal particle size distribution. Approximately 75% of the total ^{51}Cr and virtually all of the ^{239}Pu activity was associated with particles having an AMAD of 2.0 μm and density equal to 10.8 gm/cm^3 . Nearly 25% of the ^{51}Cr activity was associated with particles having an AMAD smaller than 0.5 μm , and of unknown density.

Nearly 85% of the ^{51}Cr initial body burden was rapidly eliminated with a half-time of 2.5 days. From day 14 through day 42 post-exposure, the whole-body retention half-time was not distinguishable from the physical half-life of ^{51}Cr . Initial deposition of the aerosol in various tissues was quite variable with respect to ^{51}Cr to ^{239}Pu ratios. For a period of about seven days post-exposure, more ^{51}Cr was eliminated from the lung than was ^{239}Pu . However, from day 14 through day 42 post-exposure the ^{51}Cr : ^{239}Pu ratio in the lung indicated no differential loss and a biological half-time of about 50 days. These animal data are all consistent with the observed bimodal particle size distribution of the aerosol.

The in vitro solubility studies on the reclaimed aerosol confirmed the existence of a fraction of the total aerosol which was rapidly solubilized in serum simulant. The short period of data collection on the solubility studies negates making accurate predictions on the long-term solubility of this aerosol.

The results of these studies indicate that the present procedures and techniques for producing ^{51}Cr labeled $^{239}\text{PuO}_2$ aerosols are not satisfactory to allow estimates of lung burden of $^{239}\text{PuO}_2$ during and soon after exposure of animals. However, for periods post-exposure greater than about 14 days, the ^{51}Cr and $^{239}\text{PuO}_2$ seem to be closely associated.

IN VIVO SOLUBILITY OF INTRAMUSCULARLY INJECTED
 ^{51}Cr - ^{239}Pu OXIDE PARTICLES

ABSTRACT

A $^{239}\text{PuO}_2$ aerosol was labeled with ^{51}Cr to enable *in vivo* measurements of initial deposition and early retention following inhalation exposure. A collection foil from a Lovelace Aerosol Particle Separator run was used as the source material for three suspensions of monodisperse ^{51}Cr -labeled particles administered by intramuscular injection into the hind leg of Syrian hamsters and rats. Two animals from each species and three dummies were injected at each particle size (median diameters of ~ 1.0 , 0.4 , and $0.1 \mu\text{m}$). A suspension was also made from the back-up filter from an inhalation run with a median diameter of $\sim 0.5 \mu\text{m}$, and a portion of this was injected as with the other suspensions. Animals and dummies were periodically whole-body counted for ^{51}Cr ; all animals were sacrificed on day 41. Tissue analyses were done for ^{51}Cr and for ^{239}Pu . The ratio of radionuclides in the injection site was compared to that in the dummies and although it varied between particle size groups it was consistent within groups. The results indicate that ^{51}Cr is a good label for $^{239}\text{PuO}_2$, as long as certain precautions are taken in delivery of aerosol to the animal.

PRINCIPAL INVESTIGATOR

R.G. Thomas

INTRODUCTION

The low energy x-rays from ^{239}Pu deposited in the body are not often conveniently or accurately detectable. This is particularly true when initial deposits in the lung are low and measurements are desirable during inhalation exposure. With relatively strong gamma emitters, the buildup of deposited material in the thorax can be detected externally and the exposure stopped when the desired dose is acquired. To accomplish this with the 17 KeV x-ray from ^{239}Pu would require an elaborate setup.

It was felt that plutonium oxide aerosols might be successfully labeled with a prominent gamma emitter so that at least initial deposition could be determined. If the label remained with the plutonium particles, data on retention in the lung could also be followed with time. An aerosol of $^{239}\text{PuO}_2$ containing small amounts of ^{51}Cr was made and monodisperse particles of various sizes were obtained. Suspensions of these were injected intramuscularly into rats and Syrian hamsters to determine if the ratio of the two radionuclides changed with time due to differential *in vivo* solubility characteristics.

METHODS

Aerosols of ^{51}Cr labeled $^{239}\text{PuO}_2$ aerosols were prepared as described elsewhere in this report (pp. 21 - 28). The particles collected on three segments of the Lovelace Aerosol Particle Separator (LAPS) foil were resuspended in distilled water by ultrasonification. The particle sizes represented in the three suspensions were widely separated (Table 1). Another suspension was prepared from a back-up filter obtained from an inhalation exposure with Syrian hamsters (see this report, pp. 50-54). All suspension characteristics are shown in Table 1.

Table 1
Suspension Characteristics for the ^{51}Cr - $^{239}\text{PuO}_2$ Particles
Injected Intramuscularly Into Syrian Hamsters and Rats

Source	^{239}Pu Concentration	^{51}Cr Concentration*	Median** Diameter
Foil Segment #5	2.1 $\mu\text{Ci/ml}$	8.4 MCi/ml	1.10 μm
Foil Segment #16	11.1 $\mu\text{Ci/ml}$	65.5 $\mu\text{Ci/ml}$	0.36 μm
Foil Segment #25	3.2 $\mu\text{Ci/ml}$	8.0 $\mu\text{Ci/ml}$	0.145 μm
Backup Filter Segments 13-14	1.2 $\mu\text{Ci/ml}$	2.2 $\mu\text{Ci/ml}$	0.52 μm

* Based upon average ^{51}Cr to ^{239}Pu dummy ratios (Table 5 in text)

** Density of 10 grams cm^3 used for calculation.

Two rats and two Syrian hamsters were injected intramuscularly in the hind leg with 0.05 to 0.15 cc of each suspension, depending on the amount of injection solution available. A similar quantity was placed into three counting vials at each particle size. Whole-body counting of the ^{51}Cr was performed at intervals on deep-well liquid scintillation counters; the dummy injections were assigned animal identification numbers and carried that way throughout the study. All animals were sacrificed at 41 days post-exposure and limited dissections were performed. Care was taken to separate the injection site muscle and the bone from that area. All tissue samples were counted for their ^{51}Cr content and ^{239}Pu analyses were done on these and on the dummy injections.

RESULTS

Whole-body ^{51}Cr counting data (including dummies) at first appeared to be from the same population. All data from each counting day were combined, expressed as percent of initial body (or dummy) burden (Table 2). The standard deviations for the combined samples appear to be quite small. An analysis of the data indicated that a slight trend between groups might prevail. Each group (suspension) was treated as an individual experiment with the hamster data and rat data being recalculated as the means, and expressed in terms of the mean dummy values for each group (Table 3). To determine trends in retention with time, the Syrian hamster and rat data in each size group were then combined and the data fitted to least squares analysis. The equation constants obtained from single exponential kinetics are given in Table 4. In analyzing these data, the measurements were made to only 40 days and the meaningfulness of half-lives of several hundreds of days is questionable at best.

The ^{51}Cr tissue analyses indicated very few samples other than injection site to contain quantities significantly different from background (at two standard deviations from the mean). There were significant quantities of ^{239}Pu found in a few other tissues. The data from ^{51}Cr and ^{239}Pu analyses of injection sites and dummies are presented in Table 5. There are obvious differences between groups, not only along the foil (geometric particle diameters of about 1.1, 0.36 and 0.145 μm) but also between that material suspended from the backup filter (0.52 μm) and that from close approximation on the foil (0.36 μm).

Tissue data of interest from the ^{239}Pu analyses were primarily from the carcass samples and the liver. The carcass ^{239}Pu was assumed to be primarily from skeletally-deposited material. Data on these samples are shown in Table 6, as percent injection site activity per total sample and per gram. This method of presentation was considered the most logical for a standardized presentation.

Table 2

Overall Means and Standard Deviation for all
Animals and Dummies at the Designated Counting
Times Post-Injection. Data are Expressed as
Percent of Initial (day zero) Values

<u>Day</u>	<u>$\bar{x}$</u>	<u>S. D.</u>
1	96.2	1.94
3	89.4	2.38
6	84.8	1.79
13	70.1	1.57
20	57.2	2.08
25	50.1	1.81
27	48.6	1.17
32	41.3	1.36
34	40.1	1.39
40	34.3	1.34

Table 3

Whole-Body Counting Data on Syrian Hamsters and Rats Injected With
 $^{51}\text{Cr}-^{239}\text{PuO}_2$ of Four Different Monodispersed Particle Sizes. Data are Expressed
as Percent of the Average Dummy Values for Each Group at Each Counting Time.
(This in essence places data back to zero time by correcting for physical decay.)

<u>Day</u>	<u>Foil</u> <u>1.1 μm</u>		<u>Foil</u> <u>0.36 μm</u>		<u>Foil</u> <u>0.145 μm</u>		<u>Filter</u> <u>0.52 μm</u>	
	<u>Hamster</u>	<u>Rat</u>	<u>Hamster</u>	<u>Rat</u>	<u>Hamster</u>	<u>Rat</u>	<u>Hamster</u>	<u>Rat</u>
1	98.1	98.6	99.1	99.5	96.8	102.3	97.8	98.0
3	102.7	103.8	97.2	99.4	98.7	101.7	99.5	93.3
6	100.0	100.3	97.2	96.5	96.9	99.5	98.3	95.1
13	100.5	97.0	98.4	98.0	97.0	96.1	99.5	95.6
20	97.9	97.2	93.4	92.1	94.6	103.8	100.0	96.2
25	100.9	98.0	96.1	93.8	95.1	98.0	93.0	91.4
27	100.6	99.3	96.5	95.9	97.2	97.6	95.9	93.2
32	98.5	99.0	95.6	93.1	97.8	96.4	97.0	97.0
34	100.0	96.0	98.0	96.3	90.6	92.6	95.8	92.7
40	100.8	95.9	94.1	95.8	95.9	98.8	97.1	96.0

Table 4

Equation Constants From Least Squares
Analyses of the Data From Table 3, With All
Animals Within a Size Group Combined.
A Single Exponential Rate of Loss From the
Injection Site was Assumed ($A = Be^{-\lambda t}$)

<u>Group</u>	<u>B (%)</u>	<u>λ (Day⁻¹)</u>	<u>T_{1/2} (Days)</u>
1.10 μm	100	0.00038	1800
0.36 μm	99	0.00122	570
0.145 μm	100	0.00138	500
0.52 μm	98	0.00090	770

Table 5
Content of ^{51}Cr and ^{239}Pu in the Injection Site and Dummies Expressed
in nCi. Sacrifices Were all at 41 Days Post-Intramuscular Injection ^{51}Cr .
Data Were Corrected to Zero Time (6 Sept. 1972)

Experiment Designation	Type Of Subject	Quantity Of ^{51}Cr (nCi)	Quantity Of ^{239}Pu (nCi)	Ratio $^{51}\text{Cr} : ^{239}\text{Pu}$	Ratio On Foil Prior To Using
1.1 μm - 01	Dummy	155	39	4.0	4.11
	02 Hamster	393	82	4.8	
	03 Hamster	427	85	5.0	
	04 Dummy	118	26	4.5	
	05 Rat	339	80	4.2	
	06 Rat	340	80	4.3	
	07 Dummy	343	90	3.8	
0.36 μm - 01	Dummy	851	194	4.4	3.59
	02 Hamster	783	153	5.1	
	03 Hamster	1000	255	3.9	
	04 Dummy	454	76	5.9	
	05 Rat	523	119	4.4	
	06 Rat	757	131	5.7	
	07 Dummy	643	83	7.8	
0.145 μm - 01	Dummy	53	23	2.4	3.23
	02 Hamster	98	26	3.8	
	03 Hamster	93	26	3.6	
	04 Dummy	111	42	2.7	
	05 Rat	97	36	2.7	
	06 Rat	108	39	2.8	
	07 Dummy	112	42	2.7	
0.52 μm - 01	Dummy	6	2	3.1*	3.74
	02 Hamster	83	47	1.7	
	03 Hamster	100	53	1.8	
	04 Dummy	35	27	1.3	
	05 Rat	109	61	1.7	
	06 Rat	96	50	1.9	
	07 Dummy	35	26	1.3	

* Sample was partially spilled during analysis.

The calculation of k was based upon ^{51}Cr data and hence, upon the assumption that the ^{51}Cr and ^{239}Pu remained intimately intact. The data in Table 5 would not prove this point, but certainly lend considerable support. For each particle size group the radioactivity remaining at the injection site appeared to bear the same relationship as that in the dummies. The ratios of ^{51}Cr to ^{239}Pu were quite consistent within each group. The fact that the ratios between groups varied considerably, particularly between those particles taken from the foil and those from the filter, is of concern. As indicated in Table 1, the $0.36\text{ }\mu\text{m}$ diameter particles came directly from segment No. 16 and those from the filter came from segments 13 and 14 combined. The ^{51}Cr to ^{239}Pu ratios from these two sources should have been somewhat comparable, according to Table 5. The basic difference before intramuscular injection into the animals, was that the particles suspended from segments No. 13 and 14 for the inhalation study were passed through an additional heating column at 1100°C before reaching the animals and the backup filter. Apparently some of the ^{51}Cr "boiled" off the particles, leaving a lower ^{51}Cr to ^{239}Pu ratio in the aerosol (breathed by the animals) and collected on the filters. However, it would appear that a sizeable fraction of the ^{51}Cr was in some free form which was apparently soluble. In the process of resuspending particles from the filter after ultrasonification, the suspension was centrifuged and the residue was resuspended for intramuscular injection. The free ^{51}Cr at this point went with the supernatant leaving the low ^{51}Cr to ^{239}Pu ratios in the particles. When corrected for the ^{51}Cr found in the supernatant the ratio was increased from an average of 1.8 to 2.6, much more in agreement with the backup filter data.

A similar argument may be used for the ratio variations along the foil. Although the ratios at $0.36\text{ }\mu\text{m}$ appear somewhat higher than at $1.1\text{ }\mu\text{m}$ (means of 4.2 and 5.2) it is doubtful if the difference is real with the small number of datum points. However, the mean ratio of 2.8 at the $0.145\text{ }\mu\text{m}$ particle diameter is a definite discrepancy. In obtaining the ^{51}Cr and ^{239}Pu on the foil of the LAPS, the generated suspension of droplets was first passed through a heating column. Apparently a similar high temperature effect was seen in this case as in the inhalation exposures. All particles in the polydisperse aerosol that entered the LAPS probably lost ^{51}Cr ; it appears however, that the smaller particles such as in the $0.1\text{ }\mu\text{m}$ range lost relatively more. This could be made to make sense through speculations based upon the shorter diffusion path for ^{51}Cr in the smaller particles. Although this is speculation, it seems to be a feasible explanation for the differences seen.

The data in Table 6 quite clearly show a preferential skeletal uptake of ^{239}Pu by rats over Syrian hamsters. The assumption has been made that ^{239}Pu in carcass was representative of ^{239}Pu in bone. With the one exception (animal $0.36\text{ }\mu\text{m} - 06$), the rat carcasses appear to concentrate ^{239}Pu by a factor of at least 10 over the Syrian hamster. Although the data are quite variable, there does not appear to be a real difference in liver concentrations between species (syrian hamster mean = $7.5\%/g$; rat mean = $7.8\%/g$ or $5.1\%/g$, omitting $0.36\text{ }\mu\text{m} - 06$). The ^{239}Pu appears to be selectively taken up by rat skeleton; in studies with ^{252}Cf in this laboratory the hamster was observed to selectively take up this radionuclide in the liver, with skeletal contents being about the same. This is a very interesting species difference between these two transuranic elements.

In conclusion, it appears that ^{51}Cr is a suitable label for $^{239}\text{PuO}_2$ particles. It does appear that high temperatures may cause ^{51}Cr to leave the particles and this should be avoided. The results presented imply that washing the particles alleviates this problem and that the ^{51}Cr and ^{239}Pu remain in a constant ratio until solubilized. There is no evidence from this work as to the tissue distribution of ^{51}Cr after dissolution.

Table 6
²³⁹Pu Analyses of Carcass and Liver Samples Expressed as Percent
of Each Animal's Injection Site Activity

Experiment Designation	Species	% / Sample		% / Gram	
		Liver	Carcass	Liver	Carcass
1.1 μm - 02	Hamster	0.44 x 10 ⁻³	1.59 x 10 ⁻³	4.81 x 10 ⁻⁵	1.42 x 10 ⁻⁵
03	Hamster	1.17	4.35	17.10	4.89
05	Rat	0.45	22.75	4.98	16.85
06	Rat	1.15	65.50	10.58	42.81
0.36 μm - 02	Hamster	0.39	1.11	4.34	1.05
03	Hamster	0.09	1.45	1.02	1.36
05	Rat	0.24	17.56	2.00	11.48
06	Rat	1.66	2.21	26.37	2.48
0.145 μm - 02	Hamster	0.81	4.23	10.99	4.60
03	Hamster	0.15	3.08	1.85*	2.99
05	Rat	0.78	21.94	7.02	16.00
06	Rat	0.26	24.36	2.01	13.69
0.52 μm - 02	Hamster	1.26	0.43*	11.75	0.39*
03	Hamster	0.19	0.57*	2.24	0.56*
05	Rat	0.64	28.20	5.71	18.19
06	Rat	0.36	29.60	3.43	21.76

* Of questionable significance - not used in subsequent calculations.

DISCUSSION

The values in Table 4 are very interesting considering the few data points from which they were derived, the scatter within the points, and the short length of the study. In fact, one should not place confidence in a half-life of several hundred days calculated from a 40-day experiment. As an exercise however, applying the values in Table 4 to Mercer's dissolution equations, some intriguing results are obtained for k, the constant of specific solubility. Using the particle sizes presented in Table 1 and half-lives from Table 4 yields the values in Table 7. The k values for the larger three particle sizes are remarkably similar. That for the lower size is ~ 2 times lower. Because of the reasons presented, it is fortuitous that the constants are at least within an order of magnitude of each other. The importance of these calculations is that the k values for this preparation of ²³⁹PuO₂ labeled with ⁵¹Cr appear to fall between 10⁻⁷ and 10⁻⁸ g cm⁻² day⁻¹. This places the aerosol made in this manner into the same category of solubility as fused clay. The common samples in which both *in vitro* and *in vivo* solubility measurements were made were from the back-up filters from the inhalation run (0.52 μm). The values obtained were identical, 6.8 x 10⁻⁸ g cm⁻² day⁻¹ (Table 7 and pp. 21-28, this report), based on ⁵¹Cr measurements. The *in vitro* solubility data for ²³⁹Pu from the same sample however, gave a value of 1.9 x 10⁻¹⁰ g cm⁻² day⁻¹.

Table 7
Values for k, the Constant of Specific Solubility
Calculated From the Particle Sizes and Half-Lives of Muscular Retention Presented. A Density of 10 g/cm³ was Used in the Calculations.

Particle Size (μm)	Retention Half-Life (Days)	k g/cm ² /Day
1.1	1800	6.1 x 10 ⁻⁸
0.52	770	6.8 x 10 ⁻⁸
0.36	570	6.3 x 10 ⁻⁸
0.145	500	2.9 x 10 ⁻⁸

EARLY DISTRIBUTION AND RETENTION OF 0.3 μ m MONODISPERSE

$^{239}\text{PuO}_2$ PARTICLES IN THE CHINESE HAMSTER

ABSTRACT

Forty-eight Chinese hamsters were intravenously injected with monodisperse 0.3 μ m $^{239}\text{PuO}_2$ particles labeled with ^{51}Cr . Twenty-four animals were injected at each activity level of 2×10^{-3} and 6×10^{-5} $\mu\text{Ci } ^{239}\text{Pu/gm}$ body weight. Four animals at each activity level were sacrificed at 0, 2, 4, 8, 16 and 32 days post-injection. The ^{239}Pu was avidly retained with a slight decrease by 32 days to 94% of the initial activity. At all sacrifice times, the liver, spleen and remaining carcass contained about 90%, 2.0% and 8.0% respectively, of the sacrifice body burden. The activity per gram of tissue was about equal for the liver and spleen, indicating equal competence of the reticuloendothelial system in these two organs and an equal dose commitment. These studies will serve as a basis for average and local dose calculations in future pathological and cytogenetic studies using monodispersed $^{239}\text{PuO}_2$ particles.

PRINCIPAL INVESTIGATORS

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INTRODUCTION

The toxicity of ^{239}Pu is of concern relative to both its military and civilian uses. Prospects for large scale development and use of fast breeder reactors utilizing large quantities of ^{239}Pu as fuel with associated opportunity for accidents in fuel fabrication, reactor operation, fuel reprocessing, and transport of fuel elements or processed material emphasizes the need for a sound understanding of the potential toxicity of ^{239}Pu . Of special concern is the toxicity of inhaled particles which may serve as intense radiation sources for small volumes of tissue. In an effort to develop a model system for studying the effects of internally deposited ^{239}Pu particles, monodisperse $^{239}\text{PuO}_2$ particles have been administered intravenously to Chinese hamsters with anticipated high deposition in liver so the cytogenetic effects of the intense local irradiation may be assessed.

This paper reports preliminary results from a study to determine the distribution and retention of the ^{239}Pu particles, research that was facilitated by the use of a ^{51}Cr tag on the particles.

EXPERIMENTAL METHODS

Forty-eight Chinese hamsters (chromosome number $2N = 22$), 80 to 120 days old, were used in an experiment in which 0.3 μ m $^{239}\text{PuO}_2$ particles tagged with ^{51}Cr were suspended in a saline solution and injected into the jugular vein. Activity levels of 2×10^{-3} and 6×10^{-5} $\mu\text{Ci } ^{239}\text{PuO}_2/\text{gm}$ body weight were injected, using a procedure described elsewhere (see this report, pp. 234 - 240).

^{239}Pu has two energetic alpha particles, 5.16 and 5.11 Mev. The higher energy particle is released 88% of the time and the lower, 11%. The range of the alpha is too small to be detected by external whole-body counting and the abundance and energy of the gamma rays produced by ^{239}Pu are insufficient to be useful in external detecting at low total activity levels. Therefore, in past $^{239}\text{PuO}_2$ injection experiments, it has been difficult to determine the amount of activity actually injected into each animal and to determine the whole-body retention pattern. Since ^{51}Cr has a 320 Kev gamma that can be detected by external whole-body counting, it was incorporated in the $^{239}\text{PuO}_2$ particles as a tracer. The methods for producing the ^{51}Cr labeled $^{239}\text{PuO}_2$ particles are found elsewhere (see this report, pp. 21 - 28).

Following injection, the high level animals were whole-body counted at frequent intervals for ^{51}Cr content on a NaI crystal well counter system to determine whole-body retention.

The distribution of the particles was determined by serial sacrifice of animals in groups of eight at 0, 2, 4, 8, 16 and 32 days post-injection. The liver, spleen, kidneys, lungs, gastrointestinal tract, defleshed left femur and humerus, bone marrow from the right femur and humerus, site of injection, and remaining carcass were carefully dissected to avoid cross-contamination, weighed and separately assayed for ^{51}Cr and ^{239}Pu . The tissues were counted with a NaI crystal system for ^{51}Cr detection, then transferred to scintillation vials, ashed, redissolved in 3 ml of 3 M HNO_3 , and suspended in 15 ml of Aquasol, * a xylene-based scintillation cocktail, making a gel. This suspends undissolved particles for more uniform ^{239}Pu analysis, using a liquid scintillation counter.

The activity levels used for the distribution and retention study, 2×10^{-3} and 6×10^{-5} $\mu\text{Ci } ^{239}\text{PuO}_2/\text{gm}$ body weight, will irradiate 100% and 3% of the liver, respectively. The activity level that would irradiate 100% of the liver will deliver approximately 400 rads local dose to the liver at 42-day sacrifice. This dose will result in an estimated 0.64 aberrations/cell in the cytogenetic study, based on recent ^{239}Pu data (see this report, pp. 234 - 240), and three aberrations/cell estimated from ^{241}Am studies.¹

RESULTS AND DISCUSSION

Whole-Body Retention:

The whole-body retention pattern (Fig. 1) indicates a slow, almost constant clearance rate of ^{239}Pu from the system with 95% of the initial body burden remaining at 32 days and 88% remaining at 56 days post-injection.

The variability present in the retention data may be a result of insufficient counting rates and counting times. In addition, the retention data represent three injection groups started at different times which also increases variability. A least squares analysis was fit to the data and yielded a line with an intercept of 103.5% and a slope of $-0.332\%/ \text{day}$.

Figure 1. Percent of initial whole-body count of monodispersed $^{239}\text{PuO}_2$ particles ($0.3 \mu\text{m}$) following IV injection in Chinese hamsters.

* Brand name of liquid scintillation counting cocktail prepared by New England Nuclear.

The high value for the intercept may possibly be due to a change in counting efficiency at the early times as the ^{51}Cr labeled particles redistribute in the animal's tissue.

The lack of clearance of the ^{51}Cr from the animals indicates that it is acting as a good tag for the particles. An *in vitro* loss of ^{51}Cr from particles has been reported (see this report, pp. 21-28) and if such is the case, it appears that this isotope clears the animal very slowly after it leaves the particles.

Distribution:

The percent of the sacrifice body burden was determined by both alpha analysis of the ^{239}Pu and gamma counting of the ^{51}Cr in each tissue. Regardless of the method of analysis, the percent of the sacrifice body burden is the same in each tissue, again indicating that the ^{51}Cr was an acceptable tracer for the ^{239}Pu particles.

The whole-body retention pattern is undoubtedly related to the insoluble nature of the $^{239}\text{PuO}_2$ particles and the affinity of the reticuloendothelial cell for these particles. The tissue distribution shown in Figure 2 supports this hypothesis, illustrating that the major sites of retention are the liver and spleen. Defleshed bones were analyzed and contained 0.12% of the sacrifice body burden. The bone marrow was also analyzed and found to contain an average of 33% of the activity in the long bones. These averages were derived using all sacrifice times and only the high level animals. Lindenbaum *et al.*² obtained similar results following injection of mice in the polymeric ^{239}Pu . These results suggest a small fraction of the plutonium was solubilized and incorporated into bone and further, that a small number of particles were incorporated into the marrow. Autoradiographic evaluation of marrow would help establish the form of the plutonium in marrow. The bone and all other tissues were found to individually contain less than 1.5% of the sacrifice body burden and in future analysis are included with the remaining carcass. Most of this activity was thought to be entrapped in reticuloendothelial cells throughout the body.

At four hours after injection, 87% of the ^{239}Pu is located in the liver; 3.3% in the spleen and the remaining activity is found in the carcass. Some of the particles at four hours are apparently still in a transitory state before lodging in the liver or spleen. The particles not yet taken up by the liver or spleen are accounted for in the carcass.

The relative ^{239}Pu content of the liver increases slightly at 2 and 4 days to a maximum of 92% at day 4. The activity in the spleen begins to decrease and the remaining carcass activity also decreases at this point. By day 8, the ^{239}Pu in the liver has decreased to 89% of the sacrifice body burden. The ^{239}Pu in the spleen also continues to decrease while the carcass accumulates more activity as time progresses. The liver has decreased to approximately 88.5% of the sacrifice body burden by day 32 with the spleen and remaining carcass containing 2.0% and 9.5%, respectively. This apparent increase in ^{239}Pu content found in the carcass and de-

Figure 2. Distribution of 0.3 μm monodispersed $^{239}\text{PuO}_2$ particles in the tissues of the Chinese hamster following the IV injection.

crease in liver and spleen may be possibly due to the release of particles by the liver and spleen and incorporation by the rest of the reticuloendothelial system, resulting in an increase in carcass body burden.

From Figure 3 it can be shown that on a per gram basis the spleen is receiving an equivalent number of particles as the liver. Thus, the average dose and possible biological effects will be as great in the spleen as in the liver. This figure also shows that after the $^{239}\text{PuO}_2$ particles lodge in the spleen or liver there is a low rate of redistribution of the particles from these organs to the rest of the body.

The ^{51}Cr tagged $^{239}\text{PuO}_2$ allows determination of a retention curve for the ^{239}Pu by whole-body counting and the detection of the gamma from the ^{51}Cr provides a rapid method for determining the distribution of the isotope, thus facilitating studies of $^{239}\text{PuO}_2$ particles.

Figure 3. Percent of the sacrifice body burden/gm tissue weight of $0.3 \mu\text{m}$ mono-dispersed $^{239}\text{PuO}_2$ particles in the liver and spleen of the Chinese hamster following the IV injection.

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A MULTISPECIES STUDY OF THE METABOLISM OF ^{252}Cf

ABSTRACT

The retention and organ distribution of ^{252}Cf citrate administered by intra-peritoneal injection were compared in Beagle dogs, Syrian hamsters, Chinese hamsters, rats and mice for 128 days post-injection. The percent initial body burden retained varied among the species studied whereas the computed effective half-life of retention (400-600 days) was not species-dependent. Principle organs of deposition for all species were skeleton and liver with small quantities of ^{252}Cf found in nearly all tissues and organs. Skeletal retention was avid in all species whereas hepatic retention varied among species. The Beagle dog and two hamster species retained 80% or more of the 8-day hepatic burden through the 128-day study period. The rat and mouse eliminated their hepatic burden rather rapidly with only 20% of the 8-day burden present at 128 days post-injection. Excretion patterns varied among species with the Beagle dog and two hamster species showing predominantly urinary excretion whereas for the rat and mouse, fecal excretion predominated. Hepatic elimination and predominance of fecal excretion in the rat and mouse were thought to be related to an ability in these two species to mobilize hepatic burdens and excrete ^{252}Cf via the bile into the intestine.

PRINCIPAL INVESTIGATORS

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INTRODUCTION

A previous study of the comparative retention and distribution of injected ^{252}Cf citrate in the rat and Chinese hamster¹ indicated that specific differences were operating in the metabolism of ^{252}Cf . Although several studies have been reported on the retention and distribution of injected ^{252}Cf in mice, rats and the Beagle dog,²⁻⁴ a comparison of the results of such independent studies is sometimes difficult due to variations in the experimental techniques. Recognizing that some differences exist among species and that a comparison of results from studies conducted in several laboratories is sometimes tenuous, a study was designed to determine whether differences in the retention and distribution of injected ^{252}Cf citrate existed among five animal species commonly used in biomedical research. Particular attention was paid to maintaining identical experimental conditions within each species group.

METHODS

Mice, Chinese hamsters, Syrian hamsters, rats and Beagle dogs were the experimental animals used in this study. The animals were carefully selected to represent young mature adult ages for each species by examination of published growth curves; mice, 6-8 wk; rats, 17-19 wk; Chinese hamster, 14-16 wk; Syrian hamster, 10-13 wk; and Beagle dog, 2-4 yr. All animals were injected on the same day from a single injection solution. The injection solution was prepared by adding Na citrate and NaOH to the stock solution of ^{252}Cf in 1 N HCl until the solution was 0.025 M in citrate at pH 6.0. The ^{252}Cf concentration was 1.0 $\mu\text{Ci/ml}$. Fifteen of the small animal species were intraperitoneally injected to receive 0.005 $\mu\text{Ci/gm}$ body weight and three Beagle dogs to receive 0.0015 $\mu\text{Ci/gm}$ body weight. After injection, the animals were placed in individual metabolism cages with food and water provided ad libitum. Five of each small animal species and one

Beagle dog were randomly assigned to sacrifice times of 8, 32 and 128 days. Complete urine and feces collections were obtained on all animals assigned to the 32-day sacrifice group.

All quantification of ^{252}Cf in animals, tissue samples and excreta was accomplished using external sample liquid scintillation well counters. Functions describing whole-body retentions were fitted using a non-linear least squares computer program.

RESULTS AND DISCUSSIONS

Whole-body retention of injected ^{252}Cf -citrate in each species through 128 days post-injection is shown in Figure 1. Three-component exponential equations were necessary to describe the retention data for mice, Syrian hamsters and rats. In contrast, the retention data for Chinese hamsters and Beagle dogs were adequately described by two-component exponential equations. The effective whole-body retention half-life for each species calculated from the last equation component is also shown in Figure 1. For the Chinese hamster, a second effective retention half-life (in parentheses) is given. This value was calculated from data obtained in another identical study which extended to 250 days post-injection. In this longer study, the whole-body retention equation for Chinese hamsters required a third component after about 140 days post-injection, or just longer than the present study extended. Figure 1 shows that whole-body retention of ^{252}Cf falls into three categories. The first, and highest, retention category includes only the Beagle dog. A second category showing moderate retention includes the rat, Chinese hamster and Syrian hamster; the third category showing the lowest retention includes only the mouse. Although confidence limits are not shown in Figure 1, if the computed statistical error of the long-term components of the retention equations are used to construct confidence intervals for each of the calculated half-lives, no significant differences in long-term retention exist. Thus, even though different quantities of the ^{252}Cf are retained by each species, the long-term retention half-life of this material is indistinguishable among the five species studied.

The tissue distribution of injected ^{252}Cf -citrate in these species show several similarities. For all species, nearly all tissues contain low levels of ^{252}Cf . Principle sites of deposition were the liver and skeleton. Initially, significant quantities, varying from 0.4 to 4.0% of the initial body burden, were found in the kidneys. In all species, however, the initial deposit in this organ followed the same pattern of rapid elimination. Depositions of ^{252}Cf in the skeletons of the various species were quite similar, ranging from 39 to 51% of the initial body burden present at 8 days post-injection to 30 to 44% present at 128 days. Skeletal deposition was avidly retained throughout the study for all species.

Significant differences are apparent in the deposition and retention of ^{252}Cf in the liver among the species studied. Figure 2 shows the percent initial body burden present in the liver of each species at three sacrifice times. The statistical limits on each datum point have been omitted to preserve clarity. At 8 days post-injection, the mouse is statistically different from the other species in having a lower deposition. At 32 days, the mouse is again different than the four other species and the rat is also statistically distinguishable from the mouse and the remaining three species. At 128 days, the mouse and rat are not distinguishable from each other but differ from the two hamster species and the Beagle dog. Thus, for the mouse and rat, the quantity of ^{252}Cf present in the liver at 128 days post-injection is 20%, and 17% of the quantity present at 8 days whereas for the Chinese hamster, Syrian hamster, and Beagle dog the same comparison shows 95%, 84%, and 82% of the 8-day liver burden present at 128 days post-injection, respectively. These apparent differences in liver deposition and retention indicate that a functional difference may exist in the metabolism of ^{252}Cf in the liver of mice and rats as compared to any of the three remaining species.

Figure 1. Whole-body retention of ^{252}Cf expressed as percent initial body burden for five animal species.

Figure 2. Liver retention of ^{252}Cf expressed as percent initial body burden for five animal species.

The underlying reasons for these differences or similarities in liver deposition and retention among these species remain unexplained. Attempts to further clarify the situation by applying gross anatomical factors such as total liver weight, or liver weight as a fraction of the total body weight have not been successful.

The data derived from excreta collections through 32 days post-injection are presented in Table 1. During the first day post-injection, the Beagle dog and both hamster species show that excretion is predominantly via the urine whereas rat excretion is nearly equal via urine and feces and the mouse excretion via the feces is slightly higher than via urine. From day 2 through day 32, urinary excretion continues to predominate for the Beagle dog and the two hamster species, while fecal excretion predominates for the rat and mouse. These latter data are shown graphically in Figure 3. These data reinforce the same specific grouping noted with liver deposition and retention data, e. g., the rat and mouse are similar but clearly different from the group consisting of the Chinese hamster, Syrian hamster and the Beagle dog.

Species differences in the rates of hepatic depletion of transuranic radionuclides have been noted. Biliary excretion constitutes a major route of hepatic loss and fecal excretion of transuranic

Table 1
Cumulative Percent of ^{252}Cf Initial Body Burden Excreted
by Five Laboratory Animal Species

	Days Post-Injection					
	0-1		2-32		0-32	
	Urine	Feces	Urine	Feces	Urine	Feces
Beagle Dog	14.1	0.7	5.2	3.7	19.3	4.4
Chinese Hamster	8.4	0.9	22.5	9.2	30.9	10.1
Syrian Hamster	15.2	2.1	17.9	6.3	33.1	8.4
Rat	9.8	8.4	9.3	15.2	19.1	23.7
Mouse	5.7	7.6	18.1	22.4	23.8	30.0

elements in the rat and mouse.⁵ Data are lacking regarding the magnitude of biliary excretion in the remaining species used in this study. The present study indicates that ^{252}Cf is rapidly excreted from the liver, probably via the bile for the mouse and rat. The much lower rate of hepatic ^{252}Cf loss and low concentrations in the feces for the Chinese hamster, Syrian hamster, and Beagle dog indicates that biliary excretion is not as important an excretory pathway for these species.

The species-related differences in retention, distribution and excretion noted in this study are of considerable significance when the results of this study are extrapolated to predict the human condition following internal deposition of ^{252}Cf . Since data are presently lacking on metabolism of ^{252}Cf in humans, choosing the "best" animal species for extrapolation must be by reference from comparisons of animal and human data on other transuranic elements, notably Pu. Such comparisons have consistently shown that the Beagle dog is a reasonable, metabolic model for humans, whereas the rat and mouse are rather poor models. Data are lacking for Syrian and Chinese hamsters.

Figure 3. Excretion of ^{252}Cf expressed as percent initial body burden for five animal species. U -- urine; F -- feces.

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NASAL ABSORPTION OF CsCl , SrCl_2 , BaCl_2
AND CeCl_3 IN SYRIAN HAMSTERS

ABSTRACT

Absorption of CsCl , SrCl_2 , BaCl_2 and CeCl_3 from the nasopharynx directly into the general circulation was studied in Syrian hamsters and compared with gastrointestinal tract absorption. More than 50% of the cesium, strontium and barium but less than 4% of the cerium was absorbed directly from the nasopharynx. For all isotopes, nasal absorption was approximately equal to or greater than gastrointestinal absorption during the first 4 hr after administration. This study emphasized the importance of direct nasal absorption of deposited materials in inhalation toxicity evaluations especially with readily soluble aerosols having mass median aerodynamic diameters greater than 5 μm when nasopharyngeal deposition predominates.

PRINCIPAL INVESTIGATORS

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INTRODUCTION

One of the first body tissues to come in contact with airborne pollutants are the nasal membranes since humans are normally nose-breathing. The upper respiratory passages perform beneficial functions for the body by adding heat and moisture to inhaled air and by removing a portion of inhaled airborne contaminants. Its efficiency as a filter depends mostly upon the physical characteristics of the inhaled contaminants, especially the aerodynamic properties of particulate material. For example, less than 10% of inhaled particles between 0.01 and 0.5 μm mass median aerodynamic diameter (MMAD) may be trapped in the nasopharyngeal region¹ whereas nearly all particles greater than 10 μm will be removed from the inhaled air. Nasal deposition also depends upon the rate of air flow through the nose during respiration and can vary by more than a factor of 10 for particle sizes between 1.5 and 8 μm MMAD.² During normal respiratory activity, 50 to 70% of inhaled vapors may be deposited in the nose and mouth.³

After deposition of material on nasal and pharyngeal membranes, both mechanical clearance and direct absorption into the general circulation can occur. Particles move toward the esophagus at a rate of about 0.6 cm per minute.⁴ Black and Hounam have reported an overall nasal clearance half-time of 30 min for vaporized iodine deposited in humans.³

The respiratory tract clearance model recommended by the Task Group on Lung Dynamics¹ accounts for both direct absorption and mechanical clearance of the nasopharyngeal region suggesting 4 min half-times for both processes. In the case of soluble aerosols, 50% of the nasally deposited material is estimated to be cleared by each pathway, whereas 10 and 1% of moderately soluble and insoluble particulates, respectively, are recommended for the absorbed fractions. The value selected for direct absorption of nasally deposited material is not of major importance in inhalation hazard evaluations involving aerosols having MMADs between 0.01 and 1 μm since nasal deposition is minimal. Likewise, it is a moot point when gastrointestinal absorption of the swallowed material is complete. Dyson and Beach,⁵ however, have pointed out that 1% nasal absorption allotted for insoluble plutonium oxide inhalation accounts for 33% of the total plutonium absorption predicted by the Task Group model for 4- μm MMAD aerosol particles. In such situations, the fraction taken for nasal absorption becomes highly significant.

The influence of direct nasal absorption of inhaled soluble particulates upon retention and dosimetry evaluations has been illustrated in previous work with BaCl_2 aerosols inhaled by Beagle dogs.⁶ About 75% of the barium body burden was absorbed into the general circulation within hours of the inhalation exposure. With 55% attributed to the total absorption of barium deposited in the pulmonary region of the respiratory tract and 2% attributed to gastrointestinal tract absorption, it was necessary to assume about one-third of the barium deposited in the nasopharyngeal region was also absorbed directly. This compares with 10% absorption predicted by the Task Group model and accounted for nearly 25% of the total barium absorbed.

This report describes further studies of direct nasal absorption of soluble elements conducted to complement the Task Group respiratory tract model in describing this important clearance process. Nasal absorption of CsCl , SrCl_2 , BaCl_2 and CeCl_3 was studied following intranasal administration in Syrian hamsters.

EXPERIMENTAL METHODS

Radioactive solutions of high specific activities of $^{137}\text{CsCl}$, $^{85}\text{SrCl}_2$, $^{133}\text{BaCl}_2$ and $^{144}\text{CeCl}_3$ in saline were administered to four groups of 20 Syrian hamsters each. The hamsters, approximately 1 year old, were housed 3 per cage and given food and water ad libitum up to time of use. Prior to isotope administration, food was withheld for 12 hr and each animal was anesthetized with 0.2 ml of sodium pentobarbital (60 mg/ml). The stomachs of all animals were surgically exposed and 15 of each experimental group were ligated at the pyloric sphincters. The external jugular veins were also exposed on 5 animals in each experimental group which had received stomach ligations.

One isotope was administered to each group of 20 animals in the following manner; (1) 5 animals with stomach ligations were injected with 20 μl of solution in the external jugular veins, (2) 5 animals with stomach ligations received 20 μl of solution on the anterior nasal membranes, (3) 5 animals with stomach ligations received 0.5 ml of solution via intragastric intubation, and (4) 5 animals without stomach ligation received 0.5 ml of solution via intragastric intubation. Injections and nasal administrations of the isotopes were accomplished through a 23-gauge hypodermic needle fitted to flexible polyethylene tubing (0.023 in. I.D.) which was attached to a hypodermic syringe. The tubing was filled with solution to a prescribed indicator before delivery and the contents of a 2-in. length of the tubing was subsequently delivered to the animals. The hypodermic needles were inserted about 1/4 in. into the right nasal passage for delivery. Intragastric intubation was accomplished through an 18-gauge 3-in. blunt tipped metal catheter affixed to a 1-cc hypodermic syringe which was easily passed through the esophagus for approximately 3 in. to ensure passage into the stomach.

All animals were assayed for total body radioactivity immediately after isotope administration and again after sacrifice at 4 hr after isotope administration. Animals were sacrificed by an overdose of chloroform. After sacrifice, the pelt, stomach, intestines, head and remaining carcass were taken for radioactivity assay. Both total body and tissue radioactivity measurements were made with large volume liquid scintillation well counters.

Nasal and gastrointestinal tract absorption of each isotope was calculated in the following manner. Since there was no excretion of the isotopes during the 4-hr period before sacrifice, the isotopic distributions in the tissues of the hamsters injected intravenously were taken as typical and representing 100% absorption. An average ratio of total body radioactivity to carcass radioactivity was calculated for these injected animals $(^{TB/C})_I$ and multiplied by the radioactivity contained in the carcasses of all other animals in each group to obtain values for total isotope absorption. For example, total gastric absorption, Abs_{GL} , was calculated for those animals with stomach ligations and intragastric intubation.

$$Abs_{GL} = C_{GL} (TB/C)_I$$

where Abs_{GL} = gastric absorption

C_{GL} = carcass radioactivity in animals with stomach ligation and intra-gastric intubation

Nasal absorption, Abs_N , corrected for gastric absorption of activity reaching the stomach was calculated from the following expressions:

$$Abs_N = \text{Total absorption} - \text{Gastric absorption}$$

$$Abs_N = C_N (TB/C)_I - Abs_{GL} (1 - \frac{Abs_N}{100} - H)$$

where C_N = carcass radioactivity in animals with nasal intubation

H = fraction of activity which was retained in the head

The radioactivity retained in the head was calculated from the expression

$$H = H_N - C_N (H/C)_I$$

where H_N = head radioactivity in animals with nasal intubation

$(H/C)_I$ = the ratio of head to carcass radioactivity in the injected animals

Therefore nasal absorption,

$$Abs_N = \frac{(TB/C)_I (C_N - C_{GL} + H C_{GL})}{1 - (TB/C)_I (C_{GL}/100)}$$

Gastrointestinal tract absorption was calculated from the expression

$$Abs_{GI} = C_G (TB/C)_I$$

where C_G = carcass radioactivity in animals with gastric intubation but without stomach ligation

These expressions assume the same absorption of radioactivity reaching the stomach after nasal intubation as in the case of gastric intubation with stomach ligation. Since the activity that cleared from the nose to the stomach resided in the stomach for less than 4 hr, this assumption tends to underestimate nasal absorption.

A corrected nasal absorption, $(Abs_N)_C$ was also calculated assuming that the fraction retained in the head of the animals with nasal intubation would have cleared in the same manner as the absorbed and swallowed activities.

$$(Abs_N)_C = \frac{(Abs)_N}{1-H}$$

RESULTS

Essentially all of the administered radioactivity was recovered in the hamster tissues after sacrifice (Table 1). Only after nasal administration of ^{144}Ce was the tissue recovery significantly less than 100%, in which case $85 \pm 6\%$ was retained by the animals. This was probably due to direct loss of ^{144}Ce from the nose rather than absorption and urinary excretion.

Total absorption of ^{85}Sr , ^{133}Ba and ^{144}Ce was always greater after intranasal administration than after gastric intubation with or without stomach ligation. Gastrointestinal tract absorption was greater than nasal absorption for ^{137}Cs . Absorption of ^{133}Ba in hamsters with stomach ligation and intragastric intubation was higher than gastrointestinal tract absorption in animals without stomach ligation. This should not be taken to indicate that normal gastric absorption is higher

than intestinal absorption because (1) holding the material in the stomach for 4 hr is not a normal situation, (2) intestinal absorption may not have been completed in 4 hr, and (3) there was a tendency for hamsters with stomach ligations and intragastric intubation to regurgitate some of the administered radioactivity back through the nasal and oral cavities. Any animals which visibly regurgitated portions of the administered solutions were removed immediately from the experiment. This occurred on 6 occasions and 3 of these animals showed significantly higher absorption than the remaining animals of each group with similar isotope administrations. Although average absorption of isotopes administered to hamsters by intragastric intubation with stomach ligation entered into the calculations of direct nasal absorption, this factor represented a minor correction.

Calculated nasal absorption (Table 2) was more than 50% for ^{137}Cs , ^{85}Sr and ^{133}Ba , but only 2% for ^{144}Ce . Gastric absorption in these animals, taken as the difference between total absorption and the calculated nasal absorption, may have been overestimated, however, this factor represented less than 17% of the total absorption for all isotopes.

Table 1
Percent Total Tissue Recovery and Calculated Internal Absorption of
Radioactivity in Hamsters Given as a Percent of the Administered Dose

Isotope	Tissue Recovery	Total Absorption in Animals Receiving			
		Injection	Nasal Intubation	Gastric Intubation	Gastric Intubation Without Ligation
^{137}Cs	103 (± 5) *	100	56 (± 8)	9 (± 0.1)	70 (± 24)
^{85}Sr	96 (± 8)	100	67 (± 8)	20 (± 12)	37 (± 17)
^{133}Ba	106 (± 5)	100	61 (± 10)	32 (± 17)	11 (± 13)
^{144}Ce	92 (± 4)	100	2.1 (± 0.9)	.5 (± 0.6)	.3 [†] (± 0.4)

* Figures in parentheses indicate one standard deviation of the individual measurements.

† Gastrointestinal absorption of cerium was not considered significant, however, the average value was higher than that of 0.01% reported for miniature swine.⁷

TABLE 2
Percent Absorption and Retention of Radioactivity in
Animals Following Intranasal Intubation

Isotope	Total Absorption	Head Retention	Gastric Absorption	Nasal Absorption	Corrected Nasal Absorption
^{137}Cs	56 (± 8)	28 (± 5)	2 (± 0.4)	54 (± 8)	75 (± 8)
^{85}Sr	67 (± 8)	18 (± 4)	4 (± 2)	63 (± 9)	77 (± 11)
^{133}Ba	61 (± 10)	17 (± 3)	10 (± 4)	51 (± 14)	61 (± 16)
^{144}Ce	2.1 (± 0.9)	46 (± 15)	0.3 (± 0.1)	1.8 (± 0.9)	3.9 (± 3.3)

Nasal absorption, corrected for material which had not cleared from the nasopharynx, indicated that with the exception of ^{144}Ce between 60 and 80% of these isotopes deposited on the nasal tissues can be absorbed directly; ^{144}Ce may be only about 4%.

DISCUSSION

Direct absorption of mono- and di-valent ions of cesium, strontium and barium from nasal tissues can represent a significant fraction of the total systemic absorption of these elements in an inhalation exposure. If the values for intestinal absorption and nasal absorption of these elements taken from Tables 1 and 2 are combined, then 93% of cesium, 85% of strontium and 65% of barium inhaled in soluble chemical forms and deposited in nasal tissues would eventually be absorbed into the body. Only 4% of CeCl_3 deposited in the upper respiratory tract would be absorbed.

Values for intestinal absorption of these elements taken from the Report of Committee II on Permissible Dose for Internal Emitters⁸ are 100% for cesium, 30% for strontium, 5% for barium and $10^{-2}\%$ for cerium. The report of the Task Group of Lung Dynamics¹ recommends nasopharyngeal absorption fractions of 50% for CsCl (Class D) and 10% for SrCl_2 , BaCl_2 and CeCl_3 (Class W). Thus, the total absorption of chlorides of these isotopes would be predicted to be 100% for cesium; 37% for strontium; 15% for barium and 10% for cerium. According to the data reported here, these predictions may be slightly high for cesium and cerium but very low for strontium and barium.

About 50% of the ^{144}Ce given to the hamsters by intranasal administration was retained in the tissues of the head after 4 hr. Since little direct absorption was observed with cerium, then 4 hr was approximately the half-time for mechanical clearance in these animals. This is considerably longer than 4 min taken by the Task Group of Lung Dynamics and the 30 min half-time suggested by Black and Hounam.³ This delay in nasopharyngeal clearance was probably due either to the effects of the administered anesthetic which were visually apparent for the duration of the experiment or to differences caused by the administration of these isotopes in solutions rather than particulates.

For all isotopes studied, the estimated total nasal absorption exceeded the 4-hr gastrointestinal tract absorption. Thus, the nasopharynx is not only a major site of absorption for inhaled aerosols of a soluble nature, but may be the most important site of absorption especially for aerosols greater than $5\text{ }\mu\text{m}$ MMAD where nasal deposition greatly exceeds deposition in all other areas of the respiratory tract.

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RETENTION AND TISSUE DISTRIBUTION OF ^{144}Ce FOLLOWING INHALATION
OF $^{144}\text{CeO}_2$ IN SYRIAN HAMSTERS

ABSTRACT

Retention and distribution of inhaled $^{144}\text{CeO}_2$ has been studied in Syrian hamsters for more than 1 year and comparisons made with similar data obtained in other species. A 110-day effective half-time of whole-body retention was established after about 200 days. Lung remained the primary organ of interest, having 88% of the sacrifice body burden at 320 days after inhalation exposure. Liver contained about 8% and skeleton 3% of the body burden at that time. Measurable quantities of ^{144}Ce were detected in tracheobronchial lymph nodes, ranking the lymph nodes second to lung in radiocerium concentration at the later sacrifice times. At 320 days, concentration ratios of lung-to-lymph nodes-to-liver were approximately 70:2:1. The infinite β -radiation dose to lungs of animals in this study was calculated to be about 4200 rads per μCi ^{144}Ce initial lung burden.

PRINCIPAL INVESTIGATORS

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INTRODUCTION

Because of its significance in nuclear operations ^{144}Ce in relatively insoluble forms has previously been used in inhalation studies with Beagle dogs (1968-1969 Annual Report, LF-41, pp. 19-35), mice¹ (this report, pp. 77-84), and rats² in this laboratory. Large scale toxicity studies have been carried out with the laboratory's major experimental animal, the Beagle dog. On bringing into the laboratory a second major species, the Syrian hamster, ^{144}Ce was considered the best choice for studies that would provide long-term retention characteristics and background data for interspecies comparison.

$^{144}\text{CeO}_2$ was chosen for a pilot study, the experimental methodology and preliminary observations of which have been reported (1970-1971 Annual Report, LF-44, pp. 90-95). This experiment is still in progress with 5 of 60 exposed hamsters remaining for whole-body counting and better definition of long-term retention characteristics. The data to be reported here include whole-body retention through 380 days post-inhalation exposure, tissue distribution through 320 days post-exposure, and an estimate of cumulative β -radiation dose to the lungs.

RESULTS

Whole-body retention data, averages of from 36 to 17 animals all of which lived longer than 4 months post-inhalation exposure, indicated three components of retention (Fig. 1). About 70% of initially deposited $^{144}\text{CeO}_2$ was rapidly cleared and a brief intermediate component existed that had an effective half-time of about 15 days. The long-term component corresponded to an effective half-time of 108 days ($T_{\text{biol.}} = 175$ days).

Initial lung burdens (ILB), 2 hours post-inhalation exposure, averaged 34% of the initial body burden (IBB), and clearance was such that, at 16 days post-exposure, lung comprised 96% of the sacrifice body burden (SBB). A gradual decrease to 88% SBB in lung at 320 days post-exposure and concomitant build-up of ^{144}Ce in liver and skeleton are shown in Figure 2. The fraction of the body burden detected in tracheobronchial lymph nodes was small and variable (Table 1), but represented significant quantities in terms of ^{144}Ce concentration. ^{144}Ce concentrations in lung, tracheobronchial lymph nodes and liver at 320 days post-exposure were 126%/gm, 3.3%/gm and

Figure 1. Effective whole-body retention of ^{144}Ce in Syrian hamsters after inhalation of $^{144}\text{CeO}_2$. Data shown as mean and range of values from 36 to 17 animals.

Figure 2. Tissue distribution of ^{144}Ce in Syrian hamsters after inhalation of $^{144}\text{CeO}_2$. Data shown are means from 2 to 4 animals and ranges of values from 4 animals.

Table 1

Distribution of ^{144}Ce in Tissues of the Syrian Hamster After Inhalation of $^{144}\text{CeO}_2$. Mean Percent Sacrifice Body Burden per Organ and per Gram

Sacrifice Time*	Lung		Liver		Skeleton	TBLN**		Kidneys	GI Tract
	%/org	%/gm	%/org	%/gm		%/org	%/gm		
2 Hrs	34		.0058		3.4	-		.0019	27
1 Day	38		.023		.92	-		.00055	42
2 Days	41		.011		.56	.038		.00050	36
4	70		.017		.40	.020		.0014	13
8	84		.0079		.34	.031		.0071	2.1
16	96		.22		.20	.049		.011	1.2
32	98	140	.41	.089	.21	.052	.69	.016	.74
64	98	140	1.1	.24	.40	.16	1.20	.030	.54
128	97	138	1.8	.36	.54	.19	1.57	.031	.22
192	95	135	3.6	.60	1.0	.21	5.25	.048	.26
256	93	133	4.5	.80	1.8	.10	2.22	.060	.23
320	88	126	8.2	1.76	3.0	.12	3.33	.13	.31

* Days post-inhalation exposure

** Tracheobronchial Lymph Nodes

1.8%/gm, respectively. Kidneys also showed build-up as a fraction of SBB with time after exposure; the presence of significant, though small, quantities of ^{144}Ce in kidneys was also noted in a study with rats in this laboratory.²

Of the 34% IBB originally deposited in lung, about one-third was cleared with a half-time of about 2.5 days; the remainder could be fit with a single exponent having a half-time of 90 days. From these two components, cumulative radiation dose was calculated (Fig. 3) based on an aver-

Figure 3. Cumulative beta-radiation dose to lung of Syrian hamsters after inhalation of $^{144}\text{CeO}_2$. Curve represents average rads/ $\mu\text{Ci ILB}$ based on lung weight of 0.7 gm; average beta energy of 1.29 MeV and 50% absorption of β energy.

age lung weight of 0.7 gm, the average β -energy of ^{144}Ce - ^{144}Pr in equilibrium (1.29 MeV) and 50% absorption of β energy in lung. Infinite dose to lung, that might be received in the lifetime of a Syrian hamster, was about 4200 rads per $\mu\text{Ci } ^{144}\text{Ce ILB}$.

Recognizing that interspecies comparisons of retention parameters are difficult, at best, following inhalation of polydisperse aerosol particle distributions, some basic correlations or trends may still be found. Following are whole-body retention equations that have been reported for the four species that have inhaled relatively insoluble forms of ^{144}Ce in this laboratory:

Mouse ¹	$81e^{-0.64t} + 14e^{-0.042t} + 5e^{-0.007t}$
Hamster (this report)	$74e^{-0.058t} + 10e^{-0.022t} + 16e^{-0.0064t}$
Rat ²	$88e^{-0.70t} + 7e^{-0.023t} + 5e^{-0.003t}$
Dog (LF-41, p 24)	$47e^{-2.5t} + 15e^{-0.011t} + 38e^{-0.0025t}$

Although the fractions cleared and their half-times of clearance varied to a great extent between the four species, it is notable that the third components correspond to half-times that increase with increased animal size, 99 days for mouse, 108 days for hamster, 231 days for rat, and 277 days for dog. Many more data must be collected and analyzed before definitive conclusions may be reached regarding relationships between the species. That some correlation can be made is encouraging in light of anticipated toxicity studies in the Syrian hamster planned to parallel those in the Beagle dog.

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RETENTION CHARACTERISTICS OF INHALED, RELATIVELY INSOLUBLE FORMS OF ^{144}Ce IN MICE

ABSTRACT

Two studies are reported, the first having provided impetus for the second. Mice were exposed to an aerosol of ^{144}Ce entrapped in fused montmorillonite clay particles. About 95% of the deposited material was cleared within one week post-inhalation exposure. Lung was the organ of highest ^{144}Ce concentration throughout the study and retained ^{144}Ce with an effective half-time of 85 days. Build-up of ^{144}Ce in skeleton and liver was also noted, such that at 256 days post-inhalation exposure the skeleton contained 48% and liver 11% of the body burden, compared to 33% in lung. These data differed from those of other studies in mice with $^{144}\text{CeO}_2$ and ^{144}Ce -fused clay, where inhaled material was held more tenaciously in the lung. Incomplete fusion of the aerosol particles was assumed responsible for the differences noted and was the basis for a second study in which groups of mice were exposed to ^{144}Ce clay aerosols produced under various conditions of temperature and air flow. Preliminary whole-body retention data from the latter study are included and the importance of aerosol characterization in the routine monitoring of potential exposure areas is discussed.

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INTRODUCTION

^{144}Ce is abundant in a nuclear reactor inventory after sustained reactor operation. Because of its relatively long physical half-life (285 days), it is an important component of spent fuel elements and in fuel element processing. Its prominence, half-life and relatively energetic beta-emission (average energy, 1.29 Mev, from decay of ^{144}Ce - ^{144}Pr) cause it to be of considerable biological importance following various potential nuclear accidents.

Results of numerous biological studies have indicated that cerium is poorly absorbed from the site of administration, particularly in adult animals.^{1,2} Because of its very low absorption from the gastrointestinal (GI) tract, the greatest potential hazard relating to radiocerium accidentally released to the biosphere is inhalation, with lung being the organ of particular interest when the inhaled radiocerium compound is relatively insoluble.

In studies to assess the effects of beta-radiation of lungs on susceptibility to influenza virus infection, mice were exposed to aerosols of $^{144}\text{CeO}_2$ and ^{144}Ce entrapped in fused montmorillonite clay particles.³ Numerous tests were performed to determine the effectiveness of beta-irradiation in altering the pulmonary response to the viral insult. To assist in estimating the radiation doses received, a whole-body counting regime was carried out with the $^{144}\text{CeO}_2$ and ^{144}Ce -fused clay exposed animals. In addition, some ^{144}Ce -fused clay exposed mice were assigned to a serial sacrifice and dissection experiment. Tissue distribution, retention and dose pattern data derived from these animals are presented here and compared with retention data from the $^{144}\text{CeO}_2$ exposed mice and with retention and tissue distribution data from a previous ^{144}Ce -fused clay inhalation experiment with mice.⁴

In light of observed differences between results of the present ^{144}Ce fused clay study and other studies, a series of experiments was designed to help define the effect of temperatures used in producing fused clay aerosols upon their retention after inhalation. Preliminary data relating to these experiments, which are currently in progress, are reported here.

METHODS

For the first study, $^{144}\text{CeCl}_3$ was cation exchanged into montmorillonite clay, a suspension of which was subsequently aerosolized and passed through a nichrome wire-wrapped quartz heating column at about 1100°C . The resultant aerosol was mixed and diluted with clean, dry air and drawn through a multiple small animal exposure apparatus. Four groups of 60 mice each were exposed to similar aerosols, prepared as described. The mice (strain C57BL/6J) were 8 to 10 weeks old and weighed 20 to 25 grams at the time of exposure. For inhalation exposure, each mouse was confined, unsedated, in a one-inch inside diameter polyethylene test tube that had a small hole drilled in the end, through which the mouse breathed. The test tubes were sealed horizontally into the exposure chamber with each animal's nose slightly protruding into the area of aerosol flow. During each exposure run, the aerosol was sampled for size distribution, using a cascade impactor and, during one of the runs, an electrostatic precipitator sample was taken for electron microscopic evaluation of the aerosol particles.

After a 15-minute inhalation exposure and clean air flushing of the exposure chamber, mice were removed from the exposure tubes and placed directly into holding cages or into ventilated pint containers for whole-body counting. Sixty of the 240 mice were whole-body counted within one hour after inhalation exposure and all animals were subsequently whole-body counted beginning seven days post-exposure. Twenty-eight mice were assigned to a sacrifice schedule as follows: post-exposure day 16 (9 mice), 32 (5), 64 (4), 128 (5) and 256 (5). Animals were sacrificed with chloroform, the head skin and remainder of the pelt removed separately, and the spleen, kidneys, liver, GI tract, lung, femurs and humeri resected. All samples, including the remaining carcass were analyzed for ^{144}Ce content. Whole-body and tissue analyses were made in a well-type counter in which scintillation fluid was the detection medium external to the well.

For the second study, specific methodology of which was based on results of the first study, stock suspension of ^{144}Ce in unfused montmorillonite clay was prepared. The suspension held 5 mg clay/ml suspension, to provide a particle size distribution with a predicted activity median aerodynamic diameter (AMAD) of about $1.6\ \mu\text{m}$. Nine inhalation exposure runs were carried out, using the Lovelace nebulizer and a multiple small animal exposure apparatus. The nebulizer was operated at 20 psig, with an output of 1.6 l/min, for each run. Basic experimental design is provided in Table 1, which also includes aerosol characteristics of the first study. During each exposure, three filter samples, two cascade impactor samples and three electrostatic precipitator samples were collected. For three exposures a smaller diameter heating column, anticipated to produce comparatively less effective particle heating because of the higher flow rate, was used.

Each exposure group consisted of 15 male C57BL/6J mice, about 3 months old. All mice were whole-body counted soon after a 30-minute inhalation exposure period and at increasing intervals thereafter. Several mice from each group will be sacrificed and dissected for tissue analysis, at 1 month, 2 months and perhaps at 3 or 4 months post-inhalation exposure.

RESULTS

The activity distribution of the ^{144}Ce fused clay aerosols used in Study I determined by radioactive measurement of cascade impactor samples, was approximately log-normal. An electron micrograph of a section of an electrostatic precipitator grid sample is shown in Figure 1. The polydispersity of the aerosol, as indicated by the σ_g of 1.7 (Table 1) is apparent.

Whole-body retention of ^{144}Ce in a group of 60 mice at early times to 5 mice at 128 days is presented in Figure 2, with a three-component equation which includes the early rapid loss of a large fraction of deposited material. Estimated initial lung burdens (ILB) amounting to about 5%

Table 1
Aerosol Preparation and Characteristics

Study	Clay Suspension Concentration	Heat Column Temperature	Diameter Of Heat Column	Diluting Air	AMAD*	σ_g^{**}
I	5 mg/ml	1100°C	0.6 in I. D.	0 1/min	$\approx 1.6 \mu\text{m}$	1.7
II - A	5 mg/ml	90°C	1.0 in I. D.	0 1/min	1.47 μm	1.48
B	5	200	1.0	0	1.52	1.53
C	5	500	1.0	0	1.52	1.47
D	5	900	1.0	0	1.84	1.55
E	5	1150	1.0	0	2.04	1.54
F	5	1150	1.0	2.4	2.14	1.55
G	5	900	0.6	0	1.52	1.47
H	5	1150	0.6	0	1.92	1.48
J	5	1150	0.6	2.4	1.90	1.52

* Activity Median Aerodynamic Diameter

** Geometric Standard Deviation

Figure 1. Electron micrograph of ^{144}Cs fused clay aerosol.

Figure 2. Whole-body retention of ^{144}Ce following inhalation of ^{144}Ce fused clay in mice. Datum points are the average of 5 to 60 mice.

of the initial body burden (IBB), ranged from 0.36 to 1.4 μCi with an average of 0.75 μCi . Relative distribution and concentration of ^{144}Ce in mouse tissues (Table 2) were greater in lung than in the other two primary organs of interest, liver and skeleton. Skeleton ^{144}Ce content exceeded that in lung at the latest sacrifice time but lung maintained a higher ^{144}Ce concentration throughout, as anticipated with inhaled relatively insoluble materials. Low levels of radiocerium were detected in the GI tract (Table 2) and traces were detected in some kidney and spleen samples.

Table 2
Tissue ^{144}Ce Content Following Inhalation of ^{144}Ce in Fused Clay (Study I)
Data Expressed as Percent Sacrifice Body Burden, Per Organ and Per Gram

Time*	Lung		Liver		Skeleton**		G. I. Tract	
	%/Organ	%/Gram	%/Organ	%/Gram	%/Organ	%/Gram	%/Organ	%/Gram
16	91	404	0.54	0.39	1.6	0.70	1.1	0.50
32	92	568	2.0	1.7	3.4	1.5	0.93	0.40
64	83	300	5.5	3.8	7.8	3.2	0.58	0.19
128	51	177	8.0	5.9	32	14	1.3	0.63
256	33	146	11	8.0	48	20	1.9	0.70

* Days post-inhalation exposure.

** Estimate from bone sample and carcass (muscle + bone) ^{144}Ce content.

A single exponent for lung retention, together with the last two exponential components of whole-body retention (Fig. 2) were used to calculate beta-radiation dose to lung. Other considerations were the assumption of an average lung weight of 0.23 grams and estimated absorption of beta-particle energy of 35%.⁵ The average calculated infinite dose to lung, which might be approached at approximately 600 days, was ~ 3500 rads per μCi ILB. Although substantial ^{144}Ce build-up occurred in skeleton and liver as a function of the sacrifice body burden (SBB) with time, the values shown (Table 2) represent a maximum of only 3% and 1% of the ILB for skeleton and liver, respectively. Beta-radiation dose to those organs could not, in the lifetime of the mouse, approach that calculated for lung.

Particle size distribution for Study II were determined from cascade impactor samples (Table 1). Larger particles were produced at the two higher temperatures using the larger heat column and at the higher temperature only using the smaller column.

Early whole-body retention data from inhaled ^{144}Ce clay produced with the larger heat column under identical air flow conditions (Fig. 3), show a grouping that corresponds to temperature and to particle size. Although individual values would show a fair degree of divergence between animals, the mean values indicate a trend towards greater retention of smaller and presumably poorly fused particles in contrast to those that were larger and assumed to be less soluble due to more complete fusion. Greater initial clearance of larger particles would be expected assuming their greater upper respiratory tract deposition.

Early retention data from exposures using the smaller heat column contrasted somewhat with those from similarly treated aerosols using the larger column (Fig. 4). The particles produced at 900°C with the small column appear to have behaved more like those produced at lower temperatures with the large column, probably indicating less thorough heating of particles at a given column temperature in the small column. Particle size distribution of the 900°C small column particles also resembled those of lower temperature, large column particles. It is speculated at this early time that particle characteristics were altered by the increased air flow using the smaller heat column.

Figure 3. Whole-body retention of inhaled ^{144}Ce clay aerosols in mice. Each datum point represents the average of 15 mice.

Figure 4. Whole-body retention of inhaled ^{144}Ce clay aerosols produced in 0.6" and 1.0" diameters heat columns. Each datum point represents the average of 15 mice.

DISCUSSION

Tissue distribution and beta-radiation dose data obtained for $^{144}\text{CeO}_2$ and ^{144}Ce fused clay (Study I) from the influenza virus infection studies,³ indicated that these two relatively insoluble materials had not been handled similarly biologically. Although serial sacrifices and dissections had not been performed in the $^{144}\text{CeO}_2$ study, sufficient lung samples assayed at later times post-inhalation exposure revealed much greater retention in lung than that observed in animals exposed to ^{144}Ce fused clay. Due to these retention differences, the average infinite beta-radiation dose to lung in $^{144}\text{CeO}_2$ -exposed mice was calculated to be 6000 rads/ $\mu\text{Ci ILB}$ attained at approximately 700 days post-inhalation exposure, compared with the 3500 rads/ $\mu\text{Ci ILB}$ attained at approximately 600 days for ^{144}Ce fused clay (Fig. 5). Data from a previous study with similarly prepared ^{144}Ce fused clay were similar to the $^{144}\text{CeO}_2$ data reported here.⁴ Tissue data from the three studies are compared in Table 3.

Aerosol characterization for the three studies referred to above was similar in terms of AMAD and σ_g and the heating column temperatures were recorded as being essentially identical. Examination of electron micrographs of particles collected by electrostatic precipitator revealed the most probable explanation of observed biological differences. Particles of ^{144}Ce oxide and particles of completely fused clay are shown in Figures 6 and 7. Note their similarity in apparent density, smooth surfaces and spherical shape. By comparison, the particles of incompletely fused clay seen in Figure 1, are less spherical, particularly the larger particles, and are less uniformly smooth. Incomplete particle fusion probably accounted for the greater degree of solubility of ^{144}Ce particles observed in the present first study, where substantial translocation of ^{144}Ce to liver and skeleton occurred. This solubility could be related to a greater surface area of poorly

Figure 5. Cumulative beta radiation dose to lungs of mice after inhalation exposure to ^{144}Ce as the oxide or in fused clay.

Table 3
Tissue Distribution of ^{144}Ce in Mice Exposed to Aerosols of ^{144}Ce Fused Clay or ^{144}Ce Oxide. Data are Average % Body Burden at Various Times Post-Inhalation Exposure.

	Present Study ^{144}Ce -Fused Clay			Morgan, et al ⁴ ^{144}Ce -Fused Clay		Lundgren, et al ³ ^{144}Ce Oxide	
	64*	128*	256*	64*	128*	60*	≈ 400*
Lung	83	51	33	82	75	97	86
Liver	5.5	8	11	1.5	1.5	**	**
Skeleton	8	32	48	14	21	**	**
Remainder	3.5	9	8	2.5	2.5	3	14

* Days-post inhalation exposure

** Lung and remainder analyzed. Remainder value assumed to primarily represent liver and skeleton content.

fused particles (Fig. 1) compared to well fused spherical particles (Fig. 7). Importance of surface area of particles relative to their dissolution rate has been emphasized by others.⁶ In addition, in the poorly fused particles, the ^{144}Ce may have been less securely trapped and have left the particles more rapidly than if well fused. It might also be speculated that ^{144}Ce in poorly fused particles would be more readily absorbed from the GI tract than that bound in well fused particles. The potential importance of this is apparent recognizing that in the mouse 90 to 95% of deposited activity is cleared via the GI tract within a few days of inhalation exposure. Because absorption of Ce, even as CeCl_3 has been observed to be less than 0.01% following oral administration,² it is doubtful that significant portions of the liver and skeleton burdens in the present study were of that origin.

Figure 6. Electron micrograph of ^{144}Ce oxide aerosol.

Figure 7. Electron micrograph of fused clay aerosol.

The technical cause of incomplete fusion of particles is assumed to have been a loose connection at the outlet of the aerosol generator, through which room air was pulled into the heating column thus increasing the total air flow through the column. This would have caused the ^{144}Ce clay particles to pass through the column at a faster than normal rate and to be heated at a lower effective temperature. It was on the basis of these assumptions and conclusions regarding results of Study I that Study II was planned to include variations of heat column temperature and the addition of diluting air, which would simulate a leak in the heat column system. Analysis of electrostatic precipitator samples and tissues (lung, liver, skeleton), as well as longer-term whole-body retention data, are anticipated to yield greater insight to interpretation of the apparent trends thus far observed in Study II.

From an experimental point of view, the sampling and characterizing of aerosols from each exposure run is important. Knowledge of specific aerosol characteristics is necessary for appropriate correlations developed between aerosol character, dose pattern and biological response. Recognition of the difference in possible infinite absorbed dose to a critical organ (~ 3500 vs ~ 6000 rads/ $\mu\text{Ci ILB}$) based on a small apparent deviation in inhaled particles, emphasizes the importance of proper air sampling and evaluation techniques in the routine monitoring of potential exposure areas in industrial operations. Filter and cascade impactor samples are helpful in determining air concentrations and particle size distributions; however, the expected biological behavior of inhaled particles may not be known based upon this information alone. A means of visualizing the contaminant, as by electrostatic precipitator sampling, would substantially aid in the interpretation of the significance of personnel contamination determined by whole-body counting or monitoring, and aid in the selection of appropriate therapy for accidentally exposed individuals.

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METABOLISM OF INHALED ^{124}Sb IN THE SYRIAN HAMSTER
AS A FUNCTION OF VALENCE STATE

ABSTRACT

Several radionuclides, such as radioantimony, that are present in a reactor inventory have potential for being present in accidentally-produced aerosols in more than one valence state. To evaluate the importance of this variable, the metabolism of inhaled trivalent and pentavalent ^{124}Sb tartrate was studied in Syrian hamsters and found to vary with valence state. Whole-body clearance of both the trivalent and pentavalent forms exhibited two phases. The first was rapid clearance of a large proportion of the initial body burden; the second was a longer term retention with a biological half-life of 16 days. Much of the activity was recovered in feces, but some urinary excretion was also observed. The trivalent antimony concentrated in the red blood cells (RBCs) with peak RBC/plasma ratios occurring in the day 1 samples. The pentavalent antimony was found mainly in the plasma in the day 0 samples, but was concentrated in the RBCs in subsequent samples. In the tissues, substantial accumulation was noted in liver, skeleton and pelt. The liver accumulated trivalent antimony to a greater extent than pentavalent antimony. However, after inhalation of the pentavalent ^{124}Sb aerosol, skeleton always contained a greater concentration of the activity than did the liver, and therefore would be more important in dose considerations. The pelt, and especially hair, accounted for a large proportion of the body burden.

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INTRODUCTION

There are several radioactive isotopes of antimony in a fission product inventory that may be released to the atmosphere in the event of a nuclear accident. ^{127}Sb is the most abundant antimony isotope in a thermal nuclear reactor but ^{124}Sb is more convenient to study because of its half-life (~ 60 days) and strong gamma-ray emissions. Radioantimony may potentially be released in the trivalent or pentavalent form. This report presents the results of a study in which hamsters were administered trivalent or pentavalent ^{124}Sb as an aerosol to determine the effect of valence state on the metabolism following inhalation.

MATERIALS AND METHODS

The trivalent aerosol was generated from a starting solution of ^{124}Sb tartrate complex. The trivalent solution was treated with bromine water to oxidize the antimony to the pentavalent state. The solutions were nebulized using a Lovelace nebulizer. Resulting droplets were passed through a heating column maintained at 100°C , cooled with diluting air, and then introduced into the small animal exposure chamber. The aerosols were characterized as to size distribution using data from cascade impactor measurements made during the inhalation exposure. Twenty-one hamsters were exposed to the trivalent and 33 to the pentavalent aerosols. After exposure, the hamsters were periodically whole-body counted in a deep well liquid scintillation counter. Urine and feces collections were made for 32 days post-exposure on seven hamsters in the pentavalent group. Collections were made on two animals for 15 days and on five animals for 32 days in the trivalent aerosol group. Animals were housed one to a cage and given food and water ad libitum.

Two or three animals were sacrificed per aerosol group at 0, 1, 5, 8, 15 and 32 days. At 32 days, however, 5 animals were sacrificed from the trivalent aerosol group and 11 animals from the pentavalent aerosol group. Up to and including the day 8 sacrifices, at least one animal per group was sacrificed by exsanguination via heart puncture to obtain a blood sample. The others

were sacrificed with chloroform. Complete dissections were performed and tissues were counted in NaI crystal gamma-ray detectors. Blood samples were centrifuged for 10 minutes at 2000 rpm and the RBC and plasma fractions were separated, weighed and counted in an automatic sample-changing NaI crystal counter.

RESULTS

Particle size of both aerosols was approximately $1.6 \mu\text{m}$ AMAD with a σ_g of 1.7. Whole-body retentions as percentages of the day 1 body burdens are plotted in Figure 1. Data were corrected for physical decay and reflect biological processes only. The median urine to feces radioactivity ratios for the 16 collections over the 32-day period were 0.60 and 0.86 for the trivalent and pentavalent states, respectively, values that are not statistically different from each other. Activity ratios of RBC/plasma on a per gram basis from individual animals are tabulated in Table 1. Selected tissue distribution data are tabulated in Table 2, as percentage of the total tissue burden per gram. Table 3 lists tissue distribution as percentage total tissue burden per organ. By 2 hours post-exposure, when the first sacrifices were made, less than 1% of the body burden occurred per gram lung with either valence state. The GI tract accounted for more than 10% of the total tissue activity out to the day 15 sacrifices. In both groups, the highest antimony accumulation was in liver, skin and skeleton. To localize the skin activity, hair was clipped from pelt samples of four animals from each group and the hair and skin were counted separately. No difference existed between average valence states with 57% in the hair and 43% in the skin. A certain amount of hair, however, remained on the skin after clipping.

Table 1
RBC/Plasma Activity Ratios in Hamsters
Exposed to Trivalent and Pentavalent ^{124}Sb .
Counts were Compared on a Per Gram Basis

Sacrifice-Days Post-Exposure	Sample	RBC/Plasma	
		+3	+5
0	a	1.44	0.33
	b	0.64	0.26
	c	1.36	0.29
1	a	7.80	2.79
	b	10.16	3.28
	c	6.38	2.64
2	a	1.92	2.64
	b	4.99	2.18
	c	2.13	
5	a	3.18	1.84
	b	2.49	3.30
	c		
8	a	2.10	3.00
	b		1.11

Figure 1. Whole-body retention as percentage of the day 1 body burden in hamsters exposed to (a) trivalent and (b) pentavalent ^{124}Sb aerosols. Means and standard deviations are plotted. Dotted lines indicate straight line approximations to the curve.

Table 2
Tissue Concentration of Inhaled ^{124}Sb in Hamsters (%T/TT/gm)*

Tissue	Valence of Aerosol	Sacrifice - Days Post-Exposure						
		0	1	2	5	8	15	32
Femur	+3	0.92	0.94	0.88	2.40	3.00	3.40	3.40
	+5	1.10	0.99	1.40	3.80	4.00	3.50	6.00
GI Tract	+3	3.80	4.40	4.60	1.40	1.10	0.68	0.17
	+5	2.60	6.00	4.00	1.10	0.71	0.34	0.18
Head Skin	+3	1.80	2.20	1.90	4.10	4.10	3.60	7.10
	+5	4.30	2.20	4.00	4.40	4.80	4.30	4.00
Liver	+3	1.20	1.50	1.60	4.20	4.20	4.10	2.10
	+5	0.73	0.66	0.76	0.94	1.10	1.10	1.00
Lung	+3	0.73	0.78	1.10	3.20	3.40	1.80	1.30
	+5	0.46	0.70	0.90	1.40	1.92	1.80	1.50
Muscle	+3	0.54	0.82	0.17	0.27	0.20	0.27	0.17
	+5	0.38	0.54	0.23	0.16	0.10	0.07	0.06
Skin	+3	0.53	0.51	1.30	0.95	1.60	1.40	1.80
	+5	1.70	2.00	0.56	2.00	1.16	1.60	1.50

* %T/TT/gm = % Total Tissue Content per gm of Tissue

Table 3
Tissue Content of Inhaled ^{124}Sb in Hamsters as Percent
of the Total Tissue Content

Tissue	Valence of Aerosol	Sacrifice - Day Post-Exposure						
		0	1	2	5	8	15	32
Femur	+ 3	1.10	0.99	0.94	2.80	3.40	3.70	3.80
	+ 5	1.20	0.95	1.50	4.40	4.00	5.80	6.90
GI Tract	+ 3	49.00	55.00	55.00	14.00	11.00	7.80	2.20
	+ 5	31.00	55.00	47.00	12.00	16.00	4.90	2.80
Head Skin	+ 3	5.80	7.20	5.80	12.00	12.00	12.00	23.00
	+ 5	14.00	7.70	14.00	15.00	16.00	14.00	13.00
Liver	+ 3	3.30	0.56	3.90	13.00	13.00	14.00	11.00
	+ 5	2.00	1.60	1.90	2.70	3.60	4.30	5.00
Lung	+ 3	0.80	0.72	0.98	2.50	3.90	1.80	1.50
	+ 5	0.40	0.50	0.83	1.10	1.70	1.80	1.70
Skin	+ 3	6.80	5.80	11.60	10.50	16.00	16.00	17.00
	+ 5	19.00	10.20	6.50	21.00	14.00	20.00	21.00

DISCUSSION

The initial rapid drop in the body burden can be partially explained by a large degree of upper respiratory tract clearance, reflected by high gastrointestinal tract burdens at the early sacrifice times. Also, the aerosols were apparently extremely soluble as little activity was detected in the lungs, even at 0 day sacrifice times. The long-term biological retention time was 16 days for both the trivalent and pentavalent aerosols. This is short compared to the biological whole-body retention of 40 to 100 days in dogs after inhalation of a ^{124}Sb -tartrate aerosol (see this report, pp. 90 - 95). Djuric et al.¹ report long-term biological whole-body retention times of 120 days in rats after inhalation of a soluble ^{124}Sb -chloride aerosol. It is possible that in this study, a longer term component to the retention curve would have become visible if the animals had been observed over a longer period post-exposure.

The inhaled ^{124}Sb tartrate of both valence states was excreted in both urine and feces. Fecal antimony may have resulted from lung clearance via the mucociliary apparatus. Also, excretion via the bile is known to occur with trivalent antimony.² Biliary excretion may also account for fecal content of the pentavalent antimony since no statistical difference was noted between the trivalent and pentavalent antimony in the mean urine/feces ratios throughout the experiment. No more rapid excretion of the pentavalent antimony, as compared to the trivalent, occurred in the urine, though this was reported by Goodwin and Page³ following intravenous injection of antimony in the two valence states in mice.

The RBC antimony levels differed between the trivalent and pentavalent forms only in the first few collections. Trivalent antimony was concentrated in the RBCs at all sampling times. The 0 day samples from the pentavalent antimony group revealed the highest activity in the plasma, with relatively low levels in the RBCs. However, in later samples the pentavalent antimony was also concentrated in the RBCs.

Most important tissues in dose considerations for these exposures would be lung, liver, skeleton and skin. The rapid loss of the lung burden attests to the solubility of the aerosol. However, a small amount of the initial lung burden was retained with an apparently long half-life, so that by the 32-day sacrifices, activity concentrations in the lung were comparable to those in body tissues accumulating ^{124}Sb . Thus, considerable dose to the lungs could occur over time as a result of exposure to these aerosols. As in the study by Gellhorn et al.⁴ and Otto and Maren,⁵ the liver had greater accumulation and retention of trivalent than of pentavalent antimony. This was taken as evidence that little in vivo reduction of the pentavalent antimony occurred. Reduction of the pentavalent antimony would have resulted in subsequent accumulation in the liver equivalent to that seen in the trivalent group. Table 4 lists estimated doses to femur, liver and lung for a 32-day period following inhalation of the trivalent and pentavalent ^{124}Sb aerosols. Doses to liver and lung were higher for the trivalent form, almost a factor of 4 for liver. The skeletal dose did not seem to be valence-dependent. In the pentavalent group, concentrations in the femur by 32 days post-exposure were consistently higher than in the liver, and therefore, the skeleton appears more important than liver in dosage considerations.

Table 4
Dose in Rads to Femur, Liver and Lung of
Hamsters Exposed to Trivalent and to
Pentavalent ^{124}Sb Aerosols. Dose was
Calculated for 32 Days Post-Exposure and was
Based Upon an Initial Body Burden of $1\ \mu\text{Ci}$

<u>Organ</u>	<u>Valence State</u>	<u>Dose in Rads</u>
Femur	+ 3	1.70
	+ 5	1.70
Liver	+ 3	2.30
	+ 5	.61
Lung	+ 3	1.40
	+ 5	.82

In the literature the skin is rarely noted to accumulate antimony. However, some studies have reported relatively high skin levels after subcutaneous⁶ and intraperitoneal² injections of trivalent organic antimonial compounds. Also, high skin levels of antimony have been reported after inhalation of ¹²⁴Sb-tartrate aerosols in mice (1969-1970 Annual Report, LF-43, pp. 66-70) and dogs (see this report, pp. 90 - 95). In this study, a large variability in the percentage of total tissue activity retained in the pelt seems to indicate that skin radioantimony was due to contamination. However, the retention in the head skin resembled that of other tissues with early rapid loss, followed by a decreased rate of loss. Thus, the skin may accumulate radioantimony in the fashion of some of the internal organs. Detailed analysis of the pelt revealed that about half of the activity was in the hair, but the manner in which it is associated is not clear.

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EFFECT OF TEMPERATURE OF AEROSOL FORMATION ON METABOLISM OF INHALED ^{124}Sb IN THE BEAGLE DOG

ABSTRACT

To evaluate the influence of varied thermal treatment on the biological behavior of radio-antimony aerosols, groups of three dogs were exposed to aerosols that had been nebulized from a ^{124}Sb -tartrate complex and passed through a heating column maintained at 100°C, 500°C, or 1000°C. One dog from each group was sacrificed at 32, 64 and 128 days post-exposure. Increasing the temperature appeared to decrease the mean particle size of the aerosol. The aerosol with largest particle size, generated at 100°C, showed the least overall whole-body retention, due primarily to rapid early clearance. Much of this aerosol was cleared from the upper respiratory tract or was solubilized in the lung and excreted in the urine. The 500°C aerosol was retained to a greater extent than the 1000°C aerosol, both in the lungs and on a whole-body basis. Average long-term biological half-lives were 100, 36 and 45 days for the 100°C, 500°C and 1000°C aerosols, respectively. Elimination occurred in both urine and feces, with urine/feces ratios for individual collections generally less than one. Red blood cells were observed to concentrate ^{124}Sb . In the tissues, ^{124}Sb concentrations were highest in the lung, thyroid, liver, spleen and pelt. For all three aerosols, the thyroid concentrated ^{124}Sb to a greater extent than any other tissue. With the 500°C and 1000°C aerosols, the lungs contained the next highest concentrations of ^{124}Sb . The pelt contained ^{124}Sb concentrations second only to the thyroid for the aerosol at 100°C. Because of its size, the pelt contained a large portion of the body burden at all sacrifice dates for all three aerosols. Skeletal concentrations of ^{124}Sb were significant only for the 100°C aerosol.

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INTRODUCTION

Since several antimony isotopes are present in the fission product inventory of an operating nuclear reactor they pose a potential hazard in the event of a nuclear accident. The most abundant antimony isotope in a thermal nuclear reactor is ^{127}Sb , however ^{124}Sb is more convenient to study because of its half-life of about 60 days and strong gamma emission.

The thermal condition may be an important variable in determining aerosol characteristics of fission products accidentally released into the atmosphere. The temperature of aerosol generation may alter the physical and chemical properties of an inhaled aerosol and thereby change lung solubility and other biological behavior. This study was undertaken to investigate the effect of temperature on the metabolism of inhaled ^{124}Sb aerosols in Beagle dogs. To simulate different thermal conditions, aerosols were passed through a heating column maintained at various temperatures, before inhalation by the animal.

METHODS

One week prior to inhalation exposure, nine dogs were moved into the same metabolism cage in which they were to be housed during the experiment. They were fed a standard diet and allowed water ad libitum. During the pre-exposure period, they were acclimated to the cloth sling in which they are confined during exposure. For 24 hours before exposure, the dogs were denied food. At 2.5 hours prior to exposure, 60 mg pentobarbital was administered; 45 minutes before exposure Vetame[®] was injected intravenously (0.5 mg/kg body weight).

A Lovelace nebulizer was used to produce aerosol droplets from a starting solution of ^{124}Sb -tartrate complex. Before entering the exposure chamber, the droplets were directed through a heating column maintained at one of three different temperatures, 100°C, 500°C and 1000°C, and subsequently cooled with diluting air. Three dogs were exposed at each temperature of aerosol generation. The particle size distribution characteristics of each aerosol were determined by cascade impactor analyses and air concentrations were determined from measurements on membrane filter samples, taken during the inhalation exposure runs.

Blood samples were periodically drawn from the jugular vein of at least one dog for each temperature of aerosol generation. Total sampling period covered 21 days. The samples, approximately 8 ml each, were centrifuged at 2000 rpm for 10 minutes to separate RBCs and plasma. Each fraction was weighed and counted for radioactive content on a solid scintillation detector. Urine and feces collections were made on three dogs, one at each temperature of aerosol generation, for 32 days post-exposure. Samples were counted in a deep well liquid scintillation counter.

At various times post-exposure, the dogs were whole-body counted and scanned with NaI detectors. One dog from each aerosol group was sacrificed at 32, 64 and 128 days post-exposure. After dissection the individual tissues were weighed and analyzed for radioactive content in the deep well liquid scintillation counter.

RESULTS

The physical effect of raising the temperature of aerosol generation appeared to decrease the aerosol AMAD and perhaps the σ_g (Table 1). This probably occurred by degradation of the organic portion of the molecule (tartrate).

Whole-body retention patterns for the 128-day sacrifice animals are shown in Figure 1. Initial clearance of the 100°C aerosol was the most rapid, involving 81 to 86% of the initially deposited material. Average long-term biological half-lives of 100, 36 and 45 days, for the 100°C, 500°C and 1000°C, respectively, correspond to effective half-lives of 88, 23 and 26 days.

Table 1

Activity Median Aerodynamic Diameters (AMAD) of Aerosols Inhaled By Dogs
in this Study (Particle Size Determined by a Cascade Impactor)

Temperature Of Aerosol Treatment	Group A *		Group B *		Group C *	
	AMAD	σ_g	AMAD	σ_g	AMAD	σ_g
100°C	1.25	1.6	1.28	1.6	1.33	1.5
500°C	0.87	1.6	1.01	1.6	1.01	1.5
1000°C	0.33	1.3	0.32	1.4	0.34	1.3

* Groups A, B and C represent dogs sacrificed at 32, 64 and 128 days, respectively.

Figure 1. Whole-body retention in Beagle dogs of inhaled ^{124}Sb -tartrate generated at three temperatures. Data adjusted to date of exposure to correct for physical decay. Curves were drawn according to least squares fit to the data.

Whole-body scans illustrate shifts in the gross distribution of inhaled material with time. Scan data of an animal which inhaled a 100°C aerosol, from the first 8 days post-exposure, are represented in Figure 2a. The large particle size of the aerosol generated at 100°C resulted in a considerable burden in the nasal area, apparent in the early scans. The peak near the tail on day zero may be gastrointestinal tract content, or, with this soluble aerosol it could represent activity in the bladder. No comparable burden was apparent with either of the other aerosols (Figs. 2b and 2c). In dogs which inhaled either the 500°C or the 1000°C aerosols, clearance of the lung burden was considerably slower than with the 100°C aerosol.

Figure 2. Whole-body scans of dogs that inhaled ^{124}Sb -tartrate aerosols generated at three temperatures. Scans from days 0, 1 and 8 post-exposure are plotted. Counts were corrected for machine efficiency and physical decay. Scans are of a dog that inhaled an aerosol at (a) 100°C, (b) 500°C, and (c) 1000°C.

Urine/feces radioactivity ratios were variable but generally less than one for the collection period. A notable exception was the first collection of the 100°C animal which exhibited a ratio of approximately nine.

A differential concentration of ^{124}Sb in the RBCs, compared to plasma, was maintained for the entire 21 days of sampling (Table 2). Ratios were similar for the 500°C and 1000°C aerosols, but were lower at 100°C over the first 24 hours.

In most cases, the thyroid concentrated ^{124}Sb to a greater extent than any other tissue (Table 3). Exceptions to this were the last two sacrifice dogs that inhaled the 500°C aerosol, in which the lung values were slightly higher. The pelt also concentrated ^{124}Sb to a considerable extent. The lungs of the 64- and 128-day sacrifice animals, which inhaled the 1000°C aerosol, contained almost the same percentage of the initial body burden per gram. On a per organ basis, the pelt is seen to contain a large portion of the body burden. Except at 500°C, it is always the tissue with the largest total ^{124}Sb .

Table 2
Red Blood Cells/Plasma Activity Ratios on a Per Gram Basis
in Beagle Dogs Following Inhalation of ^{124}Sb

Animal	Temperature of Aerosol Generation	Time Post-Exposure													
		1 hour	4 hours	8 hours	12 hours	1 day	2 days	6 days	7 days	9 days	10 days	14 days	15 days	20 days	21 days
842-01	100°C	0.6	1.5	7.2	--	4.6	6.8	--	4.4	--	5.6	--	8.6	--	5.21
842-02	500°C	16.1	16.1	12.5	10.6	8.3	5.5	--	5.0	--	5.4	--	10.2	--	8.41
843-01	500°C	21.3	19.2	--	--	8.2	7.4	6.8	--	6.5	--	6.8	--	--	--
843-02	500°C	15.5	14.5	--	--	6.3	5.7	4.6	--	5.0	--	6.4	--	19.0	--
842-03	1000°C	18.9	19.5	10.0	10.0	5.8	5.5	--	5.1	--	5.4	--	6.6	--	6.4
843-03	1000°C	22.2	--	--	--	6.7	6.5	5.9	--	7.7	--	7.2	--	4.9	--

Table 3
Tissue Retention of Inhaled ^{124}Sb Tartrate in Beagle Dogs at 32, 64 and 128 Days
Post-Exposure, Presented as % Initial Body Burden Per Organ and Per Gram

Tissue	100°C			500°C			1000°C		
	32*	64	128	32	64	128	32	64	128
<u>Percent Initial Body Burden Per Organ</u>									
Femur	0.02	0.01	0.01	0.02	0.01	0.01	0.03	0.02	0.01
Liver	0.30	0.10	0.08	1.12	0.62	0.53	1.28	0.70	0.38
Lung (Total)	0.09	0.02	0.01	14.85	11.16	3.41	3.19	1.25	1.46
Pelt	1.51	1.34	1.17	4.35	2.17	3.38	8.60	3.18	2.74
Thyroid	0.09	0.06	0.01	0.21	0.12	0.02	0.27	0.04	0.04
<u>Percent Initial Body Burden Per Gram</u>									
Femur	0.0090	0.00050	0.00034	0.0012	0.00070	0.00042	0.0012	0.00078	0.00039
Liver	0.0017	0.00085	0.00024	0.0054	0.0033	0.0018	0.0053	0.0034	0.0012
Lung (Total)	0.011	0.0018	0.00069	1.60	1.18	0.304	0.312	0.104	0.103
Pelt	0.0023	0.0018	0.0013	0.0052	0.0031	0.0031	0.0096	0.0047	0.0026
Thyroid	0.185	0.059	0.011	0.349	0.198	0.033	0.485	0.079	0.049

* Sacrifice days, post-exposure

DISCUSSION

Whole-body retention and tissue distribution patterns following inhalation of the ^{124}Sb aerosols depend greatly on chemical and physical form of the aerosol. The main differences among the aerosols generated at 100°C, 500°C and 1000°C correlated with the differences in particle size and lung solubility. The larger particles at 100°C resulted in a greater deposition in the upper respiratory tract as revealed by a large amount of activity in the nasal area in the whole-body scans. Also, this aerosol was readily absorbed through the lungs, resulting in high blood ^{124}Sb levels and rapid urinary excretion.

The aerosol generated at 500°C cleared less rapidly from the lungs than the aerosol generated at 1000°C. A greater retention at lower temperatures of aerosol generation is not unprecedented; for example, ^{60}CoO , generated at 1400°C was found to be more soluble than $^{60}\text{Co}_3\text{O}_4$, generated at 850°C, when inhaled in dogs (1970-1971 Annual Report, LF-44, pp. 70-75). However, when ^{124}Sb aerosols generated in the same manner as in this study were used for inhalation in mice, the 1000°C aerosol was the least soluble (1969-1970 Annual Report, LF-43, pp. 66-70). The differences between findings in mice and dogs were perhaps due to the size of the passageways of the respiratory tract, resulting in different deposition patterns for aerosols of the same AMAD.

The Task Group on Lung Dynamics¹ classifies oxides and hydroxides of antimony as "moderately soluble", exhibiting lung half-lives of 10 to 100 days. In this experiment, the higher temperatures of aerosol generation were expected to result in the oxide form of the antimony and a longer lung retention. Accordingly, as shown in the scan data, the lung half-life of the 500°C aerosol was approximately 48 days. The 1000°C aerosol cleared somewhat more rapidly but a small fraction did have a slow turnover. The 100°C aerosol, as expected, was very soluble.

The excretion of ^{124}Sb in both urine and feces in these dogs was a different finding from that reported by several investigators who have reported that trivalent antimony is excreted primarily in the urine in dogs.^{2,3} However, excretion of an inhaled substance reflects respiratory tract deposition patterns and aerosol solubility as well as normal excretory paths of the substance when administered by other routes. Continuing lung clearance via the respiratory tract accounts for a considerable portion of the fecal ^{124}Sb . This is especially likely in view of the low bile ^{124}Sb content, even at 32 days post-exposure, negating bile excretion into the GI tract. However, except for early samples, urine/feces ratios were similar in all three dogs, regardless of percentage lung retention of the aerosol.

The RBC contents of these dogs maintained a concentration differential over the plasma for the entire 21 days of sampling. The initial low RBC/plasma ratios in the 100°C dog were the only exception. According to whole-body scan and excretion data, this aerosol resulted in the greatest entry of activity into the blood stream at early times post-exposure. Hence, high plasma levels may have saturated RBC binding sites, preventing creation of a concentration gradient. More rapid elimination of plasma than of RBC ^{124}Sb may have resulted in the increase in RBC/plasma ratios in this animal. Blood retention of ^{124}Sb is very important in determining overall whole-body retention in the rat,⁴ but in the dog, retention of ^{124}Sb in the RBC was much shorter than the overall whole-body retention.

Important tissues in ^{124}Sb accumulation were lung, thyroid, liver, spleen and pelt. Liver and spleen accumulations of ^{124}Sb were expected. Concentrations of ^{124}Sb in the pelt equalled or exceeded liver at all sacrifice dates. Therefore, because of size of this organ, pelt retention of ^{124}Sb is a major factor in whole-body retention. The thyroid exhibited the greatest concentration of ^{124}Sb , and therefore would be quite important in dose considerations following exposure to radioisotopes of ^{124}Sb . The lung would be considered an important target organ after inhalation of

the aerosols generated at the higher temperatures. In some instances it would be questionable whether the lung or thyroid should be considered as the organ of greatest radiobiological importance. To emphasize this point, an estimate of radiation dose to 128 days post-inhalation exposure has been made (Table 4). Each temperature of aerosol formation resulted in its unique dosage pattern. The thyroid would appear to be most important, radiobiologically, at the lowest temperature with the lung receiving the greater dose by far, with the 500°C aerosol. With particles formed at 1000°C both lung and thyroid receive about the same rad dose, at least to 128 days. These doses emphasize the importance of the physical-chemical nature of an aerosol upon its ultimate toxicological properties.

Table 4
Estimated Radiation Doses to the Lung and
Thyroid of Dogs Exposed to ^{124}Sb Aerosols
Formed at 100, 500 and 1000°C*

Temperature Of Formation	Rads/ $\mu\text{Ci IBB}^*$	
	Lung	Thyroid
100°C	0.3	1.5
500°C	26.7	1.7
1000°C	5.2	4.3

* Doses calculated in rads per 1 μCi initial body burden (IBB), to 128 days post-inhalation exposure using an average β energy of 0.35 Mev per disintegration.

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IN VITRO SERUM PROTEIN BINDING OF SOME CITRATED LANTHANON
NUCLIDES AND THE BEHAVIOR OF ^{144}Ce (III) AND ^{91}Y (III) AS
CITRATED AND SERUM PROTEIN BOUND FORMS IN BEAGLE DOGS
AFTER INJECTION

ABSTRACT

The distribution and excretion patterns of injected actinides and lanthanons show a relationship to their ionic radii. The capacity of these metallic ions to form serum protein chelates, may depend on the ionic radii and may be a factor in the distribution and excretion patterns of these radionuclides. The dog serum protein binding of ^{144}Ce (III), ^{160}Tb (III), ^{91}Y (III), and ^{171}Tm (III) in citrate form were determined by a gel filtration method. In fresh dog serum, the percent of ^{144}Ce , ^{160}Tb , ^{91}Y and ^{171}Tm bound to serum proteins was 81, 64, 41, and 2, respectively. In vivo serum protein binding of ^{144}Ce and ^{91}Y was determined after injections of the citrate and serum protein bound forms of ^{91}Y and ^{144}Ce . In all cases the percent of ^{91}Y and ^{144}Ce bound to protein was similar to that observed in vitro. Tissue distribution and excretion patterns of injected ^{144}Ce and ^{91}Y in citrate and protein bound forms were studied in eight Beagle dogs, two in each group. No major difference in the behavior of the same nuclide injected as citrate or protein form could be observed. A possible explanation for these observations may be the rapid formation of equilibrium conditions in the blood after injection of either chemical form. The differences in serum protein binding capacities may be a possible explanation for the general trend in distribution and excretion patterns of lanthanons injected as citrate.

PRINCIPAL INVESTIGATORS

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INTRODUCTION

Tissue distribution and excretion patterns of citrated actinide, lanthanon and yttrium (an element which is chemically similar to the heavier lanthanons) radionuclides after injection show a relationship to their ionic radii.^{1,2} With a decrease in radii, higher urinary excretion, longer retention in blood, higher fractional deposition in the bone, and lower fractional deposition in the liver and other soft tissues were reported.² These differences may be related to the in vivo interaction of the injected chemical specie with the constituents in the blood. Among the many possible reactions, serum protein binding of these radionuclides may be a very important one. The extent of protein binding and the stability of the metal protein compound could affect the transport, tissue distribution and excretion pattern of the injected metallic radionuclides.

Study of the serum protein bound forms of lanthanons may improve our understanding of the general behavior of radionuclides entering in blood. Such information may be used to improve models for calculating dose distributions of radionuclides entering blood and to improve methods for the therapeutic elimination of radionuclides from blood.

In vitro serum protein binding of citrated ^{144}Ce (III), ^{160}Tb (III), ^{91}Y (III) and ^{171}Tm (III) was determined by gel filtration chromatography.³ The tissue distribution and excretion patterns of intravenously injected ^{144}Ce (III) and ^{91}Y (III) as citrate and serum protein bound forms in Beagle dogs were also determined. The in vivo serum protein binding in Beagle dogs after injection of ^{144}Ce (III) and ^{91}Y (III) as citrate and protein bound forms was also determined by gel filtration.

EXPERIMENTAL METHODS

The radionuclides, $^{144}\text{Ce(III)}$, $^{160}\text{Tb(III)}$, $^{91}\text{Y(III)}$ and $^{171}\text{Tm(III)}$ in their chloride forms were mixed with their respective carrier solutions in citrate form, pH adjusted to 7.3 and filtered through 25 μm pore size membrane filters. The concentration of the lanthanons was 1.0×10^{-3} M and the citrate concentration was 0.10 M.

Fresh Beagle dog serum samples were prepared by collecting blood in non-heparinized vacutainers, allowing it to clot for 10 minutes and centrifuging at 2000 rpm. The isotope solutions were added into the fresh serum, dropwise, while stirring, and stirring was continued for one hour. The equilibration was conducted under 5% CO_2 in N_2 atmosphere so that the pH of the system remained at 7.3.

Bio-Rad P-30 (100-200 mesh) gel was used unless noted otherwise. The bed volumes were 50 ml and when samples were eluted in this system, substances with molecular weight $> 50,000$, were collected between 16th to 22nd ml of the eluant volume. The eluant used in these experiments contained 0.13 M NaCl, 0.02 M NH_4Cl and 0.005 M NaHCO_3 . This eluant maintained a stable pH of 7.3 to 7.4 up to 24 hours and contained no chelating agents which could interfere with the binding of the lanthanons with the serum proteins. The flow rate of the eluant in the columns was above 15 ml per hour and the experimental temperature between 22 to 24°C .

Eight purebred Beagle dogs of 1 to 6 years of age were used in this experiment. Four groups of two dogs each were injected with ^{91}Y or ^{144}Ce in citrated or protein bound form. The citrate form of the injection solution consisted of 1.8×10^{-5} M of the yttrium or cerium and 1.8×10^{-3} M citrate in saline solution at pH 7.3. The estimated concentration of the yttrium or cerium in the protein bound form of the injection solution was also 1.8×10^{-5} M. The ^{91}Y and ^{144}Ce in the serum protein bound form were prepared separately by obtaining 5 ml of fresh serum from each dog, equilibrating the serum with $^{91}\text{Y(III)}$ or $^{144}\text{Ce(III)}$ citrate and separating the ^{91}Y or ^{144}Ce in the protein bound form by gel filtration. The protein bound ^{91}Y or ^{144}Ce were collected within 6 ml of the eluant at the exclusion volume. The radionuclide solutions, 2 ml, were injected into the cephalic vein and infused with sterile physiologic saline at pH 7.4. Prior to injection the dogs were given 1 ml of Innovar-Vet.

The dogs were whole-body counted immediately after injection and daily thereafter. Ten ml of blood was drawn from each dog at 20 minutes, 1 hour, 4 hours, 24 hours and 4 days post-injection. Dogs were kept in metabolism cages and excreta were collected daily. The dogs were sacrificed at eight days post-injection and distributions of radioactivities in tissues determined.

The radioactivity of gel filtration samples were determined by counting beta emissions. ^{144}Ce , ^{160}Tb and ^{91}Y were planchettetted and counted in a 2 π proportional counter. ^{171}Tm was counted in a liquid scintillation spectrometer. All tissues and excreta were analyzed by radiochemical procedures and counted in the 2 π proportional counter.

RESULTS AND DISCUSSION

In Vitro Protein Binding Studies:

The results of in vitro serum protein binding studies are summarized in Table 1. In these experiments, 1.0 ml of the serum-radionuclide equilibrium mixture were loaded on the column. The protein bound activity was eluted within the exclusion volume (16 to 22 ml) and is expressed as the percent of loaded activity. Also included in the table are the ionic radii and K , the formation constant of the lanthanons with ethylene diamine tetraacetic acid (EDTA) dentate.⁴

$^{144}\text{Ce(III)}$, the largest of these lanthanons, generally forms weaker chelates than the smaller lanthanons. The stability constants of these lanthanons with other chelates vary very much like the

Table 1
In Vitro Serum Protein Binding of Citrated Lanthanons

Isotope	Ionic Radii Å	K*	% Serum Protein Binding
$^{144}\text{Ce(III)}$	1.034	2.4×10^{15}	81 ± 4
$^{160}\text{Tb(III)}$	0.923	1.8×10^{17}	64 ± 4
$^{91}\text{Y(III)}$	0.880	2.4×10^{17}	41 ± 9
$^{171}\text{Tm(III)}$	0.869	3.8×10^{18}	1 to 2

*K is the formation constant of the lanthanon with EDTA.

K values shown in the table.⁴ The K values for the EDTA complex were included in this table since they are reliable and that of citrates are not available.

The general trend in the percent protein binding shown in Table 1 was unexpected. Since the binding of the lanthanon ions with serum protein was expected to involve chelation, the reverse order was expected. However, the serum protein binding may involve a competitive equilibrium reaction of the lanthanon (Ln) nuclide between two chelating agents, the citrate and the serum protein.

where M represents a metal ion present in the serum protein, Pr. Such a reaction could account for the experimentally observed order. With an increase in Ln-citrate stability the equilibrium reaction may shift to have more Ln-citrate and less Ln-Pr.

The ^{91}Y -protein binding showed considerable variations and only a very small percentage of the ^{171}Tm was found to bind with the protein. Experiments with Bio-Gel P-200 were also performed. The results indicated that both the $^{144}\text{Ce(III)}$ and $^{91}\text{Y(III)}$ bind with protein molecules of molecular weight about 70,000. However, studies with commercial serum protein showed very little (< 5%) binding with either ^{91}Y or ^{144}Ce . These studies included dog, bovine and human serum protein fractions of albumin, alpha globulins and beta globulins.

In Vivo Protein Binding Studies:

The percent protein binding of injected ^{91}Y citrate, ^{91}Y serum protein, ^{144}Ce citrate and ^{144}Ce protein in dog serum were determined by gel filtration method. The results are summarized in Table 2. The percent protein binding of the nuclides seems to be independent of time post-injection. However, it should be noted that elapsed time between drawing the blood and separation on the column were about one hour. The average percent of ^{144}Ce existing in serum as ^{144}Ce -protein after injections of ^{144}Ce -protein and ^{144}Ce -citrate were 84 and 78, respectively. The observed difference in percentages of ^{144}Ce existing in dog serum as ^{144}Ce -protein after the injection of the two forms is within the experimental variations. The average percent of ^{91}Y existing in serum as ^{91}Y protein after the injection of ^{91}Y protein (59%) was higher than the average percent of ^{91}Y -protein in serum after injection of ^{91}Y -citrate (43%). In both the ^{91}Y protein and ^{144}Ce -protein injections, the nuclides were originally 100% in the protein bound form. The lower observed percentage of protein bound forms of the nuclides suggest in vivo reactions which tended to diminish the protein bound form.

Both the in vitro and in vivo protein binding studies show that the citrated ^{144}Ce has higher capacity to bind with serum proteins than citrated ^{91}Y and the percent serum protein binding of these two nuclides in both in vitro and in vivo systems are comparable.

Table 2

In Vivo Serum Protein Binding of ^{91}Y and ^{144}Ce Injected
in the Citrate and Protein Bound Form (% Protein Bound)

Injection Solution	Dog No.	Time Post-Injection			
		20 min	1 hr	4 hr	24 hr
^{144}Ce Protein	1	84	85	79	
	2	87	85	89	83
^{144}Ce Citrate	3	72	74	84	
	4	78	81	79	78
^{91}Y Protein	5	62	61		
	6	55	58		
^{91}Y Citrate	7	49	47	46	
	8	38	40	43	42

Tissue Distribution and Excretion Patterns of
Injected ^{144}Ce and ^{91}Y in Citrate and Protein-
Bound Forms:

The whole-body retention data, obtained from whole-body counting, of the injected ^{144}Ce and ^{91}Y are shown in Figure 1, expressed as the percent of initial body burden (% IBB) versus the days post-injection. In all cases, the ^{144}Ce retentions were considerably higher than that of ^{91}Y . At eight days post-injection all four dogs injected with ^{144}Ce showed about the same (95% of IBB) whole-body retention. Except for one dog which was injected with ^{91}Y citrate, the ^{91}Y whole-body retentions at eight days post-injection were about 80% of IBB. The decrease in whole-body burden in dogs injected with the citrate form of ^{144}Ce or ^{91}Y occurred mostly during the first day while the reductions in body burdens of ^{144}Ce and ^{91}Y after injection of the protein forms of these nuclides occurred over a longer time period.

More than 95% of the ^{144}Ce and ^{91}Y activities in blood were present in the serum fraction after the injection of the two nuclides in citrate or protein forms. The decreases of ^{91}Y and ^{144}Ce activity in the serum with respect to time post-injections are shown in Figures 2 and 3, respectively. The data are expressed as the fraction of initial body burden per ml of serum at hours post-injection. Rapid decrease of ^{144}Ce and ^{91}Y activities in serum occurred. Slightly higher ^{91}Y activity than

Figure 1. Whole-body retention of ^{91}Y and ^{144}Ce injected as citrate and serum protein bound forms.

Figure 2. ^{91}Y activity in dog serum after injection of ^{91}Y in citrate and protein bound forms.

Figure 3. ^{144}Ce activity in dog serum after injection of ^{144}Ce in citrate and protein form.

^{144}Ce activity in the serum, between 1 and 24 hours post-injection was noted. The serum samples taken at 20 minutes post-injection showed higher fraction of ^{91}Y per ml of serum after injection of ^{91}Y citrate than that observed after the injection of ^{91}Y protein form. The fractions of ^{144}Ce per ml of serum taken at 20 minutes post-injection were higher after injection of ^{144}Ce protein form than after injection of ^{144}Ce citrate.

The cumulative excretion of injected ^{91}Y (Fig. 4) and ^{144}Ce (Fig. 5) are shown as the percent of IBB versus days post-injection. As expected from the whole-body retention data, the ^{91}Y excretions were higher than those of ^{144}Ce . During the first day the excretions of ^{91}Y and ^{144}Ce were higher from dogs injected with the citrate form of the nuclides than the corresponding dogs injected with the protein form of the nuclides. There were no other significant differences seen between the excretion pattern of the same nuclide injected as citrate or protein forms.

The tissue distributions at eight days post-injection of ^{91}Y and ^{144}Ce in citrate and protein bound forms are shown in Table 3 as the percent of sacrifice body burden (SBB). Liver and bone contained most of the radioactivity. The differences in distribution between ^{91}Y and ^{144}Ce were similar to those reported by Durbin.^{1,2} Consistent differences in the distribution of ^{91}Y after injection of ^{91}Y citrate and ^{91}Y protein were not seen. Fractional retention of ^{144}Ce in the liver was slightly higher and slightly lower in the bone after injection of ^{144}Ce -protein than after the injection of ^{144}Ce citrate.

The whole-body retention, retention in blood, tissue distributions and excretion patterns of ^{91}Y and ^{144}Ce after injection of ^{91}Y citrate and ^{144}Ce citrate were generally similar to values reported in the literature. Only minor differences could be observed in the behavior of the citrate and protein forms of the two nuclides after intravenous injection. The fractions of ^{91}Y and ^{144}Ce in the blood serum existing in the protein bound form, after injections of ^{91}Y and ^{144}Ce as citrate

Figure 4. Cumulative excretion of ^{91}Y after injection of ^{91}Y citrate (curves 1 and 2) and ^{91}Y protein (curves 3 and 4).

Figure 5. Cumulative excretion of ^{144}Ce after injection of ^{144}Ce citrate (curves 1 and 2) and ^{144}Ce protein (curves 3 and 4).

Table 3
Tissue Distribution of Injected ^{91}Y and ^{144}Ce as Citrate
and Protein Bound Form in Beagle Dogs at 8 Days Post-Injection
(% Sacrifice Body Burden)

Nuclide and Form	Bone	Liver	Spleen	Kidneys	G. I.
^{91}Y Citrate	89	7.6	0.2	1	2
	72	22	0.3	3	2
^{91}Y Protein	60	28	2	5	4
	76	14	1	4	3
^{144}Ce Citrate	30	66	0.2	2	1
	44	49	0.2	3	2
^{144}Ce Protein	22	71	2	3	1
	19	76	2	1	1

and protein bound form, tend to approach equilibrium values which were similar to those observed in in vitro studies. Thus, an in vivo transformation of the injected chemical form of the radionuclide into an equilibrium system is indicated and may explain the lack of major differences between the behavior of the injected citrate and protein forms of the two nuclides. A possible explanation for the general trend in lanthanon distribution and excretion, after injections in animals, may be due to the differences in their serum protein binding capacities. The larger lanthanons exist in blood with a larger fraction being bound to the serum proteins and may be a factor in the higher fractional deposition in the liver. Excretion and deposition in the skeleton of larger lanthanon nuclides is consistent with higher fraction of these radionuclides existing in the protein bound form. Higher fractional deposition in the skeleton and higher fractional excretions of small lanthanon radionuclides could be related to the existence of a major fraction of the radionuclide in blood in a diffusible form, such as the citrate form of the radionuclide. A possible role of this form in the uptake of yttrium in bone has been suggested by Neuman et al.⁵ and Dearmaley.⁶

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TREATMENT OF SCANNING DATA FOR DETERMINATION OF IN VIVO SOLUBILITY CHARACTERISTICS

ABSTRACT

Inhaled particles that are deposited in the lower respiratory tract are partly cleared by dissolution. This process is dynamic, in that an ion released from a particle surface normally acts independently of the particle, and behaves according to its chemistry. The ion may bind to materials in the lung or enter the blood. In studies of inhalation toxicology, the solubility characteristics of inhaled aerosols are of interest. One technique used to obtain these data is that of measuring the retention pattern of aerosol particles following intramuscular injection of a suspension of the particles. This report is a brief description of an approach used in analyzing small animal scanning data following intramuscular injection of various forms of a radionuclide, to determine values that describe its solubility characteristics.

PRINCIPAL INVESTIGATOR

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INTRODUCTION

Knowledge of the in vivo solubility characteristics of an inhaled aerosol is important to estimate its fate following deposition in the lung. With sufficient information, an air sample in a potentially contaminated area may be taken, its solubility characteristics determined, and the allowable levels that should prevail in that area established. In vitro solubility studies are faster and perhaps more accurate, but are not yet proven to yield results comparable to in vivo measurements. Until suitable media and methods are assessed as proper substitutes for in vivo systems, methods such as those described in this report will continue to be used.

The ultimate use of the solubility data is to apply it in Mercer's¹ formulation to arrive at a value for k , the constant of specific solubility. This is related to particle composition and has units of mass of material dissolved per unit of surface area per unit of time. Normally, this is expressed as grams per square centimeter per day. If the size distribution characteristics of a group of particles is known, and the in vivo dissolution rate can be determined as a retention half-life, then k can be calculated. Examples of small animal scanning data as obtained in this laboratory will be described and the method of analysis will be presented.

METHODS

Experimental Details:

A whole-body scanning device, consisting of a shielded NaI crystal (2 inch x 2 inch diameter) past which the rat or hamster was moved in increments slightly over 1 cm each, was used. At various times following intramuscular injection of the γ -emitting radionuclide, the animal was placed in a lucite holder under 50% CO₂-O₂ anesthesia flowing through the holder at a low rate. A motor, operating the cart upon which the holder rested, was energized through a timer on the scaler. The animal passed in one-minute increments past the crystal and the counts were recorded at the end of each pre-set time. Data in each interval appeared as printed material on a computer typewriter and on punched paper tape. The data from the punched paper tape were processed by a computer and analyzed either as the whole-body burden, the peak channel, or the peak channel plus or minus two channels, as a fraction of what these values were at injection time.

Data Analysis:

The solubility constant k , is generally most useful with relatively insoluble materials. Ideally, however, it is important to also have scanning data on a very soluble form of the same cation being followed. The soluble material will leave the muscle injection site quite rapidly and a fraction will deposit in internal organs. An example of scanning data from an intermediately soluble compound, BaSO_4 , is shown in histogram form in Figure 1. This shows how the ^{133}Ba leaves the injection site and goes, in part, to skeleton or is excreted, with time. The highest channel is taken as 1.0 in each of the three cases. Note how the barium spreads out in the body with time and also how the peak channel has shifted to over the pelvic region by 93 days. A very soluble barium compound would give a histogram like the 93-day pattern soon after injection, and an insoluble compound would take a long time to show evidence of spreading out. By analyzing such data with time, a retention pattern is obtained which, when extrapolated back to zero time, will give a value for the fraction of initial injection site localized in the internal organ(s). Thereafter, the assumption is made that any of the cation leaving the relatively insoluble material at the injection site will behave in a similar manner, and that the same fraction of mobilized material will localize in the internal organ. One important factor which must be borne in mind in analyzing these data, is that material at the injection site will count with a higher efficiency than that internally deposited, due to the proximity to the crystal face. Thus, all calculations should deal with data corrected to either injection site or non-injection site geometry.

One step in the analysis is to determine what fraction of non-injection site deposited radionuclide (following some dissolution) is contributing to the injection site scanning data. With the more insoluble substances, the injection site generally appears in the same channel (segment along the body) or plus or minus one channel. It is necessary to determine this channel and then analyze the scans from the soluble form. The total scan is summed to obtain what is essentially a body burden. Then the fraction of this total scan that appears in the injection site channel is calculated,

Figure 1. Histograms representing the whole-body scanning pattern at times 0, 57 and 93 days after intramuscular injection of $^{133}\text{BaSO}_4$ in rats.

and represents the contribution of the whole internal deposit to the injection site. This could be done using histograms A and C in Figure 1. The peak injection site channel in A is number 18. All the ^{133}Ba left the injection site by 93 days and histogram C represents skeletal uptake. The fraction of the total histogram that appears in channel 18 would give the value for the skeletal ^{133}Ba reflected in the injection site count in histogram A.

If one assumes the actual loss from the injection site to be exponential, as Mercer's theory predicts, then an equation for the injection site may be derived as follows:

$$I = I_0 e^{-\lambda_1 t} + F_1 Q \quad (1)$$

where

I = amount of radioactivity detected at the injection site at $t = t$,

I_0 = amount of radioactivity detected at the injection site at $t = 0$,

λ_1 = rate constant of loss from I ,

Q = amount of radioactivity detected from the internal organs at $t = t$,

F_1 = fraction of internal deposit reflected in the injection site, determined as just described.

The next step is to arrive at a value for Q . A first order differential may be set up as follows:

$$\frac{dQ}{dt} = F_2 \lambda_1 I - \lambda_2 Q \quad (2)$$

where

F_2 = fraction of material having left the injection site and detected as Q ,

λ_2 = rate of loss of Q .

The value for F_2 is taken from the retention data of the soluble form, as described above. The rate constant λ_2 , is also obtained from the retention kinetics of the internally deposited soluble form. It is assumed to be a first order kinetic rate of loss. Solution of this equation yields,

$$Q = \frac{\lambda_1 F_2 I_0}{\lambda_2 - \lambda_1} (e^{-\lambda_1 t} - e^{-\lambda_2 t}). \quad (3)$$

Substitution of this into the injection site equation results in the following,

$$I = e^{-\lambda_1 t} + \frac{\lambda_1 F_1 F_2}{\lambda_2 - \lambda_1} (e^{-\lambda_1 t} - e^{-\lambda_2 t}), \quad (4)$$

where I_0 has been taken as 1.0.

RESULTS AND DISCUSSION

The data obtained from the computer analysis of the scanner input are plotted as fractions of their initial values versus time and are subjected to standard curve fitting. When expressed as a fraction of the initial peak (injection site) value with time, an empirical expression for I is obtained. This is generally a single exponential for relatively insoluble substances. With all other constants determined as described, the only unknown in equation (4) is λ_1 , the rate constant of loss due to in vivo solubility. To date, this had been calculated by substitution of values for λ_1 , until both sides of the equation are equal. Inasmuch as this is done only at the end of a study, it has not been feasible to program the procedure into a computer analysis.

The use of λ_1 can be demonstrated using Mercer's theory. He has derived a relationship that equates the injected material and its biological properties with the constant of specific solubility (k):

$$k \sim \frac{D_M}{t_{1/2}} \quad (5)$$

where

D_M = mass median diameter of the injected particle distribution,
 $t_{1/2}$ = half-life of loss from the deposition site by dissolution.

In our studies, the relatively insoluble particles have been obtained from actual animal inhalation exposures. The aerosol is collected on a membrane filter and subsequently resuspended in distilled water by ultrasonification. A portion of this has been injected intramuscularly. From sampling of the aerosol, D_M is known, and $t_{1/2}$ is obtained from λ_1 . Thus, k can be calculated directly.

For inhaled materials, k may be determined from intramuscular injection as described, and the $t_{1/2}$ for deep lung retention determined from whole-body counting and serial sacrifice tissue distribution. Using these two parameters a value for D_M may be calculated to represent the particle distribution that may have initially been deposited in the pulmonary spaces. The main problem with this procedure at this point is that the fraction of clearance by dissolution is not well known, so the relative importance of this process is yet to be judged. Results with monodispersed aerosols currently underway should shed considerable light on this matter.

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CORRECTION OF RECTILINEAR SCANNER DATA
TO DETERMINE THE LUNG BURDEN OF ^{90}Sr - ^{90}Y
FUSED CLAY PARTICLES IN THE BEAGLE DOG

ABSTRACT

A rectilinear scanner is used routinely in this laboratory to determine the lung burden in Beagle dogs exposed to aerosols containing relatively insoluble radioactive particles such as ^{90}Sr - ^{90}Y in fused montmorillonite clay. Determination of the lung burden of ^{90}Sr - ^{90}Y in fused clay particles has the associated problem of a background level of ^{90}Sr resulting from dissolution of a portion of the lung burden and deposition in skeleton. Acceptable estimates for lung burden of ^{90}Sr - ^{90}Y in fused clay particles are possible using the following procedure: (1) determine the net radioactivity content per channel for the 184 channels (extra-lung) comprising the scan regions anterior and posterior to the 61 channels defined as the lung peak region of the scan, (2) subtract 1.43 times the net radioactivity content per channel in the extra-lung region of the scan from each channel of the lung peak portion of the scan, and (3) sum the net corrected lung peak channels to yield the value for lung burden of ^{90}Sr - ^{90}Y in fused clay.

PRINCIPAL INVESTIGATOR

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INTRODUCTION

A rectilinear dog scanner is used in this laboratory to determine the lung burden in dogs exposed to a number of radionuclides in fused clay. At early times post-inhalation exposure (0 to 3 days), quantitation of the lung burden is difficult due to the presence of radioactivity in the gastrointestinal tract primarily because of upper respiratory tract clearance. Swallowed clay particles have a short residence time in the esophagus and stomach; after a few days post-exposure this problem diminishes to the point where it has a negligible effect on the resolution of the lung peak.

The tracheobronchial lymph nodes (TBLN) accumulate particles from the lung at a rate of approximately 0.01% of the lung burden per day. Therefore, a true representation of the lung burden must consider the TBLN burden. The TBLN burden can become a significant fraction of the lung peak as determined using the rectilinear scanner.

The most troublesome factor in resolving the lung peak is determination of background for the scanner channels representing the lung peak region of the scan. This includes radioactivity in the thorax but not associated with lung tissue. For ^{90}Sr - ^{90}Y in fused clay this problem is magnified due to deposition of ^{90}Sr in bone subsequent to dissolution of the particles. The half-life for skeletal retention of ^{90}Sr is approximately 4 or 5 times that for lung retention. After several hundred days the skeletal burden of ^{90}Sr becomes a sizeable fraction of the retained body burden and in sufficient time could surpass the lung burden. It is therefore imperative, if scanner data are to be meaningful, to recognize and compensate for the contribution to the lung scan peak due to ^{90}Sr in skeleton.

Previous procedures have used the average counts per channel for the sum of the channels anterior and posterior to the lung peak channels to approximate the background in the lung peak region of the scan. Although this procedure is probably reasonable for early times post-aerosol exposure (PAE), it may not be appropriate at later times when the skeletal burden of ^{90}Sr becomes significant. The lung peak observed at later times represents the sum of the lung burden and the

bone burden in the thoracic skeleton of the dog. A scan of the skeleton may not yield a constant count rate per channel over the entire length of the scan.

This experiment was designed to determine the accuracy of resolving the lung peak of the scanner data under conditions of low, moderate and high skeletal burdens of ^{90}Sr . The ratio of skeletal burden to lung burden was calculated for what might be present in the ^{90}Sr -fused clay dogs in the longevity experiment two or three thousand days PAE (this report, pp. 177 to 188). An attempt was made to produce this ratio in the Beagle dog over a relatively short time period of 65 days. As 2000 days post-exposure may be the limit for resolution of the lung peak using the rectilinear scanner, this approach to the problem seemed satisfactory.

METHODS

Nine Beagle dogs, 7.5 to 10.5 kg body weight and 19.8 to 20.4 months of age, were randomly assigned to one of three experimental categories (A, B and C). All nine dogs were exposed to ^{90}Sr - ^{90}Y fused clay particles (AMAD 1.3 to 1.9 μm ; $\sigma_g \sim 1.5$ to 1.8) to achieve initial lung burdens which averaged 170 μCi (Table 1). After the aerosol exposure, dogs were whole-body counted and scanned on a rectilinear scanner at frequent intervals until 16 days PAE.

TABLE 1
Protocol for Aerosol Exposure and Intravenous Injection of Beagle Dogs

Tattoo No.	Weight (kg)	⁹⁰ Sr- ⁹⁰ Y Clay Particles		Duration of Aerosol Exposure (minutes)	ILB* (μCi)	⁹⁰ Sr- ⁹⁰ Y Citrate** (μCi)
		AMAD (μm)	σ _g			
Category A:						
474V	8.7	1.3	1.8	25	130	0
474B	10.5	1.7	1.5	18	190	0
475V	9.5	1.7	1.5	15	190	0
Category B:						
472S	10.3	1.7	1.5	13	170	41
475U	9.3	1.8	1.5	19	160	34
478A	8.3	1.9	1.5	13	160	51
Category C:						
475B	8.4	1.7	1.6	22	180	420
475A	8.9	1.6	1.5	17	210	500
481T	7.5	1.8	1.5	21	150	500

* Determined by computer fitting lung peak data between day 3 and day 65 post-aerosol exposure.

** Determined as the difference between body burden before and after I. V. administration of ^{90}Sr - ^{90}Y citrate.

On day 16 PAE the three dogs in category A were administered an intravenous injection of sterile physiological saline solution. Dogs in categories B and C received an intravenous injection of approximately 50 and 500 μCi of ^{90}Sr - ^{90}Y citrate, respectively. The injection solution contained 2% sodium citrate, pH ~ 6.0 , specific activity 36 Ci/gm. Following IV injection, all dogs were immediately scanned and whole-body counted and returned to their metabolism cages. Counting and scanning were continued until day 65 PAE. These data were subsequently used to estimate lung burdens and to make comparisons between the results from the rectilinear scanner and whole-body counting unit. On day 65 the dogs were intravenously administered an anesthetic dose of pentobarbital sodium, placed in a sling and exsanguinated by external heart puncture. After whole-body counting and scanning, the dogs "dead and intact," were positioned ventral side up on a dis-

section table and the legs secured in an extended position. A small incision was made in the skin of the neck and thorax for exposure of the trachea, lungs and heart, all of which were removed. A styrofoam "phantom" was inserted into the thoracic cavity to fill the void made by removal of the heart and lungs. This phantom was patterned after a fume-fixed lung mass; its main function was to maintain the orientation of the ribs, sternum and viscera as they were in the *in vivo* condition. A plastic bag surrounded the phantom to minimize blood absorption by the styrofoam. After closure of the incisions, the dogs were again positioned in counting boxes, counted and scanned, and then placed in a freezer. They were scanned approximately every other day for another week.

Dissections were completed on day 72 PAE. The skeletal samples were of particular interest and were segregated into (1) skull and jaws, (2) neck, (3) all ribs, (4) thoracic vertebrae, (5) femur, (6) humerus, (7) lumbar vertebrae, (8) anterior skeletal remains, and (9) posterior skeletal remains. Bones were defleshed as cleanly as possible using a scalpel. All tissue samples except blood, pelt and soft tissue remains were analyzed for ^{90}Sr - ^{90}Y .

The last five scans for each intact animal were analyzed together to represent the scan data for live dogs or dogs after sacrifice but prior to dissection. Likewise, the five scans accumulated for each dog between removal of the heart and lungs and final dissection were analyzed together to represent the scan of the skeletal burden for each dog. Scans contained 245 channels. Data were converted to μCi per channel based on net counts per channel and the calibration factor, 17 counts per μCi .

RESULTS AND DISCUSSION

One reason for making five scans during the week between sacrifice and final dissection was to detect the presence of a liver peak of ^{90}Y , if it existed. Liver is known to accumulate ^{90}Y and the presence of a significant amount of ^{90}Y in the liver can obscure the lung peak in scanner data. There was no indicator of such a peak in these data.

Table 2 presents a comparison of weight and activity distribution in three groups of skeletal components. Groups I, II and III represent scanner channels corresponding to the head plus neck, thorax, and posterior portion of the scan, respectively. The skull, jaws and neck were assumed analogous to channel Group I; all bones between the neck and lumbar vertebrae were assigned to Group II; the remainder of the skeleton comprised Group III. Data in Table 2, based on tissue analysis, must be recognized as being only approximations. It is very difficult to equate arbitrary channel groupings from the scanner data to distinct tissue groupings. Values for percent of total skeletal weight per channel and percent of total skeletal activity per channel are inversely proportional to the number of channels assigned to Groups I, II and III. This is a major source of error. There was uniform tissue sampling among these three groups as indicated by small values (less than 5% coefficient of variation) for standard deviation for percent of total weight per channel (Table 2). Of particular interest in this table is the difference between data for percent of skeletal burden per channel for scanner and radiochemistry data. Explanations for these results include a higher scanner efficiency or a greater concentration of ^{90}Sr - ^{90}Y in the thoracic skeleton. The radiochemistry data indicate ^{90}Sr - ^{90}Y concentrations in bone were essentially the same for all three groups when expressed as percent of skeletal activity per channel. The conclusion is that counting efficiency for ^{90}Sr - ^{90}Y was higher in the rectilinear scanner for the thoracic group of bones.

Ribs, scapulae and front leg bones are positioned several centimeters closer to the radiation detectors during the scanning procedure than the head, neck vertebral column and pelvis. Using the inverse square law this contribution to the total skeletal activity is counted with a higher efficiency than other portions of the skeleton which are nearer the body axis. It seems clear that correcting gross lung peak burden to net lung peak burden of ^{90}Sr - ^{90}Y must involve subtraction of

TABLE 2
Distribution of Skeletal Wet Weight and ^{90}Sr - ^{90}Y Activity Among Three Groups of Beagle Dog Skeletal Components*

Tattoo	Group I			Group II			Group III		
	Skeleton Weight	Skeleton Burden Scanner	Radio-chemistry	Skeleton Weight	Skeleton Burden Scanner	Radio-chemistry	Skeleton Weight	Skeleton Burden Scanner	Radio-chemistry
<u>Category A:</u>									
474V	0.458	N. A.	0.459	0.415	N. A.	0.413	0.360	N. A.	0.360
474B	0.465	N. A.	0.430	0.408	N. A.	0.406	0.362	N. A.	0.393
475V	0.456	N. A.	0.511	0.399	N. A.	0.373	0.378	N. A.	0.361
<u>Category B:</u>									
472S	0.419	0.309	0.379	0.436	0.551	0.454	0.369	0.336	0.384
475U	0.452	0.264	0.308	0.413	0.631	0.465	0.367	0.288	0.430
478A	0.496	0.346	0.411	0.389	0.578	0.435	0.356	0.272	0.378
<u>Category C:</u>									
475B	0.467	0.314	0.306	0.414	0.494	0.481	0.353	0.398	0.415
475A	0.446	0.282	0.312	0.413	0.494	0.457	0.372	0.420	0.435
481T	0.468	0.271	0.414	0.420	0.595	0.435	0.347	0.312	0.376
Mean	0.459	0.298	0.392	0.412	0.557	0.435	0.363	0.338	0.392
S. D.	0.021	0.031	0.072	0.013	0.055	0.034	0.010	0.060	0.028

* Data are expressed as percent of total weight or activity per channel for the three respective groups. Scanner data are for lungless dogs.

more than the average count per channel for the sum of channel Groups I and II. This is particularly true for long times PAE when skeletal burdens can be large due to dissolution of a portion of the lung burden and deposition in bone.

The scan data in Figures 1 and 2 include the mean values for the five scans of dogs in category C with and without lungs; values for the three dogs in category C are plotted together. All data for these figures were normalized by the multiplication factor: (average skeletal burden for the pertinent category)/(skeletal burden for the animal). Scans made with the lungs in place illustrate the position of the lung peak in the 245-channel scan. Changes in position of the various peaks of activity are assumed to be the result of dog size and position in the counting box. Category A dogs, which were not administered ^{90}Sr - ^{90}Y citrate and category B dogs, which had skeletal burdens which averaged 24 μCi , are not represented in the figures.

The head corresponds to the left side of the scans depicted in Figures 1 and 2. Here the skeletal burden of the radionuclide was significant (the average value was 212 μCi). There are essentially three regions of intense activity in the scan with lungs removed (Fig. 2). The head, thoracic and pelvic regions have apparent high concentrations of ^{90}Sr - ^{90}Y . Part of this may be due to the concentrated masses of bone in such regions as the head and pelvic girdle. Another factor, however, is the closer proximity of some bones to the radiation detectors.

The position of the lung peak may change if the position of the dog is shifted in the counting box. To compensate for this source of error, the following procedure was adopted for dividing scan data in the groupings presented in this experiment. For categories A and B there were no peaks in the lungless dog scan data; the position of the lung peaks in the two scans made after sacrifice of the dogs was used to define the channel groups. The assumption was made that dogs in categories A and B were positioned in the counting boxes the same way before and after removal of the lungs. Category C, containing the dogs with relatively high body burdens of ^{90}Sr - ^{90}Y , had three peaks in the scans, as discussed previously. For these dogs, the "head peak" was used as a reference point to identify the channels which would correspond to the lung peak region of the intact dog. Scanner channels 101-161 are normally used to define the lung peak. In some cases the lung peak is shifted slightly. Sixty-one channels are used regardless of the baseline.

Figure 1. Category C (02-992; 03-993; 02-994) Mean scanner data with lungs; skeleton burdens normalized to 212 μCi for each dog.

Figure 2. Category C (02-992; 03-993; 02-994) Mean scanner data without lungs; skeleton burdens normalized to 212 μCi for each dog.

TABLE 3
Activity Per Channel For Channel Groupings Used to Determine the
Lung Peak of Activity in Beagle Dogs Using a Rectilinear Scanner.
Lungs Were Removed Prior to Scanning 5 Times For Each Dog.

Tattoo	$\mu\text{Ci } ^{90}\text{Sr}-^{90}\text{Y}$ Per Channel				Thorax/
	Anterior	Thorax	Posterior	Extralung*	Extralung
Category B:					
472S	0.117	0.138	0.098	0.108	1.28
475U	0.056	0.080	0.040	0.049	1.63
478A	0.076	0.094	0.063	0.071	1.32
				Mean	1.41
				S. D.	0.19
Category C:					
475B	0.890	1.029	0.906	0.896	1.15
475A	0.861	1.193	0.992	0.939	1.27
481T	0.724	1.307	0.639	0.676	1.93
				Mean	1.45
				S. D.	0.42
				Overall Mean	1.43
				S. D.	0.29

* Anterior channels plus posterior channels.

Scanner data for the six dogs in categories B and C are presented in Table 3. These data pertain only to scans of dogs taken after the lungs had been removed. Scanner data for category A dogs were omitted; the body burdens for these three dogs were essentially zero after removal of the lungs and could not be quantitated using the rectilinear scanner. As indicated in Table 3, an average value of 1.43 was observed for $\mu\text{Ci } ^{90}\text{Sr}-^{90}\text{Y}$ per channel for the lung peak portion of the scan divided by $\mu\text{Ci } ^{90}\text{Sr}-^{90}\text{Y}$ per channel for the extra-lung region of the scan. This indicated that multiplying the extra-lung burden of $^{90}\text{Sr}-^{90}\text{Y}$ per channel by the factor 1.43 should yield a reasonable approximation for the contribution to total counts in the gross lung peak due to the skeletal burden of $^{90}\text{Sr}-^{90}\text{Y}$.

A valid comparison was possible between lung data from whole-body counting and scanning. On day 65 PAE the dogs were scanned three times intact, once before and twice after sacrifice. These three scans were used to determine the lung peak burden for this day. The difference between whole-body counts made prior to and after removal of the lungs was the basis for the lung burden estimate based on whole-body counting. The comparison, presented in Table 4 indicates good agreement between lung peak estimates based on whole-body counting and the scanner estimate. Results were more erratic for category C dogs than for categories A and B dogs, as might be expected. A high amount of activity in the skeleton is analogous to having a high background. Counting statistics become quite poor when a low-counting sample is counted under conditions of high background.

Subtracting 1.43 times the mean extra-lung body burden of $^{90}\text{Sr}-^{90}\text{Y}$ per channel from the portion of the scan representing the lung peak burden per channel yields reasonable results. The dogs in category C probably represent the extreme condition that would be seen in longevity dogs exposed only to $^{90}\text{Sr}-^{90}\text{Y}$ fused clay. As approximations, the [lung burden (145 μCi)]/[extra-lung burden (24 μCi)] ratio for dogs in category B was equal to 6; the same ratio for category C was

TABLE 4
Comparison Between Lung Burden on Day 65 Post-Aerosol-Exposure
as Determined From Whole-Body Counting and Rectilinear Scanner Data

Tattoo	Lung Burden On Day 65			
	Whole-Body Counting (μ Ci)	Scanner Data*		Difference**
		Lung Peak (μ Ci)	Range (μ Ci)	
<u>Category A:</u>				
474V	99	103	99-110	4.0
474B	162	169	164-174	4.3
475V	156	157	152-161	- 0.1
Mean				2.7
<u>Category B:</u>				
472S	139	153	137-163	10.1
475U	143	140	130-148	- 2.1
478A	149	155	149-159	4.0
Mean				4.0
<u>Category C:</u>				
475B	143	147	125-158	2.8
475A	186	180	169-186	- 3.2
481T	108	133	117-146	23.1

* Mean value and range for 3 scans made on day 65.

** $\left(\frac{\text{Scanner LB value} - \text{Whole-body counter LB value}}{\text{Whole-body counter LB value}} \right) \times 100$

The whole-body counter lung burden estimate was the difference between the whole-body counts made before and after removal of lungs.

equal to $136/212 = 0.64$. Based on another series of approximations for the rate of loss of a lung burden of ^{90}Sr - ^{90}Y in fused clay, skeletal uptake, and skeletal loss of ^{90}Sr , additional estimates were made for the above ratios as a function of time PAE. A ratio for (lung burden)/(skeletal burden) equal to ~ 6 would be observed two years PAE; a ratio of ~ 0.6 would be seen between 1500 and 2000 days PAE. This latter time period represents 3 to 5 half-lives for ^{90}Sr - ^{90}Y fused clay in the lung. The combination of increasing skeletal burden and decreasing lung burden of ^{90}Sr - ^{90}Y in the Beagle may result in loss of accuracy in resolution of the lung burden, as based on the rectilinear scanner, long before dogs reach the condition observed in category C.

The conclusion is that the method used with scanner data to determine estimate for the lung peak is acceptable. Using the factor 1.43 for the ratio (lung peak skeletal burden per channel)/(extra-lung skeletal burden per channel) should yield reasonable estimates for net lung peak. In this study, the lung burden as estimated from scanner data was 4.8% high for nine dogs relative to lung burden estimates based on whole-body counter data. The range was 2.7 to 7.6% for the three categories (Table 4). Such a degree of accuracy is acceptable. This method can be readily adapted to our existing methods for reduction of scanner data. No new definitions of channel groups are necessary and results should be as accurate as the scanning method, at least for the period of time between exposure and accumulation by the dog of skeletal burden which is large relative to the lung burden.

CONTROLLED ADMINISTRATION OF RESPIRATORY TRACT BURDENS IN INHALATION TOXICITY STUDIES WITH BEAGLE DOGS

ABSTRACT

Two methods for the control of initial lung burdens of inhaled particulate material in inhalation toxicity studies are discussed with reference to studies conducted at this laboratory with Beagle dogs exposed to radioactive aerosols. In the first method, the use of a thorax radioactivity monitor to measure respiratory tract deposition of the radioactive aerosols enabled desired initial lung burdens to be achieved within a standard deviation of $\pm 20\%$. If the second method of assuming aerosol air concentrations and respiratory ventilation rates of the exposed animals had been used to predict aerosol deposition rates and to control the inhalation exposures, the desired initial lung burdens would have been within a standard deviation of $\pm 40\%$.

PRINCIPAL INVESTIGATORS

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INTRODUCTION

Studies designed to investigate the toxicity of an aerosol often have unique problems in controlling the administration of specific initial quantities of the test material to the experimental animals. When the toxicity of a material is determined through administration by injection or gavage, it is usually possible to quantitate and control the administered amount through careful assay of the injection or feeding apparatus before and after administration. When the test material is administered via inhalation, however, only the exposure time and the air concentration of the test material in the exposure chamber are normally controlled. One method used to attain a given initial lung burden in an experimental animal is to calculate the duration of exposure based upon the expected mean air concentration of the test material in the exposure chamber and to assume values for the animal's inspiratory minute volume and the percentage of the inhaled aerosol that will be deposited in the lower respiratory tract. Even with a more sophisticated exposure apparatus³ that permits measurement of respiratory parameters during the exposure period, the aerosol concentration and lower respiratory tract deposition must be assumed.

More recently, another technique has been developed to determine the desired quantity of the test aerosol deposited in the lower respiratory tract of the dog. This technique involves the use of a collimated scintillation radiation detector mounted outside the exposure apparatus and pointed at the dog's thorax. With gamma- or beta-emitting radionuclides, the buildup of inhaled radioactive material in the lung can be observed and the exposure terminated when the desired level is reached.

This paper compares results obtained from the control of inhalation exposures using a thorax monitor with those that would have been obtained from control based on an assumed rate of deposition in the dog's lung.

METHODS

The dogs used in these experiments were purebred Beagle dogs from this laboratory's colony. Each dog was given a single inhalation exposure to a radioactive aerosol when they were 12 to 14 months of age. The different radionuclides used for the series of exposures were ^{90}Y , ^{91}Y , ^{144}Ce - ^{144}Pr and ^{90}Sr - ^{90}Y , but in each case the form of the aerosol was the same. Each radionuclide was produced in a relatively insoluble form by encapsulating it within fused montmorillonite clay. Details of the production and characterization of these aerosols are given elsewhere.^{1,2}

Particle sizes of the aerosols were distributed log-normally with activity median aerodynamic diameters (AMAD) ranging from 0.8 to 2.8 μm and geometric standard deviation (σ_g) of 1.4 to 2.7.

Each dog was exposed once in an apparatus that permitted a "nose-only" exposure and measurement of the dog's respiratory pattern during the exposure period by whole-body plethysmography.³ The dogs were sedated with 60 mg of phenobarbital sodium given orally 2.5 hr pre-exposure and with 0.55 mg of Vetame / kg body weight given intravenously at 0.75 hr pre-exposure. Exposures lasted from 1 to 76 min after which initial body and lung burdens were determined by longitudinal scanning and/or whole-body counting at various times post-exposure.

A thorax monitor consisting of a 2"x2" NaI (Tl) scintillation detector mounted in a cylindrical lead shield with 2" thick walls was used to monitor the buildup of radioactivity in the thorax during the exposure period. The monitor was placed directly outside of the whole-body plethysmograph. (Fig. 1) A small light bulb with a reflector attached to the front of the shield produced a spot of light on the dog to assist in aiming the monitor at the dog's thorax. The

signal from the monitor was amplified, passed through a single channel analyzer and count rate meter and read out on a strip chart recorder. Calibration was accomplished with live dogs and/or various counting phantoms containing known quantities of the radionuclide. Reference sources were also counted to determine the temporal stability of the equipment.

RESULTS

The usefulness of the thorax monitor in obtaining desired initial lung burdens was assessed by comparing the calculated ratios of the actual initial lung burdens, ILB, to the desired initial lung burdens, ILB_D, for all inhalation exposures with the ratios of the theoretical lung burdens, ILB_T, to the desired lung burdens that would have been obtained if the inhalation exposures had been controlled through the use of calculated rates of aerosol deposition.

Theoretical lung burdens were calculated for all dogs in the following manner. Theoretical exposure times for obtaining the desired initial lung burdens were calculated using (1) each dog's mean inspiratory minute volume, V_E , measured during the inhalation exposure period, (2) the measured air concentrations, C , of the radioactive aerosols, and (3) an assumed value for the fraction of the inhaled aerosols deposited in the pulmonary regions, F . The theoretical lung burdens were then the products of the actual initial lung burdens and the ratios of the theoretical to actual exposure times, ET_T/ET , or;

$$ILB = ILB (ET_T/ET)$$

$$\text{where } ET_T = (ILB_D)/(V_E \times C \times F)$$

Figure 1. Schematic diagram of Beagle dog aerosol exposure apparatus, viewed from above. Monitor is shown directed toward thorax of Beagle dog.

For aerosols of ^{90}Sr - ^{90}Y , ^{91}Y and ^{144}Ce - ^{144}Pr labeled clay particles, the value for F was assumed to be 0.25 since the aerosols were about 1.9 μm AMAD. For ^{90}Y labeled aerosols, F was assumed to be 0.35 since the average particle size was about 1.0 μm AMAD as shown in Figure 2.

Results obtained with the thorax monitor (Table 1) compared favorably with the desired initial lung burdens; mean values ranged from 0.96 to 1.1 of the desired initial lung burdens. The average standard deviation associated with the individual values was 20%. Thus, with a properly calibrated thorax monitor a series of inhalation exposures may be conducted such that 95% of the initial lung burdens fall within 60 to 140% of the desired burdens.

Controlled inhalation exposures by theoretically predicting the rate of aerosol deposition would have resulted in average initial lung burdens essentially identical to the average desired initial lung burdens in the ^{91}Y and ^{144}Ce - ^{144}Pr inhalation exposure series. However, in the ^{90}Y and ^{90}Sr - ^{90}Y inhalation exposure series, the initial lung burdens would have been 16 and 55% higher than the desired, respectively. This indicates that the assumed pulmonary deposition fractions were lower than actually observed in these studies. The standard deviations of the ratios of the initial lung burdens

Figure 2. Respiratory tract deposition of particulate aerosols in Beagle dogs as a function of aerosol particle size. Solid lines indicate average aerosol deposition and shaded areas represent one standard deviation of individual deposition fractions from mean values observed in a single group of 5 dogs exposed repeatedly to various polydisperse sized aerosols.

TABLE 1

Comparison of the Actual and Theoretical Initial Lung Burdens with the Desired Initial Lung Burdens Observed in Beagle Dogs Exposed to Aerosols Labeled with Radioactive Isotopes During Four Major Inhalation Toxicity Studies

Isotope	AMAD (μm)	No. of Animals	Actual L. B. Desired L. B.*		Theoretical L. B. Desired L. B.**	
			Mean	S. D.*	Mean	S. D.
^{90}Sr - ^{90}Y	2.04*** (1.5-2.8)	71	1.02	0.24	1.55	0.53
^{90}Y	1.06 (0.8-1.4)	87	0.96	0.14	1.15	0.45
^{91}Y	1.91 (1.2-2.4)	96	1.10	0.19	1.02	0.39
^{144}Ce - ^{144}Pr	1.86 (1.5-2.7)	72	0.99	0.22	0.98	0.48

* S. D. = Standard Deviation.

** Theoretical initial lung burdens were calculated for each animal based upon respiratory activity and aerosol concentration data during inhalation exposure and then compared to the desired initial lung burdens.

*** Average particle size and particle size range for all aerosols used in these studies.

to the desired initial lung burdens averaged about 40% of the mean values. Therefore, this method for the control of inhalation exposures in Beagle dogs could result in initial lung burdens between 20% and 180% of the desired lung burdens in 95% of the exposures.

DISCUSSION

When the duration of the exposure period is determined on theoretical grounds, two values are normally assumed: (1) the mean exposure air concentration during the exposure period, and (2) the percentage of the inhaled aerosol that will be deposited in the region of interest in the respiratory tract. The validity of the first assumption rests on the reproducibility of the aerosol generation system. Even with good systems, the generator output occasionally is less than desired.

Less control can be exerted over the second assumption, the deposition of the aerosol within the dog. A summary of aerosol deposition in the respiratory tract of Beagle dogs by Cuddihy *et al.*⁴ is shown in Figure 2. Pulmonary, tracheobronchial and total body deposition measurements had average percentage standard deviations of 31, 85 and 28%, respectively. Nasopharyngeal deposition (not shown in Fig. 2) was calculated from the difference between pulmonary and tracheobronchial deposition and total body deposition. Nasopharyngeal deposition measurements had an average standard deviation of 52%. Nasopharyngeal deposition increases markedly for aerosols greater than 1 μm AMAD and varies widely depending upon respiratory rate and respiratory tract architecture during inhalation. Since these parameters cannot be controlled during animal inhalation exposures, specific or consistent upper respiratory tract burdens of inhaled toxic materials are difficult to produce. Material deposited in the upper respiratory tract is rapidly cleared to the gastrointestinal tract. This can interfere with external monitoring of thoracic radioactivity during inhalation exposures.

The data presented in Table 1 indicate that use of the thorax monitor can reduce variability in final lung burdens. There are, however, several limitations of this system: (1) low sensitivity, (2) interference from radioactivity in the gastrointestinal tract, and (3) solubility effects.

The low sensitivity is a function of the counting geometry derived from the size and shape of the radiation detector, its distance from the thorax and the physical nature of the radiation emitted. The exposure apparatus used in these studies necessitated the placement of the thorax monitor outside the whole-body plethysmograph. In this configuration, the counting efficiencies were 5.3 counts per second (cps) per μCi for ^{144}Ce - ^{144}Pr , 0.02 cps per μCi for ^{91}Y and 0.4 cps per μCi for ^{90}Sr - ^{90}Y and ^{90}Y . Other isotopes with higher energy gamma emissions, such as ^{137}Cs and ^{140}La , have given about 10 cps per μCi with a counter background of about 20 cps in this system.

Data on the deposition sites and subsequent translocation of a fused clay aerosol containing ^{144}Ce are illustrated in Figure 3. These data were obtained after the exposure was over, however, clearance of an inhaled aerosol is a continual process that occurs even while the exposure is in progress. Thus, depending on the duration of the exposure period, some of the material originally deposited in the nasopharyngeal region can be swallowed and passed into the gastrointestinal tract during the exposure.

The solubility of the deposited aerosol in body fluids is also a critical factor in determining the usefulness of the thorax monitor. With aerosol forms that are quite soluble in body fluids, absorption from the respiratory and gastrointestinal tract begins within minutes after the start of the exposure period. In such a case, the thorax monitor would be of little use because it would record not only the activity remaining in the lung, but also fractions of the absorbed activity circulating in blood and the activity deposited in muscle, bone and liver.

Figure 3. Radioactivity distribution profile in a Beagle dog following inhalation of a fused montmorillonite clay aerosol labeled with ^{144}Ce . Numbers in parentheses indicate thoracic area count integrals showing the retention of 87% of the initial thoracic burden at 2 days after inhalation.

The preceding information has shown that monitoring the rate of accumulation of a radioactive material in the thorax can be a useful technique for producing desired initial lung burdens of an insoluble test aerosol. Certain radioactive elements, however, do not have particulate or electromagnetic emissions which can be detected outside of the animal or exposure apparatus. Also, many inhalation toxicity studies are conducted with non-radioactive materials of both particulate and gaseous forms. In these situations it is necessary to use a radioactive tracer in the aerosol to allow for external monitoring.

In addition to benefits gained in the control of initial respiratory tract doses, the use of radioactive tracers in aerosols provides an opportunity to measure other respiratory tract and gastrointestinal tract doses of the inhaled toxic material subsequent to the initial inhalation exposure. Radioactivity profile scanning as illustrated in Figure 3 is useful for these measurements.

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TISSUE BLOOD VOLUMES AND WEIGHTS IN ADULT BEAGLE DOGS

ABSTRACT

Tissue blood volumes and weights were measured in adult Beagle dogs to provide basic information relevant to tissue dosimetry in this species of experimental animal. Dogs were administered ^{51}Cr or ^{59}Fe labeled red blood cells or ^{131}I labeled human serum albumin and sacrificed by exsanguination via cardiac puncture while under pentobarbital anesthesia or by inducing circulatory arrest with intravenous injection of KC1, both with and without prior anesthesia. Surgically implanted, blood vessel occluders were used to isolate certain lung lobes from the general circulation immediately prior to sacrifice in order to assess terminal changes in the pulmonary blood distribution. Beagle dogs used in this study weighed about 10 kg and had an average blood volume of 1000 ml. Lungs, liver, spleen and kidneys were determined to be composed of about 50% blood by weight and hence would be most affected by the mode of sacrifice. Sodium pentobarbital anesthesia exerted a marked effect upon blood cell pools in the spleen. Exsanguination removed blood rather uniformly from all tissues in proportion to the total amount withdrawn with the exception of the skeleton.

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INTRODUCTION

Tissue or organ weight data are necessary items for the expression of most basic dosage terms in pharmacological and toxicological studies. Often, administered, absorbed or time integrated doses of such agents are given in terms of a quantity per gram of affected tissue, and are determined from animal tissues recovered after sacrifice or death. Death due to the administration of some agents is frequently accompanied by substantial tissue weight changes. Death produced by some mode of sacrifice is also a traumatic procedure for experimental animals which can have profound effects upon tissue blood volumes and hence tissue weights. For example, sacrifice by exsanguination under anesthesia exerts a double effect upon recovered tissue weights. First, the anesthesia can change blood distribution within body pools depending upon the type and dose.¹ Secondly, exsanguination generally results in the removal of more than 50% of the total blood volume. Since some tissues can be more than 50% blood by weight,² then the recovered tissue weights can be in substantial error. Similar considerations apply to most any other type of animal sacrifice procedure. Thus, any tissue weight changes produced by the specific agent under study during animal sacrifice can result in proportional uncertainties in specific dose estimations.

Additional problems result from tissue weight changes occurring over extended periods of experimental observation when time integrated dose estimates are meaningful. Here neither the initial normal tissue weight nor the final tissue weight at death represents the most appropriate value for dose calculations. Although it may be impossible to arrive at the correct tissue weight change function during the experiments, dose calculations can be modified to include a practical dose range due to uncertainties in this often neglected factor. In addition, the tissue weights which are employed in dose calculations should be carefully described with regard to mode of sacrifice and blood content.

The purpose of this report is to provide information concerning relatively normal and blood free tissue weights for Beagle dogs sacrificed by several methods. These included exsanguination while under sodium pentobarbital anesthesia and circulatory arrest by intravenous introduction of KCl, both with and without sodium pentobarbital anesthesia. Blood vessel occluders were also used on some pulmonary blood vessels prior to sacrifice in an attempt to isolate certain lung lobes from terminal circulatory changes. Tissue weight data from a much larger group of Beagle dogs used in various other non-destructive experiments conducted at this laboratory are also summarized in this report.

EXPERIMENTAL METHODS

Adult male and female Beagle dogs were used in these studies as described in Table 1. Blood volume measurements in Experimental Groups A through D were made following the administration of ^{59}Fe labeled red blood cells and ^{131}I labeled human serum albumin or ^{51}Cr labeled red blood cells. No blood volume distribution measurements were made in Experimental Group E where only the volume of blood recovered during exsanguination was recorded.

Red blood cells labeled with ^{59}Fe were obtained from a donor Beagle dog injected with 500 μCi of ^{59}Fe . After one week, no ^{59}Fe was detected in the plasma fraction of blood from the donor dog and red blood cell radioactivity had risen to 0.5 μCi ^{59}Fe per ml of whole blood. Total plasma volumes were determined in Experimental Groups A and B by using ^{131}I labeled human serum albumin. Between 5 and 10 μCi of ^{131}I in 10 ml of solution was used in each animal.

Red blood cells labeled with ^{51}Cr were obtained by withdrawing 20 ml of blood from each animal in Experimental Groups C and D and equilibrating with ^{51}Cr as Na_2CrO_4 and A.C.D. anti-coagulant solution at 37°C for 10 minutes. Ascorbic acid, 50 mg, was then added to reduce any free chromium and the cells were washed twice with saline and resuspended in 20 ml of saline for injection back into each donor animal.

At 13 days prior to the blood volume studies, the dogs in Group B were surgically treated to implant blood vessel occluders in the thorax. Halothane gas was used as anesthetic while an incision was made in the right thoracic wall. The fifth intercostal space was opened and inflatable

Table 1
Experimental Design and General Characteristics of Beagle Dogs
Used for Blood Distribution Studies

Experimental Group	Total Body Weight (kg)	Age (mo)	Number of		Measurements Accomplished
			Males	Females	
A	10.0	20	1	2	Red cell volume (^{59}Fe)
	(8.7-11.1)	(14-24)			Plasma volume (^{131}I)
B	10.4	22	1	2	Red cell volume (^{59}Fe)
	(8.5-11.8)	(15-27)			Plasma volume (^{131}I)
					Occluded lung lobes
C	9.5	47	1	2	Red cell volume (^{51}Cr)
	(5.2-11.8)	(45-49)			
D	8.6	55	0	3	Red cell volume (^{51}Cr)
	(7.1-10.2)	(54-56)			Exsanguination
E	9.7	26	79	71	Tissue weights
	(5.7-14.6)	(14-57)			Exsanguination

blood vessel occluders were placed around the arterial and venous vessels to the right apical and right cardiac lung lobes in one animal and to the right cardiac and right diaphragmatic lobes of the remaining two dogs. Catheters to each occluder balloon were brought through the intercostal incision, tunneled under the skin, exteriorized midline between the scapulae and sutured in place. The incision was closed by layer. Blood vessel occluders were used in three experimental animals since it was anticipated that the pulmonary blood volume would be most susceptible to change during animal sacrifice and that the lobes isolated by occlusion just prior to sacrifice would experience fewer changes due to this experimental procedure.

Food was withheld from all dogs on the day of the blood volume measurements. Labeled blood cell suspensions (20 ml) were injected into the cephalic vein of each animal and a 10 ml blood sample was taken from the jugular vein after 12 minutes. Dogs in Groups A and B then received 10 ml of ^{131}I labeled human serum albumin and a second 10 ml blood sample was taken after 12 minutes.

All dogs were sacrificed at 30 minutes after the initial injection of radiolabeled blood cells. Experimental Groups A and B received 10 ml of saturated KCl via the cephalic vein. Blood vessel occluders in Group B animals were closed seconds prior to sacrifice. Experimental Groups C and D were sacrificed after being anesthetized with sodium pentobarbital (2.5 mg/kg of body weight). Dogs in Group E were sacrificed by maximum blood withdrawal after being anesthetized with sodium pentobarbital.

After sacrifice all major organs were taken for immediate weighing and subsequent radioactivity analysis. All major blood vessels were ligated during the dissections of animals in Groups A, B and C in order to prevent drainage of blood to or from the tissues during removal. Blood vessels were severed between double ligations and there was a minimum of leakage and cross-contamination between tissues. No attempt was made to prevent blood drainage from tissues obtained in Experimental Groups D and E. Therefore, data pertaining to these tissues should be considered to be related to exsanguinated and drained body organs as is often true of most common experimental sacrifice and necropsy procedures.

Blood and tissues were assayed for ^{59}Fe and ^{131}I using a NaI scintillation crystal detector. Blood samples and tissues were assayed for ^{51}Cr using a large volume, liquid scintillation, well counter.

Large vein hematocrits, H_{LV} , were determined for all dogs in Experimental Groups A, B, C and D using a red cell packing correction factor of 0.96. Total body red cell volumes, RC_{TB} , and total plasma volumes, P_{TB} , were determined for Groups A and B from the following expressions

$$RC_{TB} = \frac{\text{Total Injected } ^{59}\text{Fe} \cdot H_{LV}}{^{59}\text{Fe per ml blood}}$$

and

$$P_{TB} = \frac{\text{Total Injected } ^{131}\text{I} (1 - H_{LV})}{^{131}\text{I per ml blood}}$$

Total body blood hematocrits, H_{TB} , were then determined from the expression

$$H_{TB} = \frac{RC_{TB}}{RC_{TB} + P_{TB}}$$

Tissue red cell volumes, RC_T , and plasma volumes, P_T , were determined for these animals by multiplying the ratios of tissue ^{59}Fe to total body ^{59}Fe by the total red cell volume and the ratios of tissue ^{131}I to total body ^{131}I by the total plasma volume, respectively.

$$RC_T = \frac{\text{Tissue } ^{59}\text{Fe}}{\text{Total body } ^{59}\text{Fe}} (RC_{TB})$$

and

$$P_T = \frac{\text{Tissue } ^{131}\text{I}}{\text{Total body } ^{131}\text{I}} (P_{TB})$$

Tissue hematocrit values were then taken to be

$$H_T = \frac{RC_T}{(RC_T + P_T)}$$

Blood free tissue weights were calculated from the total tissue weights obtained at necropsy and tissue blood volumes assuming unit density for all blood components.

Total body and individual tissue red cell volumes were calculated from ^{51}Cr radioactivity measurements in Experimental Groups C and D in the same manner as was done for ^{59}Fe described above. Total body plasma was calculated for these dogs from the total red cell volume and the corrected large vein hematocrit times 0.96. (The ratio of total body to large vein hematocrits determined in Experimental Groups A and B was 0.96 as will be discussed later.) Individual tissue whole blood volumes were calculated by dividing the measured tissue red cell volumes by the total body hematocrit multiplied by R_H , the ratios of tissue hematocrits to total body hematocrits determined from Experimental Groups A and B. Thus,

$$\text{Tissue Blood Volume} = \frac{RC_T}{H_{TB} \cdot R_H}$$

where

$$R_H = \frac{H_T}{H_{TB}}$$

Experimental Group E provided the average tissue weights obtained from a group of 150 dogs which were sacrificed by exsanguination while under sodium pentobarbital anesthesia. The total volume of blood withdrawn was recorded. Blood free tissue weights were calculated from the exsanguinated tissue weights by subtracting the tissue blood volumes after sacrifice by exsanguination as determined from Experimental Group D. Finally, normal tissue weights were calculated by adding the normal tissue blood volume weights to the calculated blood free tissue weights.

EXPERIMENTAL RESULTS

Some general whole-body characteristics of the Beagle dogs in each Experimental Group are given in Table 2. Within Groups A through D, the animals of each group were all similar in age and more than 20 months old. Animals in Group E were all more than 13 months of age and ranged upward to 50 months. Total body weight showed an average value of nearly 10 kg which was about 10% blood. The average total body hematocrit for all animals in Groups A through D was estimated to be 0.46.

Table 2
Total Body Weights and Measured Blood Volumes
for All Experimental Groups

Experimental Group	Age (mo.)	Body Weight (kg)	Red Cell Volume (ml)	Plasma Volume (ml)	Total Blood Body Weight (ml/kg)
A	21	10.0	480	635	110
B	22	10.4	405	475	85
C	47	9.6	550	620	122
D	55	8.5	385	440	97
Average	21-55	9.6	455	540	103
E	26 ± 12	9.7 ± 1.6	-	-	-

Data in Table 3 show the total body and individual tissue hematocrit values in dogs in Groups A and B in which red cell volumes and plasma volumes were measured independently. Liver, spleen and heart showed noticeably higher hematocrit values than the total body. This may be due to the large blood cell pools in liver and spleen as related to their functions as blood cell reservoirs. The high value for the hematocrit of blood contained in the heart is in apparent contrast with skeletal muscle which is only 70% of the total body hematocrit. This could be related to blood in the major chambers of the heart clotting with a subsequent preferential loss of serum during the excision. There was noticeably more mechanical manipulation of the heart before ligation of all of the associated blood vessels and removal.

Individual tissue blood volumes are shown in Table 4 as a percent of the total blood volume. The total lung blood volume in dogs in Group A was about 6% whereas in Group B it was 8.6% corresponding to more than 15 gm difference in tissue mass. The greatest differences occurred in the non-occluded diaphragmatic lung lobes with less evident changes in any of the occluded lobes. A similar picture is seen in the blood free tissue weights of the lung lobes given in Table 5. More than 80% of the increased blood free lung tissue weight in Group B could be attributed to the non-occluded diaphragmatic lobes. These observations may be attributed to (1) the development of adhesions and increased connective tissue as a result of the occluder implantations, and (2) compensatory changes in blood volume distributions in non-occluded lung lobes immediately following vessel occlusion but prior to sacrifice. It is interesting to note that the occluded lung lobes were similar to comparable non-occluded lung lobes in blood content suggesting that the lung blood volume distributed was not greatly altered by the sacrifice procedures employed with dogs in Experimental Group A. Apart from the lung the blood contents of the remaining tissues listed in Table 4 for Groups A and B were very similar. The spleen blood volumes in animals of Group C were markedly higher than in Groups A and B. This was attributed to the use of the sodium pentobarbital anesthetic just prior to sacrifice,¹ however, this was the only significant difference noted in this comparison. Data showing the blood volume contents of tissues obtained from dogs in Group D when expressed as a percent of residual blood following exsanguination were similar to data from all other experimental groups. The only exception may have been the skeleton which might not have lost a proportional amount of its normal blood content.

Data for Experimental Group E are presented in Table 6 including estimations of tissue weights free of blood with a normal content of blood and those which were obtained following exsanguination under sodium pentobarbital anesthesia. Blood free tissue weights were obtained from Group E dog tissue weights obtained at sacrifice with an exsanguinated tissue blood volume subtracted

Table 3
Total Body and Tissue Hematocrit Values
for Experimental Groups A and B

Sample	Hematocrit	R_H^*
Large vein	0.465	1.05
Total body	0.445	
Lung	0.495	1.10
Skeleton	0.345	.80
Liver	0.585	1.30
Spleen	0.575	1.30
Kidney	0.335	0.75
Pancreas	0.450	1.00
Thyroid	0.442	1.00
Adrenals	0.260	0.60
Salivary Gland	0.290	0.65
Brain	0.420	0.95
GI Tract	0.240	0.55
Mesentery	0.300	0.65
Heart	0.520	1.20
Muscle	0.306	0.70
Pelt	0.363	0.80

$$* R_H = \frac{\text{tissue hematocrit}}{\text{total body hematocrit}}$$

Table 4

Tissue Blood Volumes Expressed as a Percent of Total Blood Volumes
for all Experimental Groups and as a Percent of Residual Blood Volume

	for Group D				
	% Total Blood				% Residual
<u>Sample</u>	<u>Group A</u>	<u>Group B</u>	<u>Group C</u>	<u>Group D</u>	<u>Blood</u>
Total Lung	5. 9	8. 6	3. 8	1. 7	4. 7
Lung Lobes					
Rt. Apical	0. 85	1. 25 0. 90*	0. 60	0. 30	0. 80
Rt. Cardiac	0. 45	0. 35*	0. 30	0. 10	0. 30
Rt. Diaphragmatic	1. 50	2. 90 1. 97*	0. 95	0. 40	1. 10
Rt. Intermed.	0. 50	0. 80	0. 30	0. 15	0. 40
Lt. Ap. Cardiac	0. 95	1. 70	0. 60	0. 25	0. 70
Lt. Diaphragmatic	1. 70	2. 30	1. 00	0. 50	1. 40
Total Skeleton	4. 80	5. 50	8. 10	3. 80	11. 00
Femur	0. 10	0. 12	. 15	. 11	. 30
Humerus	0. 11	0. 14	. 25	. 12	. 35
Skull	1. 70	1. 20	. 95	. 47	1. 35
Liver	28. 20	29. 20	26. 90	7. 70	22. 20
Spleen	1. 85	1. 00	7. 25	2. 05	5. 90
Kidney	2. 80	2. 60	1. 05	0. 70	2. 05
Pancreas	0. 40	0. 40	0. 40	0. 07	0. 20
Thyroid	0. 01	0. 04	-	-	-
Adrenals	0. 04	0. 04	0. 05	. 01	0. 03
Salivary Glands	0. 10	0. 10	-	-	-
Brain	0. 20	0. 25	0. 20	0. 15	0. 40
GI Tract	3. 25	3. 35	4. 55	1. 07	3. 10
Mesentery	2. 00	3. 10	1. 60	0. 25	0. 70
Heart	3. 75	2. 40	6. 30	1. 90	5. 40
Pelt	7. 10	7. 10	7. 15	2. 40	6. 95
Blood Withdrawn	-	-	-	65. 30	
Remainder	46. 75	43. 50	40. 00	15. 00	-

* Occluded lung lobes.

Table 5

Tissue Weights (TW) and Estimated Blood Free Tissue Weights (BFTW)

Given in Grams and Normalized to a 10 kg Beagle Dog

Tissue	Group A		Group B		Group C		Group D	
	TW	BFTW	TW	BFTW	TW	BFTW	TW	BFTW
Lung Lobes								
Rt. Apical	16	7.2	18	7.1	17	9.8	9.7	7.1
			20	12.0*				
Rt. Cardiac	8.6	4.0	8.7	5.8*	7.5	4.1	4.2	3.1
Rt. Diaph.	27	11	47	21	23	11	15	11
			34	18*				
Rt. Interm.	9.6	4.0	12	5.0	7.4	43	4.9	3.6
Lt. Ap. -Card.	17	7.5	24	9.6	16	87	8.7	6.4
Lt. Diaph.	30	12	34	15	24	12	18	13
Total Lung	108	46	135	62	95	51	60	45
Skeleton	1390	1340	1510	1460	1600	1490	1380	1350
Femur	29	28	31	30	31	30	27	26
Humerus	29	27	31	29	31	30	21	20
Skull	410	390	285	275	240	235	210	205
Liver	510	200	505	260	460	135	275	200
Spleen	41	20	28	19	57	-	20	-
Kidney	74	44	69	47	56	43	43	36
Pancreas	26	22	23	20	24	20	23	22
Thyroid	0.76	0.61	0.69	.39	0.75	-	0.65	-
Adrenals	1.21	1.17	1.35	1.05	2.5	2.0	1.30	1.20
Testes	12	-	26	-	24	-	-	-
Ovaries	1.4	-	1.3	-	0.9	-	1.00	-
Salivary Glands	12	10.5	11	10	-	-	-	-
Brain	74	72	83	81	82	80	90	87
GI Tract	520	485	530	500	440	390	400	390
Mesentery	215	195	210	180	98	78	160	155
Heart	120	79	95	76	150	73	100	85
Pelt	1570	1500	1490	1420	1400	1230	1580	1560

* Occluded lung lobes.

Table 6
Tissue Weights for Beagle Dogs (Experimental Group E)
Normalized to a Total Body Weight

Tissue	Exsang. Tissue Weight (gm)	Standard Deviation (gm)	No. Observed	Blood Free Tissue Weight (gm)	Weight with Estimated Normal Blood (gm)	Concentration (ml/gm)
Total Lung	73	16	107	57	116	0.510
Lung Lobes						
Rt. Apical	11.5	2.7	109	8.8	17.2	0.490
Rt. Cardiac	6.7	1.7	108	5.6	10.0	0.440
Rt. Diaph.	17.7	3.9	107	13.8	29.0	0.525
Rt. Interm.	5.9	1.5	107	4.5	9.7	0.535
Lt. Ap. -Cardiac	12.0	2.4	107	9.6	19.0	0.495
Lt. Diaph.	19.1	3.8	107	14.3	31.0	0.540
Skeleton	1200	200	46	1160	1210	0.041
Femur	32	6	128	31	32	0.034
Humerus	31	6	128	30	31	0.032
Skull	225	49	46	220	240	0.080
Vertebrae						0.031
Ribs						0.057
TBLN	0.5	0.2	127			
Liver	266	33	128	190	470	0.600
Spleen	25	12	128	20*	41*	0.510
Kidney	45	7	128	38	66	0.425
Pancreas	23	4	57	22	26	0.155
Thyroid	0.7	0.2	61			
Adrenals	1.2	0.3	119	1.1	1.5	0.270
Ovaries	1.4	0.7	42			
Testes	15	7	47			
Salivary Glands	11	2	59	10	11	0.091
Brain	79	13	60	78	80	0.025
GI Tract	410	50	110	400	430	0.070
Pelt	1700	250	110	1675	1750	0.043
Estimated Muscle				4500	4600	0.024
Blood Withdrawn	650	120				
Total				7150	8800	

*Data taken from Experimental Group A.

as determined from Experimental Group D. The values for normal tissue blood volumes determined from Experimental Group A were then added to the blood free tissue weights to obtain the normal tissue weights including blood. The standard deviations included with the exsanguinated tissue weights ranged between 12 and 50% of the mean tissue weight values with an average of $\pm 25\%$.

DISCUSSION

The determination of "normal" tissue weights in experimental animals presents a formidable problem for studies requiring such information as an integral part of physical dose measurements. No methods are available for obtaining animal tissues whose weights or compositions accurately correspond to normal weights in intact animals. For many tissues there are no single normal tissue weights in intact animals. This is especially true for heart and lungs whose weights are more affected by cardiac cycles than other body organs. Anesthetics which are generally applied before sacrifice were seen to increase spleen weights by more than 50%. Thus, although the appropriate "normal" tissue weights for dosimetry purposes may be difficult to define and even more difficult to measure experimentally, an important need exists for these critical parameters and an understanding of their impact upon experimentally determined dosage measurements.

Attempts to measure lung blood volumes in experimental animals and man have been reported as early as 1852.³ Many of these studies employed a dye indicator dilution technique with heart catheterization. Such blood volumes measured have then included large pulmonary vessels and either all or the left side of the cardiac spaces. Values for pulmonary blood volumes in dogs have ranged from 4.8⁴ to 15 ml/kg⁵ total body weight depending upon the method of measurement. However, deducting 5 ml/kg body weight for left heart volume,^{6,7} reduces the upper range of these values to 10 ml/kg. The recommended lung blood volume obtained in this study is 6 ml/kg and an average total lung blood volume of 60 ml for 10 kg Beagle dogs. There was no indication that sacrifice by intravenous injection of KCl altered pulmonary blood volumes since the occluded lung lobes in dogs in Experimental Group B were identical in blood volumes to comparable lung lobes in Group A animals. After sacrifice by exsanguination the lung blood volume was reduced to 2.5 ml/kg reducing the total lung weight by about 35 gms or 30%.

Administering a sodium pentobarbital anesthetic was seen to increase the red cell volume of the spleen markedly. When the hematocrit value for the spleen determined from animals in Experimental Groups A and B was used to calculate the total spleen blood volumes and tissue weights for animals in Group C, a negative blood free tissue weight resulted. This indicated that the effect of the anesthetic caused pooling of blood cells predominately with a greatly elevated tissue hematocrit.

Total body muscle mass was not measured in these studies, however, it has been estimated to constitute 46%⁸ of the total body mass in Beagle dogs. Thus, the total weight including the estimated muscle which was accounted for is about 8.8 kg (Table 6). The remaining mass would be associated with adipose and connective tissues and large vessel blood reservoirs.

Of the tissues studied, the lung, liver, spleen and kidney tissues have the greatest blood concentrations with about 50% of the normal tissue masses composed of blood. Hence the weights obtained for these tissues would be most dependent upon the mode of sacrifice employed in experimental toxicity studies. Exsanguination as employed in these studies, resulting in the recovery of 65% of the total blood, reduced these tissue weights to about 65% of the estimated tissue weights. Although it may be a difficult matter of judgement to select a series of organ weights for application to basic tissue dosimetry considerations, it should be noted that individual whole organ weights can vary by as much as a factor of 2 depending upon blood content and careful attention should be paid to describing the conditions under which such values may be obtained experimentally.

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SECTION III

LONG-TERM DOSE RESPONSE STUDIES

TOXICITY OF INHALED $^{90}\text{SrCl}_2$ IN BEAGLE DOGS. VI

ABSTRACT

Studies on the metabolism, dosimetry and effects of inhaled $^{90}\text{SrCl}_2$ in the Beagle dog are continuing in an effort to provide a basis for assessing the consequences of inhaling ^{90}Sr such as might be released in certain nuclear accidents. Seventy-two dogs have been exposed to aerosols containing ^{90}Sr resulting in initial body burdens ranging from 2.6 to 280 $\mu\text{Ci } ^{90}\text{Sr/kg}$. Forty-eight of these dogs are being maintained for lifetime observation. The long-term retained burdens in these dogs range from 1 to 120 $\mu\text{Ci } ^{90}\text{Sr/kg}$. Twenty-four dogs with a mean long-term retained burden of 38 $\mu\text{Ci } ^{90}\text{Sr/kg}$ have been assigned to a sacrifice study. Two of these dogs were sacrificed at 5 days, 2 at 1 month, and 2 at 1 year after inhalation of ^{90}Sr . To date, 34 ^{90}Sr dogs have died or been euthanized, 6 during the first 31 days after inhalation of ^{90}Sr with bone marrow aplasia and 28 between 585 days and 2628 days after inhalation of ^{90}Sr . The latter group includes 11 dogs with hemangiosarcomas, 3 with fibrosarcomas, 8 with osteosarcomas, 3 with osteochondrosarcomas, 1 with osteochondrofibrosarcoma, 2 with leukemia, 1 with a baso-squamous carcinoma, 1 with an epileptic seizure, 1 with pneumonia and 1 with cerebellar hemorrhage (4 dogs had both a hemangiosarcoma and an osteosarcoma). The skeletons of the dogs dying with neoplasms received initial radiation dose rates of 4 to 55 rads/day and cumulative doses to death of 3300 to 22,000 rads. Two control dogs have died; one at 2615 days with autoimmune hemolytic anemia and one at 2638 days with a fibrosarcoma. Serial observations are continuing on 32 surviving ^{90}Sr dogs and 20 control dogs.

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INTRODUCTION

$^{90}\text{Sr}^*$ is one of the fission product radionuclides that predominates in a nuclear reactor inventory after a period of sustained operation. For this reason and because of its high probability for release in certain types of reactor accidents as well as its long half-life and energetic beta emissions, it was one of the radionuclides selected for intensive study in this program. Research is being performed with two forms of ^{90}Sr , $^{90}\text{SrCl}_2$ and ^{90}Sr in fused clay.

The experimental protocol for the $^{90}\text{SrCl}_2$ study includes two groups of dogs: (1) a longevity study consisting of graded exposure levels in which dogs are being maintained for lifespan observation, and (2) a sacrifice study in which dogs are being serially sacrificed to provide specimens for histopathologic and other studies. Details of the experimental methodology have been presented previously (1966-1967 Annual Report, LF-38, pp. 1-18; 1967-1968 Annual Report, LF-39, pp. 1-13). Briefly, the dogs were given a single inhalation exposure to an aerosol of CsCl to which $^{90}\text{SrCl}_2$ was added.

* ^{90}Sr as used in this text refers to ^{90}Sr in equilibrium with its daughter, ^{90}Y .

For the longevity study, a total of 48 dogs have been exposed and are being maintained along with 15 control dogs. To aid in visualizing the design of the longevity and sacrifice studies, the experimental designs are presented in Figures 1 and 2, respectively. Although the dogs are presented in block design format, it should be noted that they were exposed to ^{90}Sr prior to the initiation of the block type design in this laboratory. It may be noted that the dogs are grouped on the basis of Long Term Retained Burden (LTRB) as contrasted to the Initial Lung Burden (ILB) used for most studies at this laboratory. However, in the case of ^{90}Sr inhaled as $^{90}\text{SrCl}_2$ the ILB cannot be readily estimated, nor is it totally representative of the ^{90}Sr absorbed and retained in the body. This condition exists because a portion of the inhaled $^{90}\text{SrCl}_2$ is deposited in the upper respiratory tract where some may be absorbed directly into the circulation; the remainder is cleared via mucociliary activity and swallowing, a portion being absorbed from the gastrointestinal tract. Thus, for inhaled $^{90}\text{SrCl}_2$, the retained body burden may represent ^{90}Sr absorbed from the upper

													Mean LTRB $\mu\text{Ci/Kg}$
	23C 750 83 E-1099	157E 1200 120 E-759	158E 1200 120 E-927	158B 960 100 E-31	159A 840 74 D-29	159B 960 98 E-18	160C 720 69 E-1142	160B 920 97 E-864	162F 1200 100 E-886	164A 1100 120 D-585	195B 1100 100 D-28	195C 990 110 D-21	99
	12F 410 50 E-1046	13A 430 51 D-1361	19B 250 40 A-2629	19C 220 28 A-2629	19D 240 27 A-2628	22A 290 28 E-2247	22F 300 34 E-1540	23B 470 59 E-1787	22E 290 44 A-2621	26A 320 41 D-1404	26F 400 52 E-1938	162A 600 50 E-1702	42
	22D 62 6.8 A-2621	28B 51 7.1 A-2574	28C 70 9.3 D-2436	30B 65 7.9 A-2573	30C 71 8.3 A-2573	30D 58 6.6 A-2573	38E 58 8.9 A-2529	39C 79 9.1 A-2529	40E 60 9.6 A-2529	42D 60 7.7 A-2528	42E 53 6.1 A-2528	42F 41 5.7 A-2528	7.8
	23E 13 1.7 D-2247	24A 8.0 1.0 A-2593	24B 14 1.6 A-2593	26B 29 3.2 A-2592	26G 13 1.9 A-2592	27A 17 2.2 A-2592	27D 23 2.2 A-2591	30E 8.1 1.0 A-2560	30F 10 0.97 A-2560	30G 16 2.2 A-2559	35E 17 2.3 A-2559	37F 8.5 1.1 A-2530	1.8
Control	19A 0 0 A-2627	21C 0 0 A-2627	24E 0 0 A-2601	26E 0 0 A-2598	28A 0 0 A-2572	30A 0 0 A-2572	31A 0 0 A-2558	32A 0 0 A-2558	33B 0 0 A-2558	35F 0 0 A-2525	40D 0 0 A-2525	42C 0 0 A-2525	0
Control	158A 0 0 A-1985	160A 0 0 A-1983	162E 0 0 A-1978										0
	23C = ANIMAL NUMBER 750 = LONG-TERM RETAINED BURDEN (μCi) 83 = LONG-TERM RETAINED BURDEN ($\mu\text{Ci/Kg}$) E-1099 = D=DEAD, E=EUTHANIZED, A=ALIVE - DAYS POST-EXPOSURE AT DEATH OR ON 9-30-72												

Figure 1. Experimental design for longevity study on the effects of inhaled $^{90}\text{SrCl}_2$ in Beagle dogs (status as of 9/30/72).

													Mean LTRB $\mu\text{Ci/Kg}$
	2A 410 60 S-5	4C 480 65 S-28	2B 360 46 S-381	7B 510 67 E-928	8A 400 51 E-1362	9B 370 39 E-1367	9C 370 37 E-1318	9D 420 47 E-1316	10A 550 55 E-1168	10B 350 44 D-2034	11B 450 47 E-31	12E 300 36 E-2380	50
	4A 270 29 S-5	4D 290 31 S-28	5A 320 35 S-379	4B 140 16 D-2628	6B 270 36 A-2776	6C 200 24 A-2776	6D 220 29 A-2776	8B 290 34 E-2264	9A 220 20 A-2748	12B 330 30 A-2706	12C 150 15 A-2706	12D 210 28 D-585	27
Control	5C 0 0 S-6	4E 0 0 S-24	2D 0 0 S-380	6A 0 0 A-2776	9E 0 0 E-2638	10C 0 0 A-2745	12A 0 0 A-2706	13B 0 0 D-2615	13C 0 0 A-2706	13D 0 0 A-2706			0
	2A = ANIMAL NUMBER 410 = LONG-TERM RETAINED BURDEN (μCi) 60 = LONG-TERM RETAINED BURDEN ($\mu\text{Ci/Kg}$) S-5 = D=DEAD, E=EUTHANIZED, A=ALIVE - DAYS POST-EXPOSURE AT DEATH OR ON 9-30-72												

Figure 2. Experimental design for sacrifice study on the effects of inhaled $^{90}\text{SrCl}_2$ in Beagle dogs (status as of 9/30/72).

respiratory tract and the gastrointestinal tract as well as that deposited in the lung. In view of these considerations, it was felt that the parameter most comparable to an initial lung burden in other studies would be that fraction of the initial body burden associated with the long-term retention components. The long-term retained burden (LTRB) was derived for each dog by multiplying his initial body burden by the retention fraction associated with all but the first, or most rapidly cleared component, as determined from a least-squares analysis of the whole-body retention data. On the average, the LTRB consisted of 40% of the initial body burden.

A ranking of the dogs based on the long-term retained burden is presented in Appendix A. Values for individual dogs ranged from 120 to 1.0 $\mu\text{Ci } ^{90}\text{Sr/kg}$ body weight. In the parallel sacrifice study, which consisted originally of 24 exposed and 10 control dogs, 2 exposed dogs and a control dog were sacrificed at 5 days, 1 month, and at 1 year after inhaling ^{90}Sr . The average initial ^{90}Sr body burden for this sacrifice series was 88 $\mu\text{Ci/kg}$ and the corresponding mean long-term retained burden was 38 $\mu\text{Ci/kg}$. (See Appendix A for additional information on individual dogs.) This report will discuss the current status of the studies.

METABOLISM AND DOSIMETRY

Observations continue to be made on long-term retention of inhaled $^{90}\text{SrCl}_2$. Dependent upon the survival time of the animals from two to four component exponential functions were required to describe the whole-body retention data. The longest term components corresponded to effective retention half-times of 5 to 16 years. The average absorbed β radiation dose to the skeleton has been calculated for each dog using his computer-fitted whole-body retention equation and the relationship derived earlier (1966-1967 Annual Report, LF-38, pp. 1-18). Results of these calculations are given in Appendix A. These calculations are based on the assumption that the skeletal weight is 10% of the total body weight at exposure.

BIOLOGICAL EFFECTS

Twenty-two dogs from the longevity study, 12 dogs from the sacrifice study as well as one unassigned dog exposed to $^{90}\text{SrCl}_2$ and 2 control dogs, died or were euthanized prior to September 30, 1972. Figure 3 relates survival time for all dogs in the longevity and sacrifice studies as a function of long-term burden of ^{90}Sr . This includes 7 dogs that died or have been euthanized during the past year. Three of these were exposed animals from the longevity series. Two were exposed and two were control dogs from the sacrifice series. Among the exposed dogs a variety of lesions were found. One dog (12E) euthanized at 2380 days post-exposure, had a hemangiosarcoma and osteosarcoma of the ileum and an osteochondrosarcoma of the rib and one dog (162A), euthanized at 1702 days post-exposure, had an osteochondrosarcoma of the maxilla. Dog 4B, that died at 2628 days post-exposure, had a baso-squamous carcinoma of the left tympanic region. This neoplasm was well-differentiated and locally invasive and did not metastasize (Fig. 4). Dog 28C died at 2436 days post-exposure with an acute myelomonocytic leukemia. This animal had a terminal myelophthisic anemia and thrombocytopenia. The gross necropsy picture was dominated by splenomegaly and diffuse lymphadenopathy. Histopathologically, the bone marrow was completely replaced by leukemic cells and leukemic infiltrates were widespread. The leukemic cells had two distinctive patterns, some showing scanty eosinophilic granulation and others having frequent cytoplasmic vacuolation (Fig. 5). Finally, dog 23E died at 2247 days post-exposure with an acute bacterial bronchopneumonia.

Of the control dogs, one (13B) died at 2615 days after being placed on experiment (with an autoimmune hemolytic anemia and renal amyloidosis) and the other (9E), was euthanized at 2638 days due to the presence of a long-standing fibrosarcoma of the thoracic wall which metastasized wide. This dog also had amyloidosis of the kidney, spleen and pancreas.

Figure 3. Survival of Beagle dogs that inhaled $^{90}\text{SrCl}_2$ (longevity and sacrifice studies).

Figure 4. Photomicrograph of the baso-squamous carcinoma in dog 4B. The tumor had both a well-differentiated basal cell and squamous component. H + E stain. X 270

Figure 5. Smear of femoral bone marrow from dog 28C which had a myelomonocytic leukemia. The cells have irregular nuclei and abundant vacuolated cytoplasm. May-Grunwald Giemsa stain. X 625

DISCUSSION

The survival pattern for the Beagle dogs that inhaled $^{90}\text{SrCl}_2$ is quite similar to that observed in Beagle dogs that were given single intravenous injections of ^{90}Sr citrate at the University of Utah (Fig. 6). The long-term retained ^{90}Sr burdens for the Utah dogs were calculated using the published injection burdens and a LTRB equal to 64% of the injected value derived from a published retention equation for ^{90}Sr in the Utah dogs. In this figure the progression of deaths at later times with lower burdens is apparent for both the Lovelace and Utah dogs. The close agreement between the Lovelace and Utah data is even more apparent in Figure 7 in which cumulative survival curves

Figure 6. Survival of Beagle dogs that inhaled $^{90}\text{SrCl}_2$ (Lovelace) or were injected with ^{90}Sr citrate (Utah). Status of Lovelace dogs as of 4/1/72 and Utah dogs as of 3/31/71.

Figure 7. Cumulative survival curves for Beagle dogs that inhaled $^{90}\text{SrCl}_2$ (Lovelace) or were injected with ^{90}Sr citrate (Utah). Status of Lovelace dogs as of 4/1/72 and Utah dogs as of 3/31/71.

calculated by the method of Kaplan and Meier² are presented for groups of dogs. This method of presentation, which allows data for surviving animals to be used until they are considered as lost to follow-up, is especially suitable since both the Lovelace and Utah studies are still in progress.

From Figures 6 and 7, and especially 7, it is apparent that, at the lowest long-term retained ⁹⁰Sr burdens, i.e., < 10 μ Ci of ⁹⁰Sr/kg, the data presently available suggest a survival pattern that is quite similar to that of the control population. The group of 12 Lovelace dogs with burdens of 5.7 to 9.6 μ Ci/kg should provide further opportunity to determine experimentally if this level of ⁹⁰Sr is without effect on survival.

The major findings associated with death are similar in the Lovelace and Utah studies. In both, the majority of deaths were associated with bone-related neoplasms, principally osteosarcomas and hemangiosarcomas. In the Lovelace study the frequency of hemangiosarcomas is approximately equal to that of osteosarcomas, whereas in the Utah study, three times as many osteosarcomas have been observed as have hemangiosarcomas. The basis for this variance cannot be readily attributed to the major differences between the two studies: the mode of ⁹⁰Sr administration, slightly older dogs administered ⁹⁰Sr at Utah, the location of the laboratories, and the parentage of the dogs. Perhaps this variance is related to the genetic background of the dogs and/or the presence of different viral agents that might be activated by chronic irradiation.

The types of neoplastic disease observed in the Lovelace and Utah studies contrast sharply with those observed in studies where there is a chronic intake of ⁹⁰Sr. At Argonne National Laboratory, neonates or relatively young dogs received multiple injections of ⁹⁰Sr, and, in addition to a high incidence of bone-related neoplasms, about 15% died with myelogenous leukemia, reticulum-cell sarcoma or lymphosarcoma.³ At the University of California-Davis,^{4,5} and Battelle-Northwest,⁶ studies are being conducted in which Beagle dogs and miniature pigs, respectively, are being orally administered ⁹⁰Sr daily, including ⁹⁰Sr exposure of some groups starting in utero. In both studies a high incidence of myeloproliferative disease has been observed at relatively young ages at the highest ⁹⁰Sr feeding levels. In animals surviving past 18 months, bone-related neoplasms have been observed. Clearly, there is a difference in biological response related to differences in the radiation-dose pattern; acute intake of ⁹⁰Sr with a steadily decreasing average dose rate to bone and marrow favors induction of bone-related neoplasms, whereas chronic intake of ⁹⁰Sr with a relatively constant dose rate to bone and marrow leads to early development of myeloproliferative disease at the highest ⁹⁰Sr intake levels and development of bone-related neoplasms at later time periods. Previously, McClellan and Jones⁷ speculated that the chronic irradiation of marrow at high dose rates with overt marrow damage was an essential element of the etiology of the ⁹⁰Sr-induced myeloproliferative disease. If such is the case, this group of hematopoietic disorders may be of limited significance at low levels of chronic intake, levels at which bone-related neoplasms may be observed. Thus, with either acute or chronic intakes at low levels, the situations most likely to be of concern for "real-life" exposures and, accordingly, the establishment of standards, the major risk would appear to be development of bone-related neoplasms.

It is also of interest to compare the results of the ⁹⁰SrCl₂ inhalation study to those being obtained in studies with inhaled ⁹¹YCl₃ and ¹⁴⁴CeCl₃. All three radionuclides are translocated to bone, where they are tenaciously retained and deliver significant radiation doses to bone in the first 30 days after inhalation exposure (Table 1). For all three radionuclides, a significant number of the dogs die at early times after inhalation and deposition of sufficient radioactivity to result in approximately 1000 rads to bone within the first 30 days. For ⁹⁰SrCl₂, ¹⁴⁴CeCl₃ and ⁹¹YCl₃, this corresponds to a long-term retained burden of 100, 210 and 260 μ Ci/kg, respectively. It is of interest to consider the potential for late-occurring effects in the long-term survivors. A factor of major concern here is the long-term radiation dose to skeleton. Because of differences in the

Table 1
Calculated Cumulative Radiation Dose to Bone,
Liver and Whole Body of a 10 kg Beagle Dog
Following Inhalation of $^{90}\text{SrCl}_2$, $^{144}\text{CeCl}_3$
or $^{91}\text{YCl}_3$ (rads/100 μCi Retained Burden)

	Bone			Liver		
	30 Days	180 Days	4700 Days	30 Days	180 Days	5000 Days
$^{90}\text{SrCl}_2^*$	100	470	4700	-	-	-
$^{144}\text{CeCl}_3^*$	48	240	690	280	1400	4700
$^{91}\text{YCl}_3^*$	38	110	130	25	68	74

* Calculation based on long-term retained burden.

effective half-time for retention in bone (related to differences in both the physical and biological half-life of the radionuclides), the potential 5000-day skeletal dose varies considerably for the highest burden dogs from each study that survive longer than one month following inhalation exposure. For example, dogs accumulating approximately 1000 rads in 30 days and potentially surviving to 5000 days would accumulate 47,000, 14,500 and 3,400 rads, depending on whether they had deposited ^{90}Sr , ^{144}Ce or ^{91}Y , respectively. Obviously, the ^{90}Sr dog would not survive sufficiently long to accumulate the total 47,000 rads. The net effect is a marked difference in the radiation dose to skeleton to death and the time of death for the earliest dog in each study dying with a bone-related neoplasm: ^{90}Sr - 19,000 rads, 759 days; ^{144}Ce - 8300 rads, 799 days; and ^{91}Y - 3000 rads, 1990+ days. A similar spectrum of doses is seen if one compares dogs that inhaled ^{90}Sr , ^{144}Ce or ^{91}Y and died with bone-related neoplasms at about the same time following inhalation exposure (Fig. 8).

Figure 8. Cumulative radiation dose to skeleton for dogs that inhaled $^{91}\text{YCl}_3$, $^{144}\text{CeCl}_3$, or $^{90}\text{SrCl}_2$ and died at similar time intervals post-inhalation exposure.

In summary, several conclusions can be drawn from the data obtained in approximately the first half of a long-term study of the effects of inhaled $^{90}\text{SrCl}_2$ in Beagle dogs. First, it would seem that a similar relationship between deposited activity and survival is observed for inhaled $^{90}\text{SrCl}_2$ (Lovelace) and injected ^{90}Sr citrate (Utah) when the activity is normalized to long-term retained ^{90}Sr burden. Second, the primary late-occurring biological response is development of bone-related neoplasms, primarily osteosarcomas and hemangiosarcomas. This response is similar to that observed in the Utah dogs although a higher frequency of hemangiosarcomas is evident in Lovelace dogs. Third, the development of bone-related neoplasms as late sequelae of acute intake of ^{90}Sr contrasts with the high incidence of myeloproliferative disease observed at high levels of chronic ^{90}Sr intake. Fourth, the bone-related neoplasms observed in the dogs that inhaled $^{90}\text{SrCl}_2$ are similar to bone-related neoplasms observed in dogs that inhaled $^{144}\text{CeCl}_3$ or $^{91}\text{YCl}_3$. However, the neoplasms in the animals receiving ^{91}Y and ^{144}Ce occurred at lower total doses to bone even when death occurred at similar time periods following inhalation exposure.

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TOXICITY OF INHALED $^{144}\text{CeCl}_3$ IN BEAGLE DOGS. VI

ABSTRACT

Studies on the metabolism, dosimetry and effects of inhaled $^{144}\text{CeCl}_3$ in the Beagle dog are continuing in an effort to provide information that will aid in assessing the biological consequences of inhaling ^{144}Ce such as might be released in certain nuclear accidents. Studies on the tissue distribution of inhaled $^{144}\text{CeCl}_3$ indicate that ^{144}Ce deposited in the lung is translocated at a moderately rapid rate to liver and skeleton. The pattern of distribution is such that significant radiation doses are accumulated by all three tissues. Fifty-five dogs that inhaled $^{144}\text{CeCl}_3$ and 15 control dogs have been placed in a longevity study and will be observed for their lifespan. The ^{144}Ce dogs had initial lung burdens ranging from 20 to 2900 μCi . Twenty-five dogs have died; 8 at 21 to 44 days post-inhalation with signs attributed to severe bone marrow damage and associated pancytopenia; 2 at 138 and 144 days with radiation pneumonitis; 3 at 309 to 874 days with hepatic necrosis; 1 at 510 days with marrow aplasia; 1 at 375 days with pulmonary fibrosis; and 10 at 799 to 2164 days with neoplasms or myeloproliferative disorders. One of the last 10 dogs had an osteosarcoma, 2 had squamous cell carcinomas of the maxilla, 1 of these also having a small papillary adenoma of the lung, 4 had hemangiosarcomas of the liver, 2 had myelogenous leukemia and 1 had myelofibrosis with myeloid metaplasia. Serial observations are continuing on the 30 surviving $^{144}\text{CeCl}_3$ dogs and 15 control dogs.

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INTRODUCTION

$^{144}\text{Ce}^*$ is one of the fission product radionuclides that predominates in a reactor inventory after a period of sustained reactor operation. It has a half-life of 285 days and decays to ^{144}Pr which has a half-life of 17.3 minutes. During their decay, ^{144}Ce - ^{144}Pr emit several gamma rays and a number of beta particles with an average energy of 1.27 Mev. Because of the probability for release of ^{144}Ce in certain types of nuclear accidents and recognizing its radiological characteristics, it was selected as one of the radionuclides for extensive study in this program. One of the forms selected for use was $^{144}\text{CeCl}_3$. Two studies are being performed: (a) a radiation dose pattern study in which 27 dogs were exposed to $^{144}\text{CeCl}_3$ and serially sacrificed at 2 to 512 days post-inhalation exposure, and (b) a longevity study to evaluate the relationship between radiation dose and biological response for 55 dogs exposed to achieve graded initial body burdens of $^{144}\text{CeCl}_3$. These dogs and 15 control dogs are being maintained for observation over their total lifespan (Fig. 1). This report will briefly summarize the current status of these studies.

METABOLISM AND DOSIMETRY

The whole-body retention of ^{144}Ce in these dogs continues to be assessed by periodic whole-body counting. The resulting data continue to be adequately described by a three-component exponential function with the last component having an effective half-life that approaches the physical half-life of ^{144}Ce , 285 days. Cumulative radiation doses for each longevity dog have been estimated using the dogs initial lung burden and curves derived from the radiation dose pattern study expressing cumulative rads to lung, liver and skeleton per μCi initial lung burden per kg body weight (1969-1970 Annual Report, LF-44, p. 121). These data are presented in Appendix A.

* ^{144}Ce as used in this text always refers to ^{144}Ce in equilibrium with its daughter, ^{144}Pr .

												Mean ILB μCi/Kg
151B 2700 E-31	152C 2900 D-144	151C 1900 E-31	156D 2100 E-22	156B 2800 E-21	198E 1800 D-375							260
62F 960 D-874	153A 1500 D-138	195A 1500 D-336	197D 1700 D-32	199E 810 D-1808	200A 1300 D-510	201C 830 E-1759	198A 1200 E-799	199A 1600 D-44	197C 1100 D-309	201G 1400 D-27	203F 870 D-36	140
60B 590 E-1632	60C 280 A-2390	61B 300 A-2385	62B 500 A-2385	62E 540 D-1735	63B 210 A-2381	63C 300 A-2381	64C 520 E-1806	64A 660 E-2164	65B 430 A-2369	66B 380 A-2369	200E 560 D-1783	50
52C 120 A-2441	53B 150 A-2441	52D 170 A-2440	53C 130 A-2440	54A 250 A-2438	54B 280 A-2438	55A 130 A-2437	55D 150 A-2437	56B 150 D-1811	57A 130 E-1826	57C 95 A-2430	60D 140 A-2390	16
49A 55 A-2452	49B 43 A-2452	49D 52 A-2440	49E 36 A-2451	50A 50 A-2447	50D 20 A-2447	50E 51 A-2447	50F 35 A-2445	51A 40 A-2445	51B 68 A-2444	52B 56 A-2444	57B 64 A-2430	5.2
Control 49C 0 A-2452	50C 0 A-2448	51C 0 A-2444	51E 0 A-2444	52A 0 A-2444	53A 0 A-2441	53D 0 A-2441	54C 0 A-2438	56A 0 A-2431	60A 0 A-2390	61C 0 A-2385	62A 0 A-2385	0
Control 153D 0 S-149	156E 0 S-149	197B 0 A-1811	198C 0 A-1811	201A 0 A-1811								0
151B = ANIMAL NUMBER 2700 = INITIAL LUNG BURDEN (μCi) 270 = INITIAL LUNG BURDEN (μCi/Kg) E-31 = D-DIED, E-EUTHANIZED, S-SACRIFICED, A-ALIVE - DAYS POST-EXPOSURE AT DEATH OR ON 9-30-72												

Figure 1. Experimental design for $^{144}\text{CeCl}_3$ longevity study. (Status as of 9/30/72)

BIOLOGICAL EFFECTS

During the last year, 4 dogs that inhaled $^{144}\text{CeCl}_3$ died or were euthanized bringing to 25 the number of dogs that have died following inhalation of $^{144}\text{CeCl}_3$ (Fig. 2). In 3 of these dogs (199E died at 1808 days post-exposure, 200E died at 1783 days post-exposure and 201C was euthanized at 1759 days post-exposure) death was directly attributable to the presence of hepatic hemangiosarcomas and all 3 had acute terminal episodes of intraperitoneal hemorrhage from the neoplasms. The fourth dog (64A) was euthanized at 2164 days post-exposure because of a destructive squamous cell carcinoma of the maxilla. The clinico-pathological findings were generally similar to those previously reported for $^{144}\text{CeCl}_3$ dogs succumbing with hepatic hemangiosarcomas or squamous cell carcinoma (1969-1970 Annual Report, LF-43, pp. 128-136; 1970-1971 Annual Report, LF-44, pp. 126-135). Two of the dogs (200E and 201C) with hemangiosarcomas had both intrahepatic and extrahepatic metastases. Dog 199E had a large solitary tumor which was locally invasive. Dog 64A had a well-differentiated, invasive squamous cell carcinoma which metastasized to regional lymph nodes. In all of the dogs with hemangiosarcomas there was severe hepatic degeneration, necrosis and fibrosis, with many of these lesions resembling fresh or old infarcts. The two dogs (201E and 201C) for which terminal samples were available had severe elevation of alkaline phosphatase and hyperbilirubinemia which were attributable to the liver changes described. Nodular parenchymal cell hyperplasia and bile duct hyperplasia were present in all four dogs.

There was severe bone marrow hyperplasia and extramedullary hematopoiesis in the spleen and liver in all of the dogs. The bone marrow hyperplasia was predominantly erythroid in dog 199E and granulocytic in 64A, 200E and 201C. Plasma cell hyperplasia was also a consistent finding in the bone marrow and this was associated with elevated beta or gamma globulins in the three dogs checked (64A, 200E and 201C).

Figure 2. Survival of Beagle dogs that inhaled $^{144}\text{CeCl}_3$.
(Status as of 9/30/72)

DISCUSSION

The occurrence of neoplasms primarily of hepatic origin at later times post-inhalation exposure of $^{144}\text{CeCl}_3$ is not unexpected. Tumors of hepatocellular, biliary and endothelial origin are known to occur after ^{144}Ce injection in rats¹ and after thorotrast injection in man.² To date, we have observed only hemangiosarcomas of the liver but it is possible that the hepatic parenchymal cell and bile duct hyperplasia observed in our dogs will ultimately be manifested by neoplasms of these elements.

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TOXICITY OF INHALED $^{91}\text{YCl}_3$ IN BEAGLE DOGS. VI

ABSTRACT

Studies on the metabolism, dosimetry and effects of $^{91}\text{YCl}_3$ in Beagle dogs are continuing with a view to providing information that will aid in assessing the biological consequences of nuclear accidents in which ^{91}Y or other radionuclides that produce a similar radiation dose pattern may be released. Forty-two dogs with ^{91}Y initial lung burdens from 64 to 1300 $\mu\text{Ci/kg}$ were placed in four groups with mean lung burdens of 310, 180, 75, and 40 $\mu\text{Ci/kg}$. These dogs and 12 control dogs are being maintained for lifetime observation. An additional four dogs with a mean initial ^{91}Y lung burden of 180 $\mu\text{Ci/kg}$ were placed in a sacrifice study. Eleven dogs within the highest exposure groups have died or were euthanized at 17 to 33 days after inhalation of ^{91}Y with changes related to severe bone marrow damage and associated pancytopenia. Two dogs died approximately one year after ^{91}Y inhalation with convulsive seizures that were presumed to be unrelated to the ^{91}Y exposure. One dog was euthanized at 2012 days post-inhalation with a squamous cell carcinoma of the maxilla having received an estimated cumulative dose to skeleton of 2900 rads. Thirty-two dogs that had initial lung burdens of 14 to 220 $\mu\text{Ci/kg}$ and all 12 control dogs are surviving at 1905-2145 days after inhalation of ^{91}Y . Serial observations are continuing on all surviving dogs.

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INTRODUCTION

^{91}Y is an important contributor to the total fission product radionuclide inventory of a reactor after periods of sustained operation. It has a physical half-life of 59 days and emits a betaparticle with a maximum energy of 1.55 Mev and a gamma ray of 1.21 Mev with a yield of 0.22%. Recognizing the potential for release of ^{91}Y in certain types of nuclear accidents, it was selected for study in this program. Two longevity studies are being conducted; one with ^{91}Y inhaled in a relatively soluble form as $^{91}\text{YCl}_3$ and the other with ^{91}Y inhaled in fused clay particles which are tenaciously retained in the lung (see this report, pp. 151 - 156). This report will summarize the current status of the research with inhaled $^{91}\text{YCl}_3$.

METABOLISM AND DOSIMETRY

As was presented previously (1967-1968 Annual Report, LF-39, pp. 26-32), each of the shipments of ^{91}Y used in this study contained a radioactive contaminant. This contaminant was $^{152-154}\text{Eu}$ in the first two shipments and ^{144}Ce in the third shipment. Because of the higher whole-body counting efficiency for the gamma rays emitted from $^{152-154}\text{Eu}$ and ^{144}Ce - ^{144}Pr than for the Bremsstrahlung and infrequent gamma rays from ^{91}Y , a small amount of contamination interferes with the whole-body counting of ^{91}Y . To remove the contribution of these contaminants from the whole-body counting data, the following procedure was used. First, all whole-body counting data obtained on each longevity dog were plotted. Each plot showed a longer-lived component corresponding to the contaminant involved. A curve was fitted to this longest component by a computerized method of non-linear least squares and subtracted from the original data. The resulting data for each dog could be described well by 3-component exponential functions. A 3-component function, representing the mean whole-body retention for the 7 living longevity dogs exposed to ^{91}Y containing $^{152-154}\text{Eu}$ is:

$$\text{BB}(t) = 64e^{-1.3t} + 7.7e^{-0.068t} + 28e^{-0.012t}$$

where $\text{BB}(t)$ is the body burden of ^{91}Y at time t expressed as a percentage of the initial body burden.

The longest-term component in this expression has an associated effective half-life very close to the physical half-life of ^{91}Y , 59 days, over a period extending more than one year post-exposure. This lends confidence to the approach used to correct for the presence of the contaminant.

Once the whole-body retention data had been improved in this manner, it was possible to determine an initial lung burden for each dog. In agreement with the procedures followed for $^{144}\text{Ce-Cl}_3$, the initial lung burden for each dog was defined as being that amount of deposited ^{91}Y activity associated with all but the first, or most rapidly cleared, component of whole-body retention. Initial lung burdens determined in this manner were used to construct the Experimental Design Chart shown in Figure 1 and are the basis for ranking all dogs as listed in Appendix A.

Tissue data were available from radiochemical analyses of tissues obtained from dogs in a radiation dose pattern study that were sacrificed serially during the first 128 days post-exposure. The data were previously presented on a relative basis, as percent of sacrifice body burden (1966-1967 Annual Report, LF-38, pp. 40-64). This showed that the major locations of ^{91}Y in the body were lung, liver and skeleton. Much of the ^{91}Y was translocated from the lung during the first week post-exposure and was deposited in skeleton and liver with the amount in skeleton being approximately 8 times greater than that in liver.

When these same data were expressed as percentages of the initial lung burden, it was possible to obtain curves describing the temporal disposition of ^{91}Y in lung, liver and skeleton in a form that could readily be used for dose calculations. The following equation was then used to calculate cumulative doses to lung, liver and skeleton in terms of rads per $\mu\text{Ci } ^{91}\text{Y}$ ILB per kg body weight.

$$\frac{\text{cum rads}}{\mu\text{Ci ILB/kg}} = \frac{(51.2) (\bar{E}) (AF)}{TF \times 10^3} \int_0^t TB(t) dt$$

where $\bar{E}$ = average beta energy of ^{91}Y , 0.59 Mev

AF = absorbed fraction of beta energy in tissue of interest, 1.0 for lung and liver, 0.85 for skeleton

TF = Tissue weight as fraction of total body weight

TB(t) = content of ^{91}Y in the tissue of interest expressed as a fraction of the initial lung burden

													Mean ILB $\mu\text{Ci/Kg}$
	118E 5100 540 D-12	118F 2300 290 E-17	119D 1600 240 D-28	122C 3000 300 D-20	119C 2100 250 D-22	164B 2300 250 D-22							310
	118B 1100 140 A-2145	118A 1800 220 E-2012	118D 1800 200 A-2143	119A 1800 220 A-2143	123A 2600 240 D-21	120A 1400 130 A-2130	120C 1900 200 E-23	164C 1100 110 D-473	164F 1800 200 D-24	165A 1700 160 A-1947	169C 1300 150 A-1937	171F 1000 160 A-1937	180
	134A 600 62 A-2111	134C 650 66 A-2111	164G 730 94 A-1949	166E 820 73 A-1947	165F 750 82 A-1947	169D 610 100 A-1941	172C 380 53 A-1940	169A 600 58 D-364	171B 820 90 A-1934	172A 600 68 A-1934	174A 880 92 A-1928	176D 550 60 A-1928	75
	164D 400 43 A-1956	165G 380 46 A-1956	166C 350 31 A-1949	167C 140 14 A-1947	169B 440 44 A-1944	171A 320 40 A-1944	171E 310 48 A-1944	167B 540 51 A-1942	173G 370 52 A-1940	176E 280 41 A-1930	174F 97 16 A-1930	174E 450 51 A-1928	40
Control	118C 0 0 A-2145	119B 0 0 A-2143	121A 0 0 A-2140	164E 0 0 A-1949	165D 0 0 A-1949	165E 0 0 A-1949	166B 0 0 A-1947	167A 0 0 A-1944	167E 0 0 A-1944	171D 0 0 A-1937	174D 0 0 A-1934	176B 0 0 A-1905	0
118E = ANIMAL NUMBER 5100 = INITIAL LUNG BURDEN (μCi) 540 = INITIAL LUNG BURDEN ($\mu\text{Ci/Kg}$) D-12 = D=DIED, E=EUTHANIZED, A=ALIVE - DAYS POST-EXPOSURE AT DEATH OR ON 9-30-72													

Figure 1. Experimental design for studying the long-term effects of inhaled $^{91}\text{YCl}_3$ in Beagle dogs (status as of 9/30/72).

With this equation, a set of normalized dose calculations for lung, liver and skeleton was made. Dose values for individual dogs in the longevity experiment were then determined by multiplying the appropriate normalized dose factors for a given time post-exposure by the respective initial lung burden values in $\mu\text{Ci/kg}$. The resulting dose values are included in Appendix A.

BIOLOGICAL EFFECTS

Eleven dogs, nine from the longevity study and two from the sacrifice study, with ^{91}Y initial lung burdens ranging from 170 to 540 $\mu\text{Ci/kg}$ died or were euthanized from 17 to 33 days post-inhalation of ^{91}Y as a result of severe bone marrow damage and associated pancytopenia. Two dogs from the longevity study died at 364 and 473 days after inhalation of ^{91}Y with convulsive seizures that were presumed to be unrelated to ^{91}Y exposure.

During the past year, one dog (118A) was euthanized. At 1697 days post-inhalation, a swelling was noted on the right maxilla which was diagnosed on biopsy as a squamous cell carcinoma. This lesion progressively increased in size resulting in destruction of bone of the maxilla. On hematological examination the dog began to show a rise in total white count with an increase in neutrophils and bands by 1698 days post-inhalation. There was also a decrease in total red cell count, packed cell volume, and hemoglobin concentration, as well as an elevation in the sedimentation rate. By 2009 days post-inhalation the dog had a total white count of 72,800/ mm^3 with 42% neutrophils, 43% bands, 5% metamyelocytes and 1% myelocytes. The total red cell count had decreased to $3.40 \times 10^6 \text{ mm}^3$ with a packed cell volume of 29.5 vols. % and a hemoglobin concentration of 8.3 gms %. By 2001 days post-exposure, the lesion had ulcerated and was exuding a purulent exudate. The animal had a blockage of the upper nasal canals and was breathing through the mouth. There was a complete absence of normal bone of the maxilla anterior to the second premolar on radiographic examination at 2012 days post-inhalation at which time the dog was euthanized due to its debilitated condition.

At necropsy there was an invasive but well-differentiated squamous cell carcinoma involving the maxillary region. The tumor did not metastasize. The dog also had nodular hepatic parenchymal cell hyperplasia with hydropic and fatty degeneration of the hyperplastic foci. There was focal alveolar wall fibrosis and focal squamous metaplasia of alveolar epithelium. Other lesions included severe granulocytic hyperplasia of the bone marrow, multiple pulmonary granulomas, chronic interstitial nephritis, lymphocytic thyroiditis and evidence of an old widespread parasitic migration, probably strongyloides.

SUMMARY

In this study there is a sharp transition in the dose-survival relationship moving from dogs dying at 17 to 33 days after inhalation of $^{91}\text{YCl}_3$ with the highest ILBs to dogs with similar or lesser ILBs that have survived for over 5 years after inhalation of $^{91}\text{YCl}_3$ (Fig. 2). Indeed, the only dog, 118A, to die of a clearly radiation-induced late effect had an ILB of 220 $\mu\text{Ci/kg}$ which was greater than that of 2 dogs that died at 23 and 24 days after inhalation of $^{91}\text{YCl}_3$, yet this dog survived to 2012 days post-inhalation of $^{91}\text{YCl}_3$. The estimated cumulative doses to its skeleton, lung and liver were 2900 rads, 2640 rads and 1900 rads, respectively, doses only 4 to 5 times those received by the dogs that died at the earliest times. This is a reflection of the relatively rapid rate of decrease of the radiation dose rate (and rate of dose accumulation) related to the 59-day physical half-life of ^{91}Y .

The steepness of the dose-survival relationship for $^{91}\text{YCl}_3$ is similar to that for $^{137}\text{CsCl}$ (see this report, p. 146) which also had a relatively short effective half-life in the body and contrasts with the much less steeper dose-survival relationship observed for $^{90}\text{SrCl}_2$ (see this report, p. 132).

Figure 2. Survival of dogs that inhaled $^{91}\text{YCl}_3$.

^{90}Sr inhaled as $^{90}\text{SrCl}_2$ is tenaciously retained in the skeleton and serves as a source of chronic irradiation with a slow decrease in dose rate. A continuous progression of fatal radiation-induced bone neoplasms are observed beginning at about 700 days post-inhalation of ^{90}Sr and progressing to later time of occurrence with lower retained burdens. An intermediate form of dose-survival relationship is observed for dogs that have inhaled $^{144}\text{CeCl}_3$, the ^{144}Ce being retained in the skeleton with an effective half-life intermediate between that of ^{91}Y and ^{90}Sr and a retention half-time in liver longer than that of ^{91}Y .

With continuing observation of the surviving dogs it will be of interest to determine if the appearance of the first radiation-induced late effect is the forerunner of additional cases in the dogs that had the highest ILBs and have accumulated over 1000 rads to skeleton and lung or was it a spurious case such that the survival pattern of even the highest ILB dogs will be difficult to discern from that of the control population.

TOXICITY OF INJECTED $^{137}\text{CsCl}$ IN THE BEAGLE DOG. V.

ABSTRACT

Studies on the metabolism, dosimetry and effects of intravenously administered $^{137}\text{CsCl}$ in the Beagle dog are being conducted to aid in assessing the biological consequences of exposure to ^{137}Cs such as might occur in the event of certain nuclear accidents. The effects of the chronic, relatively uniform whole-body exposure produced by ^{137}Cs are being compared with other diverse radiation dose patterns resulting from inhalation of radioactive aerosols. Sixty-six dogs were entered into the study; 6 with a mean initial ^{137}Cs body burden of 3700 $\mu\text{Ci/kg}$, and 12 dogs each with mean initial ^{137}Cs body burdens of 2820, 1940, 1420, 970 and 0 $\mu\text{Ci/kg}$. All six of the highest level dogs died at 19 to 33 days post-injection with cumulative whole-body doses of 950 to 1400 rads. Three dogs in the 2820 $\mu\text{Ci/kg}$ level died at 24 to 27 days post-injection with cumulative whole-body doses of 860 to 910 rads. One dog in the 2820 $\mu\text{Ci/kg}$ level and one dog in the 1940 $\mu\text{Ci/kg}$ level died at 77 and 81 days after injection with cumulative whole-body doses of 1300 to 1400 rads. These early deaths were attributed to severe bone marrow damage which was reflected in an early dose-related pancytopenia. A dog injected with 1900 $\mu\text{Ci } ^{137}\text{Cs/kg}$ died acutely at 693 days post-injection with necropsy findings attributed to shock. A control dog died 647 days after being placed on the study with clinicopathological manifestations of an auto-immune hemolytic anemia. Forty-two ^{137}Cs dogs and 11 controls are surviving at 1317 to 1571 days after being placed on experiment. The surviving ^{137}Cs dogs had initial body burdens that ranged from 880 to 3000 $\mu\text{Ci/kg}$ and received cumulative whole-body doses of 550 to 2200 rads. Serial observations are continuing on all survivors.

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INTRODUCTION

$^{137}\text{Cs}^*$ is one of the fission product radionuclides that predominates in a reactor inventory after a period of sustained operation. It has a 30-year half-life and decays to $^{137\text{m}}\text{Ba}$ which has a 26-min half-life. With decay, these radionuclides emit a 0.662 Mev gamma photon and 0.51 and 1.17 beta particles. Biologically, ^{137}Cs behaves as an analog of potassium. If it gains entry into the body in a soluble form, such as CsCl , it distributes throughout most of the tissues in relatively uniform fashion, the distribution being similar regardless of the mode of entry, i.e., ingestion, injection or inhalation. This pattern of distribution and the radiologic characteristics of the radionuclide result in relatively uniform whole-body exposure. Because of the above characteristics and recognizing the probability for release of ^{137}Cs in certain nuclear accidents, ^{137}Cs was selected as one of the radionuclides to be studied intensively in this program. The effects of the relatively uniform whole-body beta and gamma radiation exposure resulting from internally deposited ^{137}Cs may be compared with the other diverse radiation dose patterns under study in this and other laboratories to provide a sound radiobiological basis for predicting the effects of chronic exposure from a variety of internally deposited radionuclides, especially those that enter the body via inhalation.

* ^{137}Cs as used in this text refers to ^{137}Cs in equilibrium with its daughter, ^{137}Ba .

Because the metabolism and dosimetry of ^{137}Cs were shown to be similar for the intravenous and inhalation routes of entry and recognizing that curie quantity aerosol generator loadings would be required to effect inhalation exposures, the intravenous route of injection was used to administer ^{137}Cs to the dogs in this longevity study. Anticipating a relatively steep dose-response relationship (that is, a large change in response with a small difference in radiation dose) initial ^{137}Cs body burdens were projected to scale downward by a factor of 0.7: 4000, 2800, 1960, 1370, 960 and 0 μCi ^{137}Cs /kg body weight. This report will summarize the current status of the study.

EXPERIMENTAL DESIGN

All experimental procedures were previously described in detail (1968-1969 Annual Report, LF-41, pp. 36-45). The ^{137}Cs was administered intravenously via the cephalic vein as the chloride. Whole-body counting was used to determine the initial body burden of ^{137}Cs and its subsequent retention in each dog. Dogs were housed individually in metabolism cages for the first 60 days post-injection and then transferred to the kennel facility where they were housed in pairs (same sex and comparable dose levels).

The experimental design for the ^{137}Cs longevity study shown in Figure 1 presents the initial ^{137}Cs body burdens. The actual mean body burdens achieved (3780, 2820, 1940, 1420 and 970 μCi /kg) were close to those projected as was expected considering the use of the intravenous route of administration. The metabolism and dosimetry have been previously reported in detail¹ as have early biological effects.²

All dogs were kept under close clinical observations, and serial hematologic, serum chemical, immunological and microbiological measurements have also been made. A thorough pathologic examination was made on all dogs that died.

RECENT CLINICAL FINDINGS AND CURRENT STATUS

No animals in this study have died during the course of the year. One animal, 282C, previously reported to have developed degenerative bone changes has continued to deteriorate during

Projected IBB $\mu\text{Ci/Kg}$	BLOCKS												Mean Actual IBB $\mu\text{Ci/Kg}$
	A	B	C	D	E	F	G	H	I	J	K	L	
4000	244B 3900 2300 D-33	241F 3900 2400	253C 3500 2200 D-22	249D 3600 2400 D-27	273A 3800 2400 D-19	271D 4000 2200 D-26							3780
2800	245B 2700 1500 D-81	241G 2800 1800 D-25	247E 2800 1800 D-27	248A 2600 1700 A-1520	266C 2800 1700 A-1405	273E 2800 1800 A-1405	279D 2700 1800 A-1381	277F 3000 1700 A-1381	284B 2900 1600 A-1341	282C 2900 1900 A-1341	292A 2900 1600 A-1317	280C 2900 1900 E-24	2820
1960	241A 1900 1300 D-77	244E 2100 1300 A-1570	253B 1800 1100 A-1521	247A 1800 1300 A-1520	271A 1900 1300 D-693	266D 2100 1200 A-1405	279B 2000 1300 A-1381	275E 2000 1200 A-1381	283A 1900 1100 A-1341	283D 2000 1200 A-1341	291A 1900 1000 A-1317	292C 1900 1200 A-1317	1940
1370	244C 1600 820 A-1571	241E 1400 1000 A-1570	252C 1200 830 A-1521	247C 1300 870 A-1520	267A 1400 940 A-1405	268C 1400 1100 A-1405	279A 1500 830 A-1381	278F 1500 950 A-1381	289D 1400 790 A-1341	280D 1600 910 A-1341	291C 1200 870 A-1317	286D 1500 800 A-1317	1420
960	241B 1000 590 A-1571	244F 1100 640 A-1570	248C 880 560 A-1521	249C 900 590 A-1520	266A 890 540 A-1405	273F 1000 710 A-1405	278B 1100 760 A-1381	278D 1000 610 A-1381	285A 920 540 A-1341	281C 1000 600 A-1341	287A 900 630 A-1317	281B 940 590 A-1317	970
Control	241C 0 0 A-1571	244D 0 0 D-647	251D 0 0 A-1521	247B 0 0 A-1520	270B 0 0 A-1405	267D 0 0 A-1405	277A 0 0 A-1381	274E 0 0 A-1381	282A 0 0 A-1341	283C 0 0 A-1341	286C 0 0 A-1317	282D 0 0 A-1317	0
244B = ANIMAL NUMBER 3900 = INITIAL BODY BURDEN ($\mu\text{Ci/Kg}$) 2300 = 14 DAY BODY BURDEN ($\mu\text{Ci/Kg}$) D-33 = D=DEAD, E=EUTHANIZED, A=ALIVE - DAYS POST-EXPOSURE AT DEATH OR ON 9-30-72													

Figure 1. Experimental design for $^{137}\text{CsCl}$ study (status as of 9/30/72).

the year. The animal is inactive, but alert and in fair condition. There is increased disuse atrophy of the hind legs. Abnormal posture has developed due to the swelling and deformities present in both carpal joints. The animal is extremely reluctant to walk and will do so only if placed in an upright position and encouraged. Radiographic examination performed at 1232 days post-injection showed increased areas of lysis in the carpals, metacarpals, tarsals, metatarsals and the distal portions of the radii and the tibia-fibulae. There were roughened surfaces present on all articular surfaces of the above bones.

DISCUSSION

Of the 54 dogs administered $^{137}\text{CsCl}$ and the 12 controls, 12 ^{137}Cs dogs have died or been euthanized and 1 control has died (Fig. 2). Nine of the deaths in ^{137}Cs dogs occurred at 19 to 33 days after ^{137}Cs administration and two occurred at 77 and 81 days. One control animal died 647 days after being placed on experimentation and 1 ^{137}Cs dog died 693 days post-injection. Although there were some differences from a clinical and pathological viewpoint in the deaths of the ^{137}Cs dogs at the two early time periods, they were sufficiently similar that they may be considered as representing one modality of death, related primarily to severe bone marrow damage. The one control animal died as the result of an overwhelming bacteremia resulting secondarily from the therapy regime that was necessary due to the autoimmune hemolytic anemia that had developed spontaneously. The ^{137}Cs dog that died at 693 days had pathological findings consistent with a diagnosis of shock although the underlying cause is not known. All other exposed animals that survived an early death are still alive at 1317 to 1571 days post-administration of $^{137}\text{CsCl}$. The infinite cumulative radiation dosage to these dogs was nearly achieved by 180 days post-injection when the cumulative doses to whole body ranged from 540 to 2200 rads. It is anticipated that the late occurring biological response anticipated in these dogs will be much more diverse than that noted for the early experimental deaths.

Figure 2. Survival of dogs injected with $^{137}\text{CsCl}$.

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TOXICITY OF INHALED ^{90}Y IN FUSED CLAY IN BEAGLE DOGS IV

ABSTRACT

Studies on the metabolism, dosimetry, and effects of inhaled ^{90}Y in fused clay in the Beagle dog are being continued with a view toward assessing the consequences of inhalation of an energetic beta emitter that has a short effective half-life in the lung. A radiation dose pattern study in which 12 dogs were sacrificed in pairs at 0, 2, 4, 6, 8 and 12 days post-inhalation exposure has been completed. A longevity study in which 89 dogs have been exposed to ^{90}Y fused clay to date with initial lung burdens ranging from 80 to 5200 $\mu\text{Ci/kg}$ and 12 control dogs were exposed to stable yttrium in fused clay is in progress. The ^{90}Y was retained in lung with a half-life that approximated the physical half-life with only small quantities translocated to tracheobronchial lymph nodes, skeleton and liver. The infinite radiation doses to lung, tracheobronchial lymph nodes, skeleton and liver, for an initial lung burden of 100 $\mu\text{Ci/kg}$ of body weight were estimated to be 1200, 170, 0.54 and 0.38 rads, respectively. Thirty-seven of 39 dogs with doses to lung from 7300 to 55,000 rads have died at 7.5 to 463 days post-exposure and the two surviving dogs in this dose range are showing signs of pulmonary insufficiency at 586 and 550 days post-exposure. All the dogs that have died have shown clinico-pathological features, consistent with radiation pneumonitis. Fifty exposed dogs with doses to lung of 990 to 6200 rads are surviving with no significant abnormalities at 548 to 1104 days post-exposure and will be studied for the remainder of their lifespan.

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INTRODUCTION

As one of a series of studies designed to better define the relative importance of total radiation dose and radiation dose rate on radiation damage to the lung following inhalation of radionuclides, dogs have been exposed to ^{90}Y in fused clay.¹ ^{90}Y , with a physical half-life of 64.2 hours was selected for study as it represents a typical short-lived, high energy beta emitter. The relative insolubility of ^{90}Y in fused clay, the short half-life of ^{90}Y and the dose levels used resulted in radiation insult to the lung and associated structures at a relatively high but rapidly decreasing dose rate. The toxicity of ^{90}Y in fused clay can then be compared to the toxicity of the longer-lived beta emitters also under study (^{91}Y , ^{144}Ce and ^{90}Sr in fused clay).

Twelve dogs were exposed to ^{90}Y in fused clay and sacrificed at 0, 2, 4, 6, 8 and 12 days post-inhalation exposure to define the radiation dose pattern following inhalation of ^{90}Y in fused clay. In addition, 89 dogs have been exposed to ^{90}Y in fused clay aerosols to achieve graded lung burdens of ^{90}Y in order to evaluate dose-response relationships over the total lifespan of the animals. Twelve dogs were exposed to aerosols of stable yttrium in fused clay as controls for the dose-response (longevity) study.

Details of the experimental procedures, the results of the radiation dose pattern studies and the early biological effects observed have been reported previously (1968-1969 Annual Report, LF-41, pp. 46-58; 1969-1970 Annual Report, LF-43, pp. 146-162; 1970-1971 Annual Report, LF-44, pp. 145-150).²⁻⁴ This report is intended to briefly summarize and update the results from the dose-response study.

EXPERIMENTAL RESULTS

The experimental design of the dose-response study is illustrated in Figure 1. The 12 blocks A through L can be grouped into 10 levels with mean initial lung burdens (ILB) of 3600, 1800, 1700, 1200, 810, 620, 390, 320, 200 and 100 μCi of ^{90}Y per kilogram of body weight and a control level in which the dogs were exposed to stable yttrium in fused clay. Two dogs per block at the 620 and 320 levels in Blocks G, H, I and J were necessary to fill in dose levels not included in the initial blocks. This provided 12 dogs at each exposure level except for the four highest levels in which dogs were not exposed in latter blocks due to early mortality.

^{90}Y , inhaled in the fused clay form, was retained in the lung with a half-life that approximated the physical half-life of ^{90}Y with only small quantities translocated to the tracheobronchial lymph nodes (TBLN), skeleton and liver. The infinite radiation doses to lung, TBLN, skeleton and liver for an ILB of 100 μCi $^{90}\text{Y}/\text{kg}$ were estimated to be 1200, 170, 0.54 and 0.38 rads, respectively.³ Doses to lung to death or infinity have been tabulated in Appendix A for each of the dogs in the dose-response study.

Projected ILB μCi/Kg	BLOCKS												Mean ILB μCi/Kg				
	A	B	C	D	E	F	G	H	I	J	K	L					
3000	333A 53,000 5200 D-7.5	333T 31,000 3600 D-12	340C 28,000 2600 D-31	347S 27,000 2800 D-47									3600				
2000	334A 19,000 1700 E-38	335S 18,000 1900 D-70	339A 19,000 1900 D-64	340U 16,000 1600 D-88									1800				
1600	334B 15,000 1400 D-23	332V 13,000 2400 D-75	341C 5000 1500 D-85	340B 15000 1400 D-90	341T 15,000 1700 D-75								1700				
1200	335A 11,000 1100 D-91	332T 11,000 1400 E-89	347B 11,000 1300 D-75	343V 7500 1100 D-92									1200				
800	333B 8000 680 D-126	339S 7800 1000 D-82	343C 7100 760 D-117	341S 6800 690 D-163	380B 6600 730 D-199	371S 4600 590 D-182	406A 12,000 980 D-108	406U 8800 1100 D-91	439A 11,000 850 D-105	437T 5600 740 D-122	451B 6900 730 D-143	448U 7600 900 D-141	810				
600					378B 5100 490 A-880	374T 3000 370 A-880	402C 4700 660 D-463	413A 7600 680 D-214	403T 4900 710 D-435	404U 3700 640 D-220	448B 6600 670 A-586	446C 6700 600 D-237	434T 4700 640 D-123	436U 5300 590 D-205	452B 6900 700 D-121	449U 4200 710 A-550	620
400	332C 3500 410 A-1101	333S 3500 460 A-1101	348C 3200 360 A-1044	343T 3100 360 A-1044	380D 2900 300 A-879	375U 2200 290 A-879	411C 3500 380 A-745	400T 3300 500 A-745	439C 3700 380 A-585	446S 3400 420 A-585	450B 4200 450 A-549	449S 3200 400 A-549	390				
300					377B 2300 250 A-879	372S 2200 230 A-879	411D 3700 380 A-753	406B 3600 300 A-753	407S 3000 320 A-753	400U 3400 400 A-753	446D 3400 300 A-584	441A 2400 270 A-584	434S 3200 340 A-584	437S 2300 280 A-584	452A 3600 380 A-549	449T 3100 380 A-549	320
200	335B 1900 190 A-1101	332S 1900 220 A-1101	339B 2100 230 A-1041	339U 1700 240 A-1041	380C 1500 140 A-878	377S 1500 150 A-878	399A 2300 260 A-752	408U 1700 190 A-752	447B 1300 180 A-583	438S 1800 190 A-583	450C 2600 250 A-548	447U 1500 220 A-548	200				
100	334C 850 100 A-1100	339T 830 130 A-1100	343A 1000 110 A-1041	340T 810 80 A-1041	379B 960 90 A-878	372T 860 83 A-878	407B 1300 130 A-752	405U 720 110 A-752	438B 840 98 A-583	436V 750 100 A-583	450E 1300 130 A-548	448T 960 120 A-548	110				
Control	333E 0 0 A-1104	334T 0 0 A-1104	349B 0 0 A-1048	348S 0 0 A-1048	378A 0 0 A-883	383U 0 0 A-883	401A 0 0 A-757	407T 0 0 A-757	441B 0 0 A-589	438U 0 0 A-589	448A 0 0 A-554	447W 0 0 A-554	0				
	333A = ANIMAL NUMBER 53,000 = INITIAL LUNG BURDEN (μCi) 5200 = INITIAL LUNG BURDEN (μCi/Kg) D-7.5 = D=DEAD, E=Euthanized, A=ALIVE - DAYS POST-EXPOSURE AT DEATH OR ON 9-30-72																

Figure 1. Experimental design for ^{90}Y in fused clay longevity study (status as of 9/30/72).

To date, of the 89 dogs exposed to ^{90}Y , a total of 37 dogs have died from 75 to 463 days post-inhalation exposure. Two dogs, 402C and 446C, died in the past year at 237 and 463 days. However, as the clinical and pathological findings in these animals were typical of the other animals that died, they will not be reported individually. The ILB of the dogs that have died ranged from 590 to 5200 $\mu\text{Ci/kg}$ with cumulative radiation doses to lung to infinity or death from 7300 to 55,000 rads. In addition, dogs 449U and 448B, with ILBs of 710 and 670 $\mu\text{Ci/kg}$ and a dose to lung of 8800 and 8300 rads, respectively, have clinical findings consistent with severe long-standing pulmonary fibrosis. The remaining 50 dogs with ILBs of 80 to 500 $\mu\text{Ci/kg}$ and doses to lung of 990 to 6200 rads and the 12 control dogs are surviving with no significant clinical abnormalities from 548 to 1104 days post-inhalation exposure. The dose-response pattern observed to date is further illustrated in Figure 2.

Figure 2. Relationship between radiation dose to lung and survival time for dogs that inhaled ^{90}Y in fused clay.

Clinical and pathological findings that have been observed to date can be related to radiation damage to the lungs and to a lesser extent the TBLN and heart. Clinically, all dogs that died showed progressive respiratory insufficiency which varied in its time course. In general, the lower the dose the longer the time between exposure and the appearance of the clinical signs and the longer the course of the disease.

The pathological lesions in the lungs were also similar in the dogs that died. They can be grouped into 5 general areas:

1. An active inflammatory reaction characterized by congestion, edema and variable leukocyte infiltration progressing to evidence of capillary injury with swelling of endothelial lining cells and, finally, intra-alveolar accumulation of dense fibrin deposits, macrophages and proliferation and bizarre hypertrophy of alveolar cells.

2. Injury to bronchiolar and alveolar duct epithelium was characterized by denudation of much of the alveolar duct epithelium. Some bronchiolar epithelium was lost in patches but more often cells of terminal bronchioles assumed a hyperchromic, peg-like configuration with focal areas of regenerative hyperplasia. Bronchial epithelium was seldom affected.

3. Conspicuous vascular and interstitial inflammation developed with time into an extensive dense fibrous accumulation around blood vessels. Ultimately, both medium and small blood arteries and arterioles developed formidable fibrous obliterative intimal thickening.

4. Development of pulmonary fibrosis. Two general patterns were observed: (a) fibrillar thickening of alveolar septal walls, and (b) large or small stellate-shaped areas of fibrosis with obliteration of the normal alveolar pattern.

5. Adenomatous proliferation of alveolar epithelium. In a few of the animals exhibiting this feature, the proliferating epithelium had undergone a distinct squamous metaplastic change filling the alveoli with nests of squamous cells.

The TBLN in early deaths showed marked lymphoid depletion while in the later deaths these nodes were enlarged and exhibited lymphoid hyperplasia. Cardiac dilatation and hypertrophy particularly of the right heart was a characteristic finding. Right atrial necrosis with varied patterns of organization and fibrosis was present in most of the animals with doses to lung from 11,000 to 29,000 rads.

DISCUSSION

In this study the majority of the dogs with cumulative radiation doses to lung of greater than 6000 rads have died at relatively early time periods with clinico-pathological findings, consistent with radiation pneumonitis and/or pulmonary fibrosis. Another study⁵ in which dogs were exposed to ⁹⁰Y in fused clay aerosols and sacrificed at one year post-inhalation has demonstrated that sub-clinical (at least by the methods employed on the dose-response study dogs) pulmonary fibrosis is present on histopathological examination with doses to lung as low as 4900 rads.

The radiation dose to lung from inhalation of ⁹⁰Y in fused clay at these levels is delivered to the lung at a relatively high but rapidly decreasing dose rate. In the other three fused clay studies (⁹¹Y, ¹⁴⁴Ce and ⁹⁰Sr) the initial dose rates are lower with higher cumulative doses due to their longer half-lives.¹ Deaths from pulmonary insufficiency have occurred in all four studies but at significantly lower doses and at earlier times in this study than any of the other three. Thus, it appears that a higher dose rate is more "efficient" in the production of acute pulmonary injury. No pulmonary neoplasms have as yet been observed in the dogs exposed to ⁹⁰Y in fused clay. However, it should be emphasized that the longest lived dogs are only 1104 days post-inhalation exposure and continued observation will be necessary to determine the potential effect of dose rate on the dose-response relationship of the ⁹⁰Y fused clay dogs relative to those exposed to longer lived beta emitters.

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TOXICITY OF INHALED ^{91}Y FUSED CLAY IN BEAGLE DOGS. III

ABSTRACT

Studies of the radiological hazards due to inhalation of ^{91}Y in fused clay are being conducted in Beagle dogs to aid in assessing the biological consequences of inhaling a relatively insoluble, high energy β emitter with a short to intermediate effective half-life in the lung. These consist of two parallel efforts: (1) a radiation dose pattern study in which 30 dogs were exposed to ^{91}Y entrapped in fused clay and were serially sacrificed from 0 to 320 days post-exposure, and (2) a longevity study in which 96 dogs have been exposed to ^{91}Y entrapped in fused clay with initial lung burdens ranging from 11 to 360 $\mu\text{Ci } ^{91}\text{Y/kg}$ of body weight and 12 control dogs exposed to stable yttrium in fused clay. The aerosols ranged in size from 1.5 to 2.6 $\mu\text{m AMAD}$. Total body deposition of ^{91}Y ranged between 40 and 50% of the inhaled activity with about 22% deposited in the pulmonary region of the lung. The ^{91}Y deposited in the pulmonary region was retained in the lung with a mean effective half life of 50 days whereas the whole-body effective half life was found to be 53 days. Small quantities of ^{91}Y were translocated to the tracheobronchial lymph nodes, liver and skeleton. Concentrations of ^{91}Y in tracheobronchial lymph nodes exceeded that of the lung by 50 days post-exposure. In the longevity study, 38 dogs have died to date between 113 and 810 days post-exposure. The cumulative radiation dose to lung to death ranged from 6500 to 47,000 rads. Several surviving dogs are presently showing clinical signs typically associated with radiation-induced pulmonary injury. Principal clinical and pathological findings in the dogs that died have been related to pulmonary edema, inflammation, fibrosis and vasculature obliterative changes. At this time it appears that only dogs with potential infinite radiation doses to lung of less than about 12,000 to 14,000 rads will be long-term survivors. All survivors will be observed over their life-span with particular attention directed toward relating ^{91}Y tissue radiation dose distribution and the resulting clinicopathological effects.

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INTRODUCTION

^{91}Y , an energetic beta-emitter with a physical half-life of 59 days was selected for inclusion in a series of dose-response studies in which beta-emitting radionuclides of varying physical half-lives have been inhaled in the fused clay form. Inhaled in the common vector fused clay, a material relatively insoluble in the lung, the effective half-life of the radionuclide in the lung is proportional to the physical half-life of the radionuclide. In this context ^{91}Y serves as model of a short to intermediate lived radionuclide. By comparing the results of this study with the results from the studies with ^{90}Y , ^{144}Ce and ^{90}Sr in fused clay, it will be possible to assess the relative importance of total radiation dose and radiation dose rate following chronic irradiation of the lung.¹ Also, ^{91}Y is an important contributor to the total fission product radionuclide inventory of a reactor after periods of sustained operation.

A radiation dose pattern study in which 30 dogs were serially sacrificed at 0, 4, 8, 16, 32, 64, 128, 193, 256 and 320 days post-inhalation exposure to ^{91}Y in fused clay has been completed. For the dose-response study 96 dogs were exposed to ^{91}Y in fused clay aerosols to result in graded initial lung burdens (ILB) and 12 dogs were exposed to stable yttrium and are serving as controls.

Detailed descriptions of the experimental procedures, the results of the radiation dose pattern study, the distribution and dosimetry of ^{91}Y in fused clay and the early biological effects have been reported previously (1969-1970 Annual Report, LF-43, pp. 163-182; 1970-1971 Annual Report, LF-44, pp. 151-163). This report briefly summarizes the previous findings and extends the results of the dose-response study to the present time.

EXPERIMENTAL RESULTS

The experimental design of the dose-response study is illustrated in Figure 1. The 12 blocks of dogs represented by the vertical columns can be grouped into 10 levels with mean ILBs of 320, 270, 210, 160, 130, 100, 82, 49, 30 and 17 μCi of ^{91}Y /kg of body weight and a control level in which the dogs were exposed to stable yttrium in fused clay. Two dogs per block in blocks E, F, I and J were exposed to fill in dose levels not included in the original blocks. This provided 12 dogs at each exposure level except for the 3 highest levels which were not completed due to evident early mortality in these animals.

The activity median aerodynamic diameters of the aerosols used ranged from 1.5 to 2.6 μm with geometric standard deviations of from 1.5 to 2.0. Of the total activity inhaled by the dogs,

Projected ILB $\mu\text{Ci}/\text{Kg}$	BLOCKS												Mean Actual ILB $\mu\text{Ci}/\text{Kg}$				
	A	B	C	D	E	F	G	H	I	J	K	L					
300	375A 3300 320 D-140	383S 3300 300 D-162	384A 3600 300 D-194	386T 4900 360 D-113									320				
250	372A 3100 270 D-185	384S 3300 300 D-202	384B 2700 260 D-236	392U 2400 260 D-153									270				
200	374A 2100 200 D-147	393S 2300 210 D-177	385A 2600 230 D-173	387V 1300 190 D-123									210				
150	374B 1500 160 D-137	385S 1300 150 D-274	385D 1500 160 D-181	390V 1100 140 E-252	423C 1500 170 D-160	426S 1300 170 D-196	420C 1700 150 D-152	419V 1100 150 D-153	491A 1700 170 D-196	484S 1200 170 D-172	489C 1500 190 D-298	483T 1100 170 D-346	160				
125					422C 1400 130 D-179	430A 1200 110 E-295	425T 940 110 A-663	420U 880 120 E-159	420B 1300 120 D-174	422S 1400 120 D-173	492A 1500 140 D-216	491B 1300 150 E-181	485U 830 130 A-382	490T 920 120 A-381	484E 1700 180 D-213	484V 680 110 A-380	130
100	376B 900 110 D-810	390T 830 97 A-843	383C 820 80 A-843	387S 800 100 A-842	422B 1200 110 A-656	419T 800 100 D-152	428A 1100 110 A-653	428S 640 89 A-660	484B 930 110 A-382	489S 890 110 A-375	489B 1300 130 A-379	490S 850 100 D-206	100				
75					420A 1000 82 D-426	432A 780 80 A-656	422T 740 75 A-653	426T 490 67 A-660	426A 910 79 A-656	425S 760 73 A-660	483D 720 94 A-380	484D 670 88 A-375	485W 500 76 A-379	492S 720 90 A-375	490A 840 92 D-124	488U 740 87 A-380	82
50	372B 400 35 A-925	396X 340 44 A-842	382B 390 57 A-843	391T 500 59 A-843	431A 500 49 A-655	421T 430 45 A-661	430C 340 42 A-661	428T 230 41 A-654	492C 340 47 D-183	491S 560 69 A-374	487B 430 59 A-374	489T 340 44 A-374	49				
25	376A 240 29 A-920	387J 260 34 A-842	386A 330 30 A-845	396S 300 33 A-842	420D 250 27 A-661	429S 270 27 A-655	422A 230 19 A-655	424S 290 31 A-655	488B 310 39 A-373	488S 230 31 A-372	489D 320 33 A-373	484T 180 27 A-372	30				
12	373A 140 15 A-920	383W 110 14 A-845	382C 130 18 A-845	383V 170 23 A-845	432B 92 11 A-653	423U 110 13 A-654	425A 180 19 A-654	420S 140 18 A-654	487A 130 16 A-373	487S 130 19 A-372	492B 140 16 A-372	485T 120 16 A-373	17				
Control	370A 0 0 A-920	385T 0 0 A-857	381B 0 0 A-857	389W 0 0 A-857	424A 0 0 A-666	428U 0 0 A-666	431B 0 0 A-666	420T 0 0 A-666	488C 0 0 A-383	485S 0 0 A-383	483A 0 0 A-383	488T 0 0 A-383	0				
	375A = ANIMAL NUMBER 3300 = INITIAL LUNG BURDEN (μCi) 320 = INITIAL LUNG BURDEN ($\mu\text{Ci}/\text{Kg}$) D-185 = D=DEAD, E=EUTHANIZED, A=ALIVE - DAYS POST-EXPOSURE AT DEATH OR ON 9-30-72																

Figure 1. Experimental design for ^{91}Y in fused clay longevity study (status as of 9/30/72).

approximately 50% was deposited in the respiratory tract and about 45% of the total deposited was retained in the lung (and considered as the ILB) following rapid clearance ($T_E < 5$ hrs) of the remaining 55% from the upper respiratory tract. The whole-body effective half-life of the retained ^{91}Y was about 53 to 58 days.

The ^{91}Y deposited in the lung was retained in the lung with an effective half-life of about 50 days. Small amounts were translocated primarily to tracheobronchial lymph nodes (TBLN), liver and skeleton. The concentration in the TBLN appeared to exceed that in the lung by about 50 days post-exposure with concentrations in the liver and skeleton about two orders of magnitude less than those of lung and TBLN. The dose to lung, TBLN, liver and skeleton per 1 μCi of ILB were estimated to be 16, 26, .038 and .082 rads, respectively, normalized to tissue weights of a 10 kg dog. Doses to lung for each of the dogs in the dose-response study are tabulated in Appendix A.

CLINICAL FINDINGS

At the present time, of the 96 dogs exposed to ^{91}Y in fused clay, 38 have died from 113 to 810 days post-inhalation. The ILB of these dogs ranged from 47 to 360 $\mu\text{Ci/kg}$ with cumulative doses to lung to death from 6500 to 47,000 rads. Of these 38 dead dogs, 12 died (420A, 483T, 484E, 484S, 489C, 491A, 491B, 492A, 490A, 490S, 492C and 376B) within the past year from 124 to 810 days post-exposure. As most of the findings in these 12 dogs were similar to those previously reported only those that differed in a significant manner will be discussed separately. Seven additional dogs with ILBs of 59 to 130 $\mu\text{Ci/kg}$ and doses to lung of 9100 to 20,000 rads have either clinically and/or radiographically detectable pulmonary damage. The 51 remaining dogs with ILBs from 11 to 110 $\mu\text{Ci/kg}$ and doses to lung from 1900 to 18,000 rads and the 12 control dogs are surviving at 372 to 925 days with no detectable abnormalities. The dose-response relationship observed to date is illustrated in Figure 2.

The clinical signs observed in the majority of the dogs that died have been very similar. The initial signs of elevated respiratory rates and abnormal sounds on auscultation of thorax were generally first seen from 3 to 4 months post-exposure. There was then a progression in the respiratory rate and increased intensity of the abnormal auscultatory findings to death. Cyanosis of the mucous membranes was generally not observed until 24 to 48 hours prior to death if at all. Increased density of the lung fields on thoracic radiographs was apparent by 4 to 6 months post-exposure.

Figure 2. Relationship between radiation dose to lung and survival time for dogs that inhaled ^{91}Y in fused clay. (Status as of 9/30/72)

In three of the dogs (490A, 490S and 492C) that died during the past year, the deaths appeared to be more sudden in nature. In fact, in two dogs (490A and 492C) no clinical symptoms had been observed prior to the death of the animal. However, only routine observations and not complete examinations had been made due to the early time of their deaths (124 and 183 days) and these two dogs had ILBs of 47 and 92 $\mu\text{Ci/kg}$ which were lower than levels at which deaths had occurred in the previous 8 blocks. Dog 490S had a respiratory rate which was generally from 60-80 min. just prior to its death while in most of the other decedents the respiratory rate had progressed to greater than 100/min. for several weeks prior to their deaths. The ILBs of these three dogs were 47, 92 and 100 $\mu\text{Ci/kg}$ and the dose to lung to death was 6500, 11,000 and 15,000 rads.

Dog 376B with an ILB of 110 $\mu\text{Ci/kg}$ and a dose to lung of 17,000 rads that died at 810 days is also of interest. Clinical signs, thoracic radiographs and pulmonary function measurements were suggestive of only a minimal degree of pulmonary damage until about 24 hours prior to the animal's death. At that time a marked dyspnea with a respiratory rate of 80/min was present and lung sounds were diminished over the entire thorax. In retrospect, the symptoms were similar to those observed in dog 391T as described below.

Of the 7 dogs that have evidence of pulmonary damage from 375 to 843 days post-exposure, 6 are showing signs of persistent pulmonary insufficiency of varying degrees. In one or two of the dogs the severity of the clinical symptoms appear to be regressing to some degree. The seventh dog, 391T, with an ILB of 52 $\mu\text{Ci/kg}$ and a dose to lung of 9100 rads is of interest in that an apparent spontaneous pneumothorax occurred at two years post-exposure. The pneumothorax was evident on thoracic radiographs and regressed slowly over a period of 4 to 6 weeks. The dog had no significant clinical or radiographic findings prior to that time and is clinically normal at this time.

PATHOLOGY SUMMARY

Complete pathological examinations have been performed on the 12 dogs that died during the past year. In 8 of the 12 dogs (420A, 483T, 484E, 484S, 489C, 491A, 491B, 492A) the gross and microscopic lesions were generally similar to those described previously (1969-1970 Annual Report, LF-43, pp. 163-182 and 1970-1971 Annual Report, LF-44, pp. 151-163). The major findings in these dogs were (1) a severe radiation pneumonitis characterized by increased numbers of alveolar macrophages, hypertrophy and hyperplasia of alveolar lining cells, degeneration and metaplasia of bronchiolar epithelium, congestion and edema, (2) a severe pulmonary fibrosis (except in 2 cases, 491B and 492A, where it was mild) characterized by widespread alveolar septal fibrosis, pleural fibrosis and large parenchymal scars, (3) a mild to moderate subintimal proliferation and fibrosis of elastic pulmonary arteries which, in one case (489C), led to small pulmonary infarction and bullous emphysema, (4) a right atrial focal degeneration and necrosis in only two cases (484E and 492A), and (5) variable TBLN lesions consisting of cortical atrophy (489C, 491B), cortical hyperplasia (491A, 420A) or germinal center atrophy with otherwise normal cortex (484S, 492A, 484E and 483T).

Three dogs had slightly different lesions than those generally seen in animals dying following inhalation of ^{91}Y fused clay. These dogs (490A, 490S, 492C) died at relatively early times post-exposure (124, 216 and 183 days, respectively) with relatively low ILB values (92 $\mu\text{Ci/kg}$; 100 $\mu\text{Ci/kg}$; 47 $\mu\text{Ci/kg}$, respectively) when compared with other animals in the study. The lesions in the three dogs were similar and were characterized by (1) a mild to moderate radiation pneumonitis with alveolar accumulations of macrophages, fibrin, edema and with mild alveolar lining cell hypertrophy and hyperplasia, (2) a mild pulmonary fibrosis with only a few foci of alveolar septal fibrosis and pleural fibrosis, and (3) a severe vasculitis which was focal and, in two cases, widespread with evidence of severe vascular damage such as widespread pulmonary hemorrhage (490A),

fibrinoid necrosis (492C) (Fig. 3) or pulmonary thrombosis (490S). The severe vascular lesions seen in these three dogs were not seen in other dogs and were apparently the reason for these dogs dying at relatively early times before severe radiation pneumonitis or pulmonary fibrosis could develop.

One dog (376B) is of special interest because of its long survival time after inhalation exposure, 810 days. Five dogs on the ^{144}Ce fused clay and ^{90}Sr fused clay longevity studies have developed pulmonary tumors prior to 810 days post-exposure. A radiation pneumonitis was present but was relatively mild, characterized by increased numbers of alveolar macrophages and some alveolar lining cell hypertrophy and hyperplasia. There was little adenomatous proliferation of alveolar cells and no metaplasia of bronchiolar epithelium as seen in some of the ^{90}Y fused clay longevity study dogs. Pulmonary fibrosis, however, was severe and widespread (Fig. 4). Fibrosis thickened the alveolar septa 10 to 50 x normal and coalesced to form thick fibrous trabeculae in a honey combed pattern. Some fibrous scars several millimeters in thickness coursed through the lung helping to form large cystic spaces. Vascular lesions were minimal and consisted of a thickened media in muscular pulmonary arteries and a few fibrous subintimal proliferations in the elastic pulmonary arteries.

DISCUSSION

At the dose levels used in this study, the initial dose rate to the lung at the higher dose levels is less than that in the ^{90}Y in fused clay study but decreases at a slower rate. The converse is true between this study and those in which the dogs have inhaled ^{144}Ce and ^{90}Sr in fused clay. The doses to lung to death in this study were about from 15,000 to 47,000 rads in the dogs dying from relatively acute pulmonary insufficiency. This is a higher cumulative dose than similar deaths which have been observed following inhalation of ^{90}Y in fused clay and lower than those following inhalation of ^{144}Ce and ^{90}Sr in fused clay.

Figure 3. Fibrinoid necrosis of muscular arteries in the lung of dog 492C. H + E stain. X 175.

Figure 4. Severe pulmonary fibrosis with minimal radiation pneumonitis and obliteration of a small muscular artery (arrow) in dog 376B. H + E stain. X 175.

Of particular interest in this study is the occurrence of the deaths of 3 dogs with pulmonary vascular lesions being the most pronounced pathological finding, contrasted with lesions of pulmonary fibrosis and/or radiation pneumonitis predominating in the remainder of the decedents and the dog that died at 810 days with extensive pulmonary fibrosis. Extensive pulmonary vasculature lesions not associated with pulmonary fibrosis have been observed in only one dog in the other three studies involving inhalation of radionuclides in the fused clay form. This was dog 303A in the ^{144}Ce fused clay study. The death at 810 days from pulmonary fibrosis is later than deaths which have occurred in the other three studies with pulmonary fibrosis being the most prominent finding. In contrast, as mentioned previously, 5 dogs in the ^{144}Ce and ^{90}Sr in fused clay studies have developed pulmonary neoplasms prior to that time. This, coupled with other dogs with clinical findings consistent with pulmonary fibrosis at later times than in the other studies, may indicate that with the radiation dose pattern and/or the dose levels selected for use in this study have resulted in more subclinical pulmonary fibrosis. If and when pulmonary tumors develop in this study, it will be interesting to see if the tumor type may bear any relation to the degree of pulmonary fibrosis.

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TOXICITY OF INHALED ^{144}Ce FUSED CLAY IN BEAGLE DOGS. V

ABSTRACT

The metabolism, dosimetry, and effects of inhaled ^{144}Ce in fused clay are being investigated in the Beagle dog to aid in assessing the biological consequences of release of ^{144}Ce in a relatively insoluble form such as might occur in certain types of nuclear accidents. Further, the toxicity of inhaled ^{144}Ce fused clay is of broad interest since it may be considered as representative of a group of intermediate-lived beta emitting radionuclides. Two major studies are being performed: (1) a metabolism and dosimetry study in which 24 dogs have been serially sacrificed over an extended period of time, and (2) a longevity study with 2 series of dogs; Series 1 with 15 dogs exposed to aerosols of ^{144}Ce in fused clay to yield initial lung burdens of 11 to 210 $\mu\text{Ci/kg}$ and 3 control dogs exposed to nonradioactive clay and Series II with 96 dogs exposed to aerosols of ^{144}Ce in fused clay to yield initial lung burdens of .0024 to 66 $\mu\text{Ci/kg}$ and 12 control dogs exposed to nonradioactive clay. The ^{144}Ce in fused clay is slowly translocated from the lung to tracheobronchial lymph nodes, liver and skeleton, however, because of the small size of the tracheobronchial lymph nodes they exceed the lung in ^{144}Ce concentration by 100 days post-inhalation. To date, 23 dogs have died or been euthanized at 143 to 1318 days after inhalation of ^{144}Ce . The prominent findings have been radiation pneumonitis in 17 dogs and in 4 cases hemangiosarcoma of the lung, in 1 case both a hemangiosarcoma and a fibrosarcoma and in 1 case both a bronchiolo-alveolar carcinoma and a hemangiosarcoma of lung. The cumulative radiation dose to the lung to time of death has ranged from 19,000 to 100,000 rads. The 103 surviving dogs include dogs with a potential for accumulating radiation exposures to lung of up to 49,000 rads. Serial observations are continuing on all survivors with special attention directed toward evaluating ^{144}Ce metabolism and dosimetry, and various clinical, pulmonary function, hematologic, serum chemical and microbiological parameters.

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INTRODUCTION

^{144}Ce is an important contributor to the fission product inventory of a nuclear reactor after a period of sustained operation. Further, because of its relatively long physical half-life, 285 days, it represents a substantial portion of the fission product activity present in spent fuel elements as well as solutions and solids associated with the chemical processing of such fuel elements. ^{144}Ce decays to ^{144}Pr which has a half-life of 17.3 minutes; during their decay, several gamma photons and a number of beta particles with an average energy of 1.27 Mev are emitted. Because of the probability of release of ^{144}Ce in nuclear accidents and the radiologic characteristics noted above, it was selected for extensive study in this program. Studies with a relatively soluble form, $^{144}\text{Ce-Cl}_3$, are reported elsewhere (see this report, pp. 137 - 139). Recognizing that relatively insoluble forms of ^{144}Ce might be released, it was decided to study ^{144}Ce entrapped in fused montmorillonite clay as a typical insoluble form. In conducting these studies, it was recognized that the information obtained on the biological response of dogs that had inhaled ^{144}Ce in fused clay will be useful in predicting the hazards associated with inhalation of ^{144}Ce but perhaps more important, the information gained would be of value in predicting the biological consequences of inhaling any intermediate-lived, insoluble, beta-emitting radionuclides.

The experimental details for these studies, including the results of a radiation dose pattern study, have previously been reported (Annual Reports LF-39, pp. 33-53; LF-41, pp. 19-35; LF-43, pp. 183-187; and LF-44, pp. 164-180). Suffice it to note that one series of longevity dogs were exposed to aerosols of per-fused ^{144}Ce fused clay particles (Fig. 1) and a second series of longevity dogs were exposed to particles fused at the time of exposure (Fig. 2). This report will detail the current status of this experiment.

Figure 1. (right) - Experimental design for ^{144}Ce fused clay longevity study (Series I). (Status as of 9/30/72).

Projected ILB $\mu\text{Ci/Kg}$	BLOCKS			Mean Actual ILB $\mu\text{Ci/Kg}$
	A	B	C	
200	210B 1500 190 D-173	208B 2000 180 D-182	228B 1700 210 D-143	190
100	209B 1700 190 D-181	211G 890 120 D-171	226C 740 96 D-189	140
50	211A 540 66 D-257	211E 440 51 D-410	217A 600 68 D-186	62
25	211D 190 27 D-1185	211F 170 19 A-1746	228A 330 34 E-1318	27
12.5	209C 100 11 A-1753	208D 91 15 A-1746	223A 150 15 A-1705	14
Control	208A 0 0 A-1747	209D 0 0 A-1747	220C 0 0 A-1703	0

210B
1500
190
D-173
 =ANIMAL NUMBER
 =Initial Lung Burden (μCi)
 =Initial Lung Burden ($\mu\text{Ci/Kg}$)
 =D=Dead, E=Euthanized, A=Alive
 Days Post-Exposure at death or on 9-30-72

Projected ILB $\mu\text{Ci/Kg}$	BLOCKS												Mean Actual ILB $\mu\text{Ci/Kg}$
	A	B	C	D	E	F	G	H	I	J	K	L	
50	298B 590 65 D-234	297S 470 46 E-750	315A 380 35 D-916	315V 470 66 D-246	327A 520 56 D-273	330S 440 53 D-279	454A 280 32 A-533	453S 230 29 A-533	465A 460 41 D-311	465S 330 41 D-185	470A 480 44 D-275	479U 360 54 A-414	47
25	303A 320 33 D-193	298S 240 23 A-1249	312B 210 24 A-1221	310T 230 26 E-765	330B 220 34 A-1157	330U 220 37 E-1077	455B 220 19 A-533	453T 110 18 A-533	464B 250 27 A-463	460S 190 24 A-463	471A 150 19 A-414	480S 200 24 A-414	26
12.5	297B 110 12 A-1249	304S 120 17 A-1249	311B 130 14 A-1222	315U 150 18 A-1221	326C 110 12 A-1157	328T 140 13 A-1157	454E 95 9.8 A-534	454S 160 10 A-534	467A 160 13 A-464	460T 70 9.5 A-464	480B 91 11 A-415	467T 81 13 A-415	13
6	303B 51 7.6 A-1250	305V 57 9.8 A-1250	308B 55 5.4 A-1222	310S 72 6.3 A-1222	327B 60 8.0 A-1158	323V 60 7.8 A-1158	454C 51 5.4 A-534	455T 130 4.9 A-534	463A 130 12 A-464	464T 37 5.0 A-464	478B 54 5.7 A-415	469S 42 5.8 A-415	7
1.2	296B 21 2.1 A-1234	304T 12 1.6 A-1234	313C 17 1.8 A-1209	313S 19 2.4 A-1209	331A 11 1.3 A-1159	324V 11 1.5 A-1159	453B 5.1 0.63 A-535	452U 4.2 0.52 A-535	461B 13 1.2 A-465	466V 15 2.0 A-465	477A 12 1.1 A-416	467U 7.5 1.2 A-416	1.4
0.23	297A 2.0 0.18 A-1235	296U 3.7 0.44 A-1235	313B 3.7 0.37 A-1212	314S 4.4 0.45 A-1212	329C 5.9 0.71 A-1156	322V 2.0 0.32 A-1159	457B 1.4 0.17 A-535	453U 1.1 0.18 A-535	461A 4.3 0.35 A-465	463S 5.5 0.53 A-465	476C 2.7 0.30 A-416	471S 1.6 0.25 A-416	0.35
0.045	304A 0.50 0.0442 A-1240	298U 1.2 0.12 A-1240	310A 0.59 0.051 A-1212	308U 0.77 0.076 A-1212	327D 0.14 0.016 A-1160	323T 0.33 0.039 A-1160	450D 0.20 0.18 A-536	451T 0.45 0.057 A-536	462C 0.75 0.083 A-466	464S 0.50 0.062 A-466	476B 0.67 0.079 A-417	472W 1.3 0.16 A-417	0.069
0.009	306A 0.31 0.033 A-1241	303S 0.68 0.077 A-1241	312A 0.27 0.025 A-1213	310U 0.33 0.041 A-1213	327C 0.090 0.0096 A-1160	324T 0.068 0.0063 A-1160	453A 0.027 0.0030 A-536	452T 0.023 0.0024 A-536	465B 0.20 0.018 A-467	462S 0.11 0.014 A-467	478C 0.081 0.0092 A-418	472U 0.17 0.020 A-418	0.022
Control	306D 0 0 A-1242	303V 0 0 A-1242	310B 0 0 A-1213	308T 0 0 A-1213	324B 0 0 A-1160	322U 0 0 A-1160	450A 0 0 A-540	452S 0 0 A-540	467B 0 0 A-470	464U 0 0 A-470	477B 0 0 A-421	479T 0 0 A-421	0

298B
590
65
D-234
 =ANIMAL NUMBER
 =INITIAL LUNG BURDEN (μCi)
 =INITIAL LUNG BURDEN ($\mu\text{Ci/Kg}$)
 =D=DEAD, E=EUTHANIZED, A=ALIVE—DAYS POST EXPOSURE AT DEATH OR ON 9-30-72

Figure 2. Experimental design for ^{144}Ce fused clay longevity study (Series II). (Status as of 9/30/72).

RESULTS

No new information was obtained during the past year on the metabolism and dosimetry of inhaled ^{144}Ce fused clay. Dosimetry calculations continued to be made with the procedures previously described (1968-1969 Annual Report, LF-41, pp. 19-35) using the dog's own whole-body retention data when available and a standardized relationship between lung burden and total retained body burden. For 24 low-level dogs, satisfactory whole-body retention data was not available so a standardized whole-body retention function has been used. Current information on initial lung burdens and dosimetry values are presented in Appendix A.

During the past year, five additional dogs died bringing to 23 the total number of dogs from the two series that have succumbed (Fig. 3); 17 with death related to radiation pneumonitis and pulmonary fibrosis and 6 with death due to pulmonary neoplasms.

Three dogs (465A, 465S, 470A) died with radiation pneumonitis and pulmonary fibrosis at 311, 185 and 275 days post-exposure with ILB values of 41, 41 and 44 $\mu\text{Ci/kg}$ and cumulative doses to lung of 22,000, 32,000 and 32,000 rads, respectively. The clinical, hematological, clinical chemistry, pulmonary physiological and pathological findings were generally similar to those seen in other dogs similarly exposed and previously described in earlier reports (1967-1968 Annual Report, LF-39, pp. 33-53; 1968-1969 Annual Report, LF-41, pp. 19-35). The dog (465S) dying at an early time (185 days) had an acute vasculitis of small muscular arteries characterized by fibrinoid necrosis. This lesion was not prominent in the other two dogs. One additional dog, 479U, with an ILB of 54 $\mu\text{Ci/kg}$ is showing signs consistent with pulmonary insufficiency at 414 days post-inhalation exposure. Of the 6 dogs that have died from pulmonary neoplasms, two dogs, 315A and 330U, with ILBs of 35 and 37 $\mu\text{Ci/kg}$ and cumulative doses to lung of 34,000 and 36,000 rads, respectively, died at 916 and 1077 days post-exposure during the past year. Both dogs had a pulmonary hemangiosarcoma. In addition, 315A had bronchiolo-alveolar carcinoma. In many respects the tumors were similar to those previously reported in the other 4 dogs with tumors in this study (1970-1971 Annual Report, LF-44, pp. 164-192). The remaining 88 exposed dogs with ILBs from 0.0024 to 34 $\mu\text{Ci/kg}$ and the 15 control dogs are surviving from 414 to 1753 days post-exposure with no significant abnormalities. These surviving exposed dogs have the potential of cumulative doses to lung of 2.7 to 35,000 rads. The dose-response relationship observed to date is shown in Figure 3.

Figure 3. Survival of Beagle dogs that inhaled ^{144}Ce fused clay. (Status as of 9/30/72).

Clinical examinations and thoracic radiographs on dog 315A from 9 months to 2 years post-exposure were consistent with mild pulmonary fibrosis in that there was a tendency toward a honey-comb pattern of the lung fields on thoracic radiographs and mild increased bronchial sounds on auscultation of the thorax. On thoracic radiographs at about 2 years post-inhalation exposure, there was no indication of a pulmonary mass. Twenty-four hours prior to the dog's death at 916 days (~ 2 1/2 years) post-exposure, the dog started coughing up large quantities of blood mixed with some mucus. At that time there was a soft tissue density mass occupying the right apical region of the lung field and evidence of severe pulmonary edema and/or multiple masses in the lung fields on thoracic radiographs. After repeated episodes of hemoptysis over 18 to 24 hours, the dog's mucous membranes became markedly cyanotic and there were marked harsh rales on auscultation of the thorax. The dog died shortly after a final episode of hemoptysis.

On dog 330U, there were no significant clinical findings prior to about 2 1/2 years post-exposure when a "coin type" lesion about 1 x 2 cm in size was present in the right mid-lung field. On subsequent radiographs up to the time of euthanasia this lesion (Fig. 4) enlarged to about 1 1/2 to 2 times in size and appeared denser. There were no other significant radiographic findings including the findings on a post-mortem skeletal survey. At about one week prior to euthanasia a posterior ataxia was noted. This progressed to complete posterior paralysis with urinary and fecal incontinence by 48 hours prior to euthanasia. The dog was euthanized at 1077 days post-exposure due to the complications of the posterior paralysis.

Figure 4. Thoracic radiographs of dog 330U at 734 days (left) and 1056 days (right) post-inhalation exposure. At 734 days there were no significant lesions, but at 1056 days a mass was visible just to the left of the heart (arrow).

At necropsy, dog 315A had one large and several smaller hemorrhagic masses present in the right apical lobe of the lung which projected above the pleural surface. The rest of the lung was congested and edematous. Pleural fibrosis was present where lobes were in contact. The tracheo-bronchial lymph nodes were small, firm and fibrotic. There was no gross evidence of metastasis outside the thoracic cavity.

Histologically, the large mass in the right apical lobe was composed of large blood-filled spaces, fibrosis and necrotic tissue. The spaces were lined by oval or spindle-shaped cells with indistinct boundaries, containing a moderate amount of cytoplasm and oval nuclei with sparse chromatin and prominent nucleoli (Fig. 5). The pattern was characteristic of a hemangiosarcoma. A few mitotic figures were found. In some places, masses of neoplastic cells were found in the trabeculae between the blood-filled spaces.

The remainder of the lobe was infiltrated with neoplastic cells of a different type that were characteristic of a bronchiolo-alveolar carcinoma. The cells, medium sized, round or cuboidal, occurred in clumps or ribbons and had distinct cytoplasmic boundaries. The individual cells had a moderate amount of eosinophilic cytoplasm and a moderate-sized round or oval nucleus with sparse chromatin and occasionally a prominent nucleolus. The distinctive pattern of the cells was their growth along alveolar walls and the tendency to fill alveoli with little alteration of alveolar architecture (Fig. 6). Similar, but smaller infiltrations of neoplastic cells, were seen in the left apical cardiac and right diaphragmatic lobes. Large clumps of these cells were found lining and filling lymphatics in all lobes (Fig. 7).

In some areas of the lung away from foci of neoplastic infiltrations, histologic changes typical of radiation pneumonitis and pulmonary fibrosis could be seen. Fibrosis of alveolar septa was prominent and occasionally large parenchymal scars could be seen. Hypertrophic and hyperplastic alveolar lining cells were frequently seen lining the alveolar septa. Many alveoli contained vacuolated macrophages and a few neutrophils. In the smaller bronchioles there was a loss of much of the epithelium with a hypertrophy of the remaining cells. The trachea was normal except for the filling of the peritracheal lymphatics with neoplastic cells of the bronchiolo-alveolar type.

The tracheobronchial lymph nodes were completely obliterated by fibrosis and metastasis from the bronchiolo-alveolar tumor. A general outline of the node could be determined by the metastasis-filled peripheral sinus and a small accumulation of radioisotope observed as an autoradiographic image. No other significant lesions or metastasis were found in the remainder of the organs.

At necropsy, dog 330U had two nodules present in the lung, one in the right apical lobe (2 cm diameter) and one in the anterior portion of the right diaphragmatic lobe (1 cm diameter). Several firm scars could be palpated in the anterior portions of both diaphragmatic lobes and in the central portions of both apical lobes (Fig. 8).

The tracheobronchial lymph nodes were so small they were difficult to accurately locate. No lesions were found in any of the other nodes of the thorax. Three reddish nodules (ranging from 0.3 to 2.5 cm diameter) protruded from the capsule of the spleen.

A red lobulated mass measuring 1 x 4.5 x 0.5 cm was present in the spinal canal at the level of the second lumbar vertebra. The mass was attached to the vertebral body and compressed the adjacent spinal cord. No gross lesions were noted in other organs.

Histologically, the nodules in the right apical and right diaphragmatic lobes had a pattern of hemangiosarcoma. The larger one was a very cellular mass that compressed and invaded surrounding tissue, including blood vessels (Fig. 9). The basic cell type had an oval nucleus with a small to moderate amount of clumped chromatin and one or two prominent nucleoli. Mitotic figures were fairly common (Fig. 10). The cytoplasm was faintly eosinophilic and had indistinct boundaries. The cells formed a syncytium, small vascular channels or, rarely, large cavernous spaces filled with red blood cells. Many areas of necrosis were present and, in the smaller nodule, occupied much of the tumor. In conjunction with the tumors were large areas of fibrosis that appeared to be pre-existing pulmonary fibrosis and not reaction to the tumor. In areas away from the tumor there was focal but widespread pulmonary fibrosis. Evidence of active radiation pneumonitis was scant.

A severe atrophy of the tracheobronchial lymph nodes with partial replacement of some lymphoid tissue was present. No tumor metastasis could be found in this or any lymph nodes. The three splenic nodules were hemangiosarcomas very similar histologically to those seen in the lung. The mass on the vertebral body also had the histologic characteristics of a hemangiosarcoma. It had

Figure 5. Cellular detail of the hemangiosarcoma in the lung of dog 315A showing the large oval nuclei and prominent nucleoli in the neoplastic cells. EM stain. x 640

Figure 6. Bronchiolo-alveolar carcinoma filling and lining the alveoli in the lung of dog 315A. H + E stain. x 250

Figure 7. Bronchiolo-alveolar carcinoma filling the lymphatics (arrow) in the lung of dog 315A. H + E stain. x 90

Figure 8. Lungs of dog 330U. Note tumor nodules at arrows.

Figure 9. Proliferation of the hemangiosarcoma in the lumen of an elastic pulmonary artery of dog 330U. (arterial wall at arrow) EM stain. x 10

Figure 10. Detail of hemangiosarcoma in the lung of dog 330U showing large nuclei, prominent nucleoli and many mitotic figures (arrows) of the neoplastic cells. H + E stain. x 500

invaded the bone, marrow cavity, muscle and spinal canal but not the spinal cord. A microscopic tumor embolus was found in one glomerulus of the kidney but no other metastatic lesions were found in other organ systems.

DISCUSSION

The beta particles from decay of ^{144}Ce and ^{144}Pr have a substantial range in tissue and when large numbers of ^{144}Ce -labeled particles are deposited in the lung, the result is a relatively uniform distribution of the radiation dose to the lung. When incorporated in fused clay, the ^{144}Ce is relatively insoluble and has an effective half-life in the lung of about 200 days resulting in protracted radiation exposure to the lung. Because of the relatively long retention time in the lung and the loss of ^{144}Ce through physical decay, only a small amount of the initial lung burden was translocated to the other tissues: tracheobronchial lymph nodes (< 2% ILB), liver (< 5% ILB) and skeleton (< 3% ILB). However, when these tissue activity levels are examined on a concentration basis, the concentration in the tracheobronchial lymph nodes soon exceeds that in the lung leading to high absorbed radiation dose for this tissue as well.

The radiation dose to the lung of any given dog in this study is dependent on three factors: the initial lung burden, the removal rate of the material from lung, and the lifespan of the dog. These relationships for the 6 dogs that died of pulmonary neoplasm are shown in Figure 11a and b.

In general, as may be seen in Figure 3, dogs with greater initial lung burdens died at early times with radiation pneumonitis and pulmonary fibrosis. The average time of survival post-exposure for this mode of death was 226 days. This is in contrast with the average time of death for dogs dying of pulmonary neoplasia which was 1002 days post-exposure. No dog died due to radiation pneumonitis after 410 days and no dog died with pulmonary neoplasms in less than 750 days.

The clinical findings indicate that the pulmonary hemangiosarcomas are rapidly growing, fatal tumors. In spite of annual clinical examinations and daily observation, the longest time a dog lived after initial diagnosis of a pulmonary tumor was three months.

Figure 11. Dose rates (a) and cumulative radiation doses (b) to the lung after inhalation of ^{144}Ce fused clay particles in six dogs with pulmonary neoplasms compared with surviving dogs (shaded areas).

The tumors found in 6 dogs exposed to ^{144}Ce fused clay particles are apparently primary to the lung. The evidence is strong that the tumors in dogs 228A, 297S, 310T, 315A and 330U are, in fact, primary to the lung while dog 211D is less clear-cut but likely in view of the other cases. In four of these dogs, there was a pulmonary tumor mass much larger than any other mass in the remainder of the lung or elsewhere in the body. In one dog, 315A, the hemangiosarcoma was restricted to the lung and no neoplasm of this type was found outside the lung at post-mortem examination or with post-mortem radiographic surveys.

The distribution of the neoplastic foci in the lungs of the 5 dogs is not compatible with the usual picture of a hematogenous metastatic origin. For example, Waller and Rubarth,¹ in a study of 49 dogs with hemangioendothelioma, found tumors in the lungs of 37 dogs. The tumors, in most cases, were multiple, scattered over the whole lung and "seldom larger than a hazelnut." In 35 of the dogs, tumors were found in other organs, usually the heart, that were considered the primary site. In the two dogs where no extra-pulmonary tumors were discovered, it was suggested because of the distribution of the pulmonary tumors, that the primary site was not found. Kleine *et al.*² in a study of 31 dogs with hemangiosarcoma of the heart, found metastatic hemangiosarcomas in the lungs of 18 dogs. The pulmonary nodules were numerous, diffusely disseminated, spherical, of similar size (0.5 to 2 cm in diameter), red to black, and oozed blood when incised.

It is difficult to accurately determine the age of a neoplastic focus but the clinical history of dog 310T is additional evidence that the tumor was primary in the lung. In this dog, a singular pulmonary mass was detected radiographically and followed 84 days later by disseminated multiple pulmonary nodules. A splenic nodule was detected 80 days after noting the lung mass and only 7 days before death, indicating the splenic tumors were probably metastatic and not primary.

The distribution and appearance of the pulmonary tumors in dog 211D are consistent with a hematogenous metastatic tumor to the lung; however, no particular extrapulmonary site can be singled out as a primary site either on a basis of size, degree of anaplasia and invasion or time of onset. It is possible and, in view of the similarities or exposure and time with the other five dogs, probable that the lung in dog 211D was the primary site and that intrapulmonic metastasis occurred. Such metastasis does occur in spontaneous canine pulmonary tumors. Ressang,³ in a study of 19 dogs with metastasis from primary pulmonary carcinomas, found intrapulmonary metastasis in 63% of the animals.

The findings of primary pulmonary hemangiosarcomas after inhalation of ^{144}Ce is a new and unexpected finding. Stuart *et al.*⁴ found no evidence of neoplasia in dogs followed for 480 days after inhalation of an aerosol of $^{144}\text{CeO}_2$. No hemangiosarcomas of the lung have been observed in any of the control dogs in this colony. In fact, primary pulmonary hemangiosarcomas have not been reported in the dog, although hemangiosarcoma of other origins is not uncommon. One hemangioma of the lung of a dog was reported by Smith and Jones⁵ in a search of the AFIP collection. They also reported finding two sarcomas, type unspecified. There have been only a few reports of primary pulmonary hemangiosarcoma in any species following inhalation exposure to radionuclides. Howard⁶ reported hemangiomas in two dogs exposed to $^{239}\text{PuO}_2$ particles. Lisco⁷ reported a hemangiosarcoma in a rat exposed to $^{239}\text{PuO}_2$ fumes and Cember⁸ reported one case in a rat exposed to $^{144}\text{CeCl}_3$ which had received a dose of over 25,000 rads. Primary lung neoplasms of other types have been found in dogs after inhalation of $^{239}\text{PuO}_2$ particles.⁶ Of 40 dogs exposed, 22 died with pulmonary lung neoplasia 1151 to 3313 days post-exposure. Most of the tumors found were bronchiolo-alveolar carcinomas and three epidermoid carcinomas. Also in this group of dogs were three with thoracic sarcomas and two with malignant lymphomas.

Although the experiment is still in progress, the dogs that have died with pulmonary tumors to date have succumbed at earlier times post-exposure (750 days) than those reported after inha-

lation of $^{239}\text{PuO}_2$ (1151 days). The relationship of the tumors in the two groups of dogs with lesions in the lungs is also interesting. The bronchiolo-alveolar carcinomas in the plutonium study are reported to arise from areas where there is severe fibrosis and much hyperplasia and the metaplasia of alveolar epithelium. In the one instance of bronchiolo-alveolar carcinoma occurring after inhalation of ^{144}Ce in our study, this was also the case. However, in most dogs with hemangiosarcomas the pulmonary fibrosis was not nearly as severe and alveolar hyperplasia and metaplasia were not as prominent as in the dogs exposed to ^{239}Pu . Some of the residual radiation pneumonitis may have been obscured by the hemangiosarcomas in these dogs as the neoplasms arose in the anterior lobes of the lung, the very lobes where, typically, radioisotope concentration is highest and damage is most severe.

It is tempting to speculate on the pathogenesis of the neoplasms in the dogs exposed to ^{144}Ce fused clay particles and compare it with that of dogs exposed to $^{239}\text{PuO}_2$. The location of the endothelial tumors in the anterior lobes where the radioisotope concentration is highest indicates that the high radiation dose is probably related to the initiation of the tumors. The vascular neoplasms may arise from a localized but severely scarred foci or from a direct effect of energetic beta particles on vascular endothelium. A direct effect should not be surprising considering nearly 50% of the cells of the alveolar portions of the lung are endothelial cells.^{9, 10} The alpha particles from the $^{239}\text{PuO}_2$, although highly ionizing, penetrate the tissue only slightly and, thus may explain the predominance of epithelial tumors in ^{239}Pu -exposed dogs. The completion of this experiment should help clarify these speculations.

If, in coming years, people develop similar hemangiosarcomas following inhalation of beta-emitting isotopes, it would bode ill for the treatment of such cases. These neoplasms in dogs were rapidly growing, frequently disseminated and would be very difficult to remove surgically or treat with radiation therapy.

In conclusion, it should be noted that it is very unlikely that people will inhale quantities or forms of ^{144}Ce which will result in lung radiation doses similar to those seen in the dogs. It remains to be seen whether similar neoplasms will be observed at later times in dogs that received lower initial lung burdens of ^{144}Ce . Such information will be of great importance in assessing the radiation protection standards for potential low levels of exposure.

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TOXICITY OF ^{144}Ce FUSED CLAY INHALED BY IMMATURE BEAGLE DOGS. I.

ABSTRACT

The metabolism, dosimetry and effects of ^{144}Ce fused clay inhaled by immature Beagle dogs (~ 3 months of age at exposure) is being investigated for comparison with information obtained in companion studies with dogs exposed at 12-14 months of age and 8-10.5 years of age. These studies will provide an assessment of possible age-related differences in the biological behavior and effects of inhaled radionuclides, differences that may be of significance in predicting the response of accidentally exposed human populations that include individuals of varied age. Eighteen immature dogs have been entered into a radiation dose pattern study to be serially sacrificed at different intervals post-inhalation exposure. Dogs have been sacrificed at 0, 8, 32 and 64 days and tissue analyses indicate a higher content of ^{144}Ce in skeleton than in liver in contrast with the reverse relationship seen in dogs exposed as young adults. The ^{144}Ce content of tracheobronchial lymph nodes was also greater in the immature dogs. Eight dogs exposed to achieve graded initial lung burdens of ^{144}Ce in fused clay and one control dog have been entered as the first block in a longevity study. Additional blocks will be entered into the study in the future.

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INTRODUCTION

Because a population of humans that might be exposed in a nuclear incident could contain individuals differing widely in age, it is important that age-related effects on the biological behavior and effects of inhaled radionuclides be examined. To examine this question, two experiments were initiated during the past year to complement the experiment already underway in Beagle dogs exposed at 12-14 months of age. One experiment involving dogs exposed at 8 to 10.5 years of age is reported elsewhere (this report, pp. 172 -176). The second involves immature dogs that were ~ 90 days of age at exposure. Cerium-144 in fused clay was selected as the exposure aerosol because it may be considered as typical of many beta-emitting radionuclides with an intermediate retention half-time in lung.

One block of longevity dogs containing 8 dogs exposed to achieve graded initial lung burdens of ^{144}Ce and one control dog exposed to a fused clay aerosol containing only stable cerium has been entered into the longevity experiment at the present time. The lung burden levels used are similar to those used in the study with dogs exposed at 12-14 months of age. In addition, 18 dogs that were 85 to 97 days of age were entered into a radiation dose pattern study for serial sacrifice in pairs at different intervals post-inhalation exposure.

EXPERIMENTAL PROCEDURES

All dogs entered in this experiment were purebred Beagle dogs produced in this colony. Each dog was transferred from the kennel facility to individual metabolism cages at least 3 weeks prior to exposure to allow cage and restraint acclimation as well as pre-exposure sampling. The dogs were supplied water ad libitum and the normal daily rations of the colony diet.

On the day of exposure, food was withheld until the inhalation exposure and post-exposure whole-body counting were complete. Pre-exposure sedation consisted of 60 mg phenobarbital orally at 2.5 hours before exposure followed by an intravenous injection of Vetame® (0.25 mg/lb body wt) at 45 minutes before exposure. Exposures were conducted individually in a previously described apparatus¹ that permitted "nose-only" inhalation exposures.

A collimated NaI scintillation detector was used to monitor the deposition of ^{144}Ce in the lower respiratory tract and indicate when the desired initial lung burden had been reached. The duration of exposure ranged from 7 to 52 minutes depending on the desired initial lung burden, the exposure air concentration and the dog's respiratory pattern. For the longevity dogs with very low initial lung burdens, this thoracic monitor was not useful. Thus, exposure times were calculated using an assumed percentage for aerosol deposition in the lung. For the different exposures, the particle size distributions of the ^{144}Ce -fused clay aerosols were log normal with activity median aerodynamic diameters (AMAD) that ranged from 1.6 to 2.2 μm for individual exposures with geometric standard deviations of 1.3 to 1.6. Concentrations of ^{144}Ce in the exposure air ranged from 0.01 to 40 $\mu\text{Ci/liter}$ for individual exposures.

A profile scan and whole-body count were obtained immediately post-exposure and continued to be taken at selected times post-exposure to quantitate lung and whole-body retention. Daily urine and feces collections were made on four dogs in the radiation dose pattern study during the first 64 days after exposure and will be made periodically in the future.

The clinical condition of all longevity dogs was critically assessed prior to exposure. This included measurement of serum biochemical, hematological and pulmonary function parameters. Special attention was directed to age differences in serum biochemical and pulmonary function parameters which are included elsewhere in this report (pp.199 -205 and pp.195 -198). Serial clinical observations will continue to be made throughout the animals' life.

RESULTS AND DISCUSSION

Initial Lung Burdens and Deposition Percentages:

Two-component exponential functions are adequate to describe the whole-body retention of ^{144}Ce in the early time period after an inhalation exposure to ^{144}Ce in fused clay particles. Previous work has shown that the initial lung burden of ^{144}Ce inhaled in this form can be estimated from the zero-time intercept of the long-term component of whole-body retention. First estimates of the initial lung burdens were made by extrapolating the long-term component obtained from the whole-body counts during the first 14 days. These values will be refined as more data are obtained and a computer is used to fit curves to the data. Computed in this manner, the initial lung burdens for the dogs in the radiation dose pattern study ranged from 9 to 18 $\mu\text{Ci } ^{144}\text{Ce}$ per kg body weight.

Values for the dogs in Block A of the longevity experiment are given in Table 1 along with the levels that were desired for each dog. In general, there is reasonably good agreement between the desired and actual initial lung burdens. Because 629B was too low to be useful in the 75 $\mu\text{Ci/kg}$ level, it will not be included in the longevity experiment. When the next block of male dogs is entered into the experiment, 2 dogs will be exposed at 75 $\mu\text{Ci/kg}$ to compensate for this deletion. An unusually high percentage of the initial body burden for 629B (85%) was associated with the upper respiratory tract. Apparently, some of this material was detected during exposure by the thoracic monitor, thus leading to an overestimate of the lung burden and a pre-mature termination of the exposure. Because of the small size of dogs at this age, a large portion of the whole body is seen by the thoracic monitor, reducing the ability to discriminate between activity in the upper and lower respiratory tracts and gastrointestinal tract.

From the initial whole-body and lung burdens determined from whole-body counting, the mean concentration of ^{144}Ce in the exposure air and the volume of air inhaled during exposure, it was possible to calculate deposition percentages for total body and lung. For the 18 dogs in the radiation dose pattern study, the total deposition had a mean of 43% with range of 25 to 69%. Of the total deposition, a mean fraction of 0.38 (range of 0.19 to 0.64) was associated with the lung. Deposition

Table 1

Initial Lung Burdens of Dogs in Block A of the Longevity Experiment
Involving Immature Dogs Exposed by Inhalation to ^{144}Ce in Fused Clay

Dog. No.	ILB $\mu\text{Ci/kg}$	
	Desired	Actual
629B*	75	40
629A	50	36
627B	25	24
630B	12.5	9.5
630A	6.0	5.7
624D	1.2	3.0
623A	0.23	0.28
624C	0.045	0.061
624A	0.009	0.014**
623B	0	0

* Because the desired ILB was not obtained, this dog will not be included in Block A. It will be replaced when another block of males is entered into the experiment.

** The data were extremely variable due to their closeness to background rates. Listed value represents average of whole-counts at 1, 4, 8 and 14 days post-exposure.

in the lung averaged 16% with a range of 9.0 to 30%. Corresponding values for the longevity dogs in Block A were total deposition = 68% (range = 41 to 88%), lung deposition = 20% (range = 8.8 to 29%) and ^{144}Ce in lung as a fraction of total body ^{144}Ce = 0.27 (range = 0.11 to 0.40).

Metabolism and Dosimetry:

To date, 8 dogs have been sacrificed in the radiation dose pattern study in the interval from 9 to 64 days post-exposure. Relative tissue distribution data, expressed as percent of sacrifice body burden, are presented in Table 2 for the dogs sacrificed from 8 to 64 days after exposure. Comparable data from an earlier radiation dose pattern experiment involving young adult dogs exposed to ^{144}Ce in fused clay were reported earlier (1969-1970 Annual Report, LF-43, p. 185); mean values are included for comparison in Table 2. The fraction of body burden associated with

Table 2
Relative Tissue Distribution of ^{144}Ce Following Inhalation of ^{144}Ce in Fused Clay
by Immature (~3 months) and Young Adult (18-22 months) Beagle Dogs

Days Post-Exposure	Age At Exposure	Percent Of Sacrifice Body Burden			
		Lung	TBLN	Liver	Skeleton
8	~3 months	95	.65	.25	1.1
8	~3 months	94	.052	.22	1.8
8	18-22 months*	95	.075	2.0	1.9
32	~3 months	95	1.1	1.0	2.6
32	~3 months	96	.28	1.2	2.1
32	18-22 months*	94	.12	2.8	2.1
64	~3 months	90	.95	1.9	6.0
64	~3 months	90	.61	2.1	6.6
64	18-22 months*	92	.28	3.4	3.1

* Average values for 3 dogs for the animals exposed at 18-22 months of age.

lung is similar in both experiments but there are differences among the other three major accumulators of ^{144}Ce activity. The immature dogs had a larger fraction of body burden in the tracheobronchial lymph nodes (TBLN) and skeleton and a corresponding smaller fraction in liver than was seen in the radiation dose pattern study with young adult dogs.

When these data are expressed as percentages of initial lung burden, as in Figure 1, the differences are more readily seen. In the many young adult dogs exposed by inhalation to various forms of ^{144}Ce in this laboratory, the ^{144}Ce content of liver exceeded that in total skeleton in almost all cases including the data shown in Figure 1b. However, in the immature dogs, whose skeletons are rapidly growing in this time period, the quantity of ^{144}Ce in skeleton exceeded that in liver by a factor of 2 to 5 times. Such an age-related effect may be very important in determining the subsequent long-term biological effects seen in liver and skeleton and emphasizes the need for studies with animals in different age ranges. It is also of interest to note the higher tracheobronchial lymph node content in these younger dogs. This route of clearance from the lung may be more active at this age.

Figure 1. Tissue distribution of ^{144}Ce following inhalation of ^{144}Ce fused clay particles by immature (~ 3 months) and young adult (18-22 months) Beagle dogs.

Because the relation between lung and total body activity was similar in this experiment to the original radiation dose-pattern experiment, an expression expressing lung burden as a percent of body burden derived from the original radiation dose pattern study, $(98e^{-0.00097t})$, was used in these dose calculations. By combining this expression with the individual whole-body retention curves for dogs in the longevity experiment, lung retention, $\text{LuB}(t)$, was estimated for each dog. Average total lung doses were then calculated using the following formulas:

$$\text{Rads/day} = \frac{(51.2) (1.27) A}{(0.014) (10^3) W} \text{LuB}(t) = 4.64 \frac{A}{W} \text{LuB}(t)$$

and

$$\text{cum rads} = 4.64 \frac{A}{W} \int_0^t \text{LuB}(t) dt$$

where

1.27 = average beta energy of ^{144}Ce - ^{144}Pr

A_0 = initial lung burden in μCi

W = exposure weight in kg

0.014 = lung weight (with blood) as fraction of total body weight

Appendix A lists all dogs entered into this longevity study to date and gives dose rate and cumulative doses calculated in this manner for the early post-exposure period. In the table, dose rate calculations are made for 0 and 60 days post-exposure using the body weight at those times. For the cumulative dose over the first 60 days, the average body weight for the time interval was used.

BIOLOGICAL EFFECTS

No significant biological effects have been observed in the longevity dogs through 60 days post-inhalation exposure. Observations will continue on these dogs and additional blocks of immature dogs will be entered in the study in the future.

REFERENCE

1. Boecker, B. B., F. L. Aguilar and T. T. Mercer, "A Canine Inhalation Exposure Apparatus Utilizing a Whole-body Plethysmograph," Health Phys. 10: 1077-1090, 1964.

TOXICITY OF ^{144}Ce FUSED CLAY INHALED BY AGED DOGS I.

ABSTRACT

The toxicity of ^{144}Ce fused clay inhaled by 8 to 10.5 year old dogs is being investigated with a view to providing information on possible age-related differences in the response of older members of the human population to accidental inhalation exposures to radioactive aerosols. The data developed on aged dogs will be compared to the results of similar studies using dogs exposed at approximately 3 months or 12 to 14 months of age. To date, 6 blocks of 5 dogs divided into 4 exposure levels with mean initial lung burdens of 7.7, 14, 24 and 58 $\mu\text{Ci/kg}$ body weight and control dogs exposed to non-labeled fused clay have been entered into a longevity study. Three dogs with initial lung burdens of 75, 42 and 28 $\mu\text{Ci/kg}$ and cumulative doses to lung of 41,000, 26,000 and 16,000 rads have died at 197, 209 and 214 days post-inhalation of ^{144}Ce with clinico-pathological findings related to radiation pneumonitis and pulmonary fibrosis. The retention and distribution of inhaled ^{144}Ce was similar to that observed in dogs exposed at 18 to 22 months of age in a radiation dose pattern study conducted at this laboratory. Serial observations are continuing on the 27 survivors.

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INTRODUCTION

The long-term dose-response studies conducted in this laboratory with experimental animals, like most toxicity studies, are being conducted to provide information that may be used to predict dose-response relationships for man. Unfortunately, most toxicity studies are conducted in young adult animals, the individuals in the population who may be the most physiologically fit. If this is the case, dose-response predictions for man based on data obtained from exposure of young adults may under-estimate the risk of exposure for older members of the population who are generally less physiologically fit. To obtain information relevant to this important problem, a longevity study has been initiated with 8 to 10.5 year-old Beagle dogs exposed to $^{144}\text{Ce}^*$ fused clay to achieve initial lung burdens of 6.0, 12.5, 25 and 50 $\mu\text{Ci/kg}$ body weight. This study is designed to complement two other ^{144}Ce fused clay studies; one that is well under way with dogs exposed at 12 to 14 months of age (see this report, pp. 157 - 166) and a recently initiated study with dogs exposed at approximately 3 months of age (see this report, pp. 167 - 171). The 50 $\mu\text{Ci/kg}$ level was selected to provide comparative information on the early pulmonary response; the majority of 12-14 month-old dogs exposed at this level died between 180 and 320 days after inhalation exposure with a few long-term survivors. The 25 $\mu\text{Ci/kg}$ level was selected since dogs exposed at 12-14 months of age to achieve burdens between 25 and 50 $\mu\text{Ci/kg}$ are known to develop lung neoplasms. It is anticipated that comparison can be made between young adult and aged dogs exposed in this range to determine if the incidence or time of appearance of radiation-induced lung neoplasms is altered. The lower levels (6.0 and 12.5 $\mu\text{Ci/kg}$) were included for similar comparisons in the event the dose-survival relationships were sharply altered due to the older dogs being more susceptible to radiation injury. During the past year, 6 blocks of female dogs have been entered into the experiment. Six blocks of male dogs will be entered into the experiment when sufficient male dogs reach the appropriate age for exposure.

* ^{144}Ce as used in this text refers to ^{144}Ce in equilibrium with its daughter, ^{144}Pr .

EXPERIMENTAL PROCEDURES

All dogs entered in this experiment to date are purebred female Beagle dogs that were originally part of the program's breeding colony. They were entered into the experiment in blocks, each of which contained 4 dogs exposed, by inhalation, to achieve graded initial lung burdens of ^{144}Ce in fused clay particles and 1 control dog exposed to fused clay particles containing stable cerium. Each dog was transferred to an individual metabolism cage at least 3 weeks prior to exposure to allow cage and restraint acclimation as well as pre-exposure sampling. The dogs were supplied water ad libitum and received the normal colony diet daily.

On the day of exposure, food was withheld until the inhalation exposure and post-exposure whole-body counting were complete. Pre-exposure sedation consisted of 60 mg phenobarbital orally at 2.5 hours before exposure followed by an intravenous injection of Vetame* (0.25 mg/lb body weight) at 45 minutes before exposure. Exposures were conducted individually in a previously described apparatus¹ that permitted "nose-only" inhalation exposure.

A collimated NaI scintillation detector was used to monitor the deposition of ^{144}Ce in the lower respiratory tract and indicate when the desired initial lung burden had been reached. Determined in this manner, the duration of exposure ranged from 5 to 62 minutes depending on the desired initial lung burden, the exposure air concentrations and the dogs' respiratory patterns. For the different exposures, the particle size distributions of the ^{144}Ce fused clay aerosols were log-normal with activity median aerodynamic diameters, AMAD, that ranged for individual exposures from 1.6 to 2.3 μm with geometric standard deviations of 1.3 to 1.6. Concentrations of ^{144}Ce in the exposure air ranged from 20 to 70 $\mu\text{Ci/L}$ for the individual exposures.

A profile scan and whole-body count were obtained immediately post-exposure and continue to be taken at selected times post-exposure to quantitate lung and whole-body retention. The dogs were housed individually in metabolism cages in the early post-exposure period and returned to kennel facility at 6 months post-exposure where they were housed 2 dogs to a run.

Detailed clinical evaluations were performed prior to exposing the dogs. This was especially important since it was desirable to document the presence of any pre-existing disease states. Special attention was directed to serum biochemical and pulmonary function parameters since it was anticipated that these might be altered in the older dogs as compared to the extensive data developed in this laboratory on 12-14 month-old dogs. Detailed analysis of the baseline pulmonary function and serum biochemical data on dogs of various ages are presented elsewhere in this report (pp. 195 - 205). Serial clinical observations are continuing on all dogs. A thorough pathological examination was performed on each dog that has died.

RESULTS AND DISCUSSION

Deposition and Metabolism:

Two-component exponential functions are adequate to describe the whole-body retention of ^{144}Ce in fused clay during the first 60 days post-exposure. Consistent with earlier experiments involving Beagle dogs exposed by inhalation to ^{144}Ce in a fused clay aerosol, the initial lung burden for each dog was considered to be the zero-intercept of the long-term component of whole-body retention. For the dogs entered into this experiment to date, this component had a mean slope corresponding to an effective half-life of 170 days with a range from 120 to 220 days. Although initial lung burdens computed in this manner ignore a small fraction (~ 13%) of the initial lung burden that clears rapidly after exposure,² they were used to construct the experimental design chart shown in Figure 1. In spite of some variability within a given dose level, the mean values for each level are near those that were desired.

*Vetame, E. R. Squibb & Co., New York, N. Y.

From the initial whole-body and lung burdens determined from whole-body counting, the mean concentration of ^{144}Ce in the exposure air and the volume of air inhaled during exposure, it was possible to calculate deposition percentages for total body and lung. For the 24 dogs exposed to date in this experiment, the total deposition had a mean of 39% with range from 15 to 49%. Of the total ^{144}Ce deposited, a mean fraction of 0.36 (range of .18 to .54) was associated with the lung. Deposition in the lung averaged 15% with a range of 3.4 to 22%. These values may be compared with values seen in the young adult dogs (12 to 14 mo.) that were exposed in the Series II longevity experiment. The mean total deposition in those dogs was 54% (range = 29 to 92%) and mean lung deposition was 26% (range = 13 to 50). Values for the aged dogs in general are lower than for the young adults. This may in part be due to the higher respiratory frequency during exposure for some of these aged dogs. These differences will be examined more closely in future work.

Three dogs (FD49, FD145 and FD12) have died to date at 197, 209 and 214 days post-exposure. Following necropsy, all tissues were radioanalyzed for ^{144}Ce content by gamma-counting. In Table 1, the tissue data are expressed on a relative basis, as percentages of body burden at death. Ranges for values observed at 128 and 256 days post-exposure in an earlier radiation dose pattern study involving young adult dogs are also listed for comparison. From these data, it appears that the relationships between lung, liver, skeleton and tracheobronchial lymph nodes are similar in the aged dogs to those in young adults at this time period post-exposure.

From relative data such as these, an expression relating lung burden as a percent of total body burden ($98e^{-.00097t}$), was derived for use in calculating absorbed beta doses to lung in the other longevity experiments with ^{144}Ce in fused clay. Since the data in Table 1 agree in this time period, this expression was also used in this experiment by multiplying it times the whole-body retention

Projected ILB $\mu\text{Ci/Kg}$	BLOCKS						Mean ILB $\mu\text{Ci/Kg}$
	A	B	C	D	E	F	
50	FD-145 440 42 D-209	FD-49 710 75 D-197	FD-98 610 56 A-228	FD-108 690 59 A-228	FD-40 800 66 A-219	FD-118 330 50 A-220	58
25	FD-31 250 23 A-234	FD-100 190 22 A-233	FD-12 400 28 D-214	FD-94 150 23 A-220	FD-7 240 24 A-219	FD-121 360 22 A-220	24
12.5	FD-30 150 13 A-233	FD-103 190 20 A-234	FD-47 110 14 A-229	FD-32 110 14 A-229	FD-153 95 11 A-221	FD-15 160 13 A-221	14
6.0	FD-104 83 6.8 A-233	FD-38 67 7.7 A-233	FD-95 65 8.8 A-229	FD-48 100 8.3 A-229	FD-150 55 5.6 A-221	FD-154 100 8.8 A-221	7.7
Control	FD-101 0 0 A-235	FD-6 0 0 A-235	FD-117 0 0 A-235	FD-147 0 0 A-235	FD-4 0 0 A-235	FD-149 0 0 A-235	0
FD-145 = ANIMAL NUMBER 440 = INITIAL LUNG BURDEN (μCi) 42 = INITIAL LUNG BURDEN ($\mu\text{Ci/Kg}$) D-209 = D=DEAD, E=EUTHANIZED, A=ALIVE- DAYS POST-EXPOSURE AT DEATH OR ON 9-30-72							

Figure 1. Experimental design for studying the effects of ^{144}Ce in fused clay inhaled by aged Beagle dogs.

Table 1
Comparison of the Relative Tissue Distribution of ^{144}Ce Inhaled in Fused Clay Particles by Young Adult and Aged Beagle Dogs
(% Body Burden at Death)

<u>Tissue</u>	<u>Days PE</u>	3				3
		Young	Dog	Dog	Dog	Young
		Adults	FD49	FD145	FD12	Adults
		<u>128</u>	<u>197</u>	<u>209</u>	<u>214</u>	<u>256</u>
Lung		86-88	84	85	82	74-83
Liver		5.5-6.7	7.5	6.9	8.5	6.8-13
Skeleton		4.0-4.5	3.0	3.0	4.5	6.1-6.8
Tracheobronchial Lymph Nodes		.79-1.3	2.2	3.2	2.5	2.9-4.0

curves to estimate lung retention, $LuB(t)$, individually for each dog. Average total lung doses were calculated using the following formulas:

$$\text{rads/day} = \frac{(51.2) (1.27) A_o}{(0.014) (10^3) W} \quad LuB(t) = 4.64 \frac{A_o}{W} \quad LuB(t)$$

and

$$\text{cum rads} = 4.64 \frac{A_o}{W} \int_0^t LuB(t) dt$$

where 1.27 = average beta energy of ^{144}Ce - ^{144}Pr

A_o = initial lung burden in μCi

W = exposure weight in kg

.014 = lung weight (with blood) as fraction of total body weight

Because some of these old female dogs were somewhat obese, the lung weight to body weight ratio used, .0140, which was determined primarily from necrotomies of young adults dogs, may tend to overestimate true lung weight. If the lung weights are overestimated, the resulting calculated absorbed beta doses will be too low. Although this fraction was used, further information is needed on this point. In Appendix A, all dogs are listed in a ranking based on initial lung burden in $\mu\text{Ci/kg}$ body weight. Also given are dose rate and cumulative doses for the early post-exposure period calculated in the above manner.

BIOLOGICAL EFFECTS

The current status of dose survival relationship for this study is presented graphically in Figure 2. Of the 24 aged dogs exposed to ^{144}Ce in fused clay, 3 dogs (FD49, FD145 and FD12) with ILBs of 75, 42 and 28 $\mu\text{Ci/kg}$ and cumulative doses to lung to death of 41,000, 26,000 and 16,000 rads, respectively, have died at 197, 209 and 214 days post-inhalation. In addition, 5 dogs at 219-228 days post-exposure with ILBs from 22 to 66 $\mu\text{Ci/kg}$ have clinical findings associated with pulmonary insufficiency of varying degrees of severity. The remaining 16 exposed dogs with ILBs from 5.6 to 24 $\mu\text{Ci/kg}$ and the 12 control dogs are surviving from 219 to 235 days post-exposure with no clinical abnormalities clearly related to their radiation exposure. Many of the dogs in this study have had mammary tumors and other superficial tumors surgically removed either pre- or post-exposure.

Figure 2. Survival of aged dogs that inhaled ^{144}Ce fused clay (status as of 9/30/72).

The clinicopathological findings in the 3 dogs that have died were related to radiation pneumonitis and/or pulmonary fibrosis, and were very similar to those observed in young mature dogs with similar lung burdens that were exposed to ^{144}Ce in fused clay and reported previously (1967-1968 Annual Report, LF-39, pp. 35-53; 1968-1969 Annual Report, LF-41, pp. 19-35; and 1969-1970 Annual Report, LF-43, pp. 183-187). As in the dogs exposed at 12-14 months of age, a dose and time related lymphopenia was evident with the earliest most severe depression occurring in the dogs with the highest ILB. There is a suggestion that the lymphopenia may be occurring earlier than in the young adult dogs and pups.

The time from exposure to the appearance of the initial clinical signs of an elevated respiratory rate (50-60/min) and abnormal sounds on auscultation in higher level dogs has generally been about 120 to 180 days. The respiratory rate has then tended to progressively increase to greater than 100/min by several weeks prior to death. There has also been a progressive increase in the intensity of the abnormal sounds on auscultation of the thorax and a progressive weight loss. A marked increased density of the lung fields was present on thoracic radiographs at 6 months post-exposure. Electrocardiographic tracing at 6 months post-exposure and/or just prior to death have not been remarkable.

Pathological examinations have been performed on the 3 dogs that died. Radiation pneumonitis was severe in dogs FD12 and FD49 but only moderate in FD145. Pulmonary fibrosis was present mainly in the form of septal fibrosis with occasional coalescence to form larger scars. Atrophy of the cortex and medulla was present in the tracheobronchial lymph nodes of all 3 dogs. No lesions in the right atrium, such as those described in the earlier reports, were seen in any of the dogs.

In addition to the pulmonary and tracheobronchial lymph node lesions, which are most certainly due to radiation damage, many other lesions were found in the 3 dogs. Most of these are fairly common findings in old dogs and included parathyroid hyperplasia, nodular-hyperplasia of the liver, nodular hyperplasia of the mammary gland, mammary tumors, an adrenal cortical tumor and a pituitary tumor. The effect, if any, of the aging lesions on the course of the radiation pneumonitis is not clear at this time. When all of the dogs in the higher levels are dead the relationships between the aging lesions and radiation pneumonitis may be more evident.

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TOXICITY OF INHALED ^{90}Sr FUSED CLAY IN BEAGLE DOGS. III.

ABSTRACT

Studies on the metabolism, dosimetry and effects of inhaled ^{90}Sr fused clay in Beagle dogs have been continued with a view toward obtaining information on the biological consequences of inhaling this important radionuclide in a relatively insoluble form. This report emphasizes (a) components of lung retention of ^{90}Sr in fused clay particles as determined using data from a radiation dose pattern study, and (b) recent observations on lung neoplasia observed in dogs in the longevity study. For the radiation dose pattern study, 26 dogs were exposed to aerosols in which activity median aerodynamic diameters ranged from 1.9 to 2.5 μm with a σ_g equal to 1.6 to 2.0. Data from this radiation dose pattern study were used to develop a model for lung loss of the burden. The model included clearance, dissolution and uptake by tracheobronchial lymph nodes (TBLN). Uptake by TBLN was considered constant at 0.01% lung burden per day (% LB/day). Respective values for clearance and dissolution were 0.3% LB/day and 0.08% LB/day and 0.04% LB/day and 0.05% LB/day for days 10 and 500 post-inhalation, respectively. The ratio for clearance/dissolution was 4.1 and 0.8 for days 10 and 500, respectively, with a ratio of 1 observed at approximately 400 days post-exposure. A total of 72 dogs were exposed to achieve graded initial lung burdens (ILB) of ^{90}Sr for the longevity study along with 12 dogs exposed to stable strontium in fused clay which are serving as controls. Of the 72 exposed dogs, 32 dogs with ILBs from 29 to 94 $\mu\text{Ci/kg}$ and doses to lung to death of 31000 to 75000 rads died from 159 to 414 days post-exposure from radiation pneumonitis and/or pulmonary fibrosis. Three dogs with ILBs of 25, 34 and 36 $\mu\text{Ci/kg}$ have died from hemangiosarcomas, one of which was probably primary in soft tissues of an intercostal space or the lung and the other two were most probably primary in the lung. These dogs died at 644, 715 and 718 days post-exposure with cumulative doses to lung of 40000, 47000 and 50000 rads. The remaining 37 dogs and the 12 controls are surviving at 339 to 977 days post-inhalation with no detectable abnormalities.

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INTRODUCTION

^{90}Sr is a major component of the fission product inventory in a nuclear reactor after a period of sustained operation. It is also an isotope of major abundance in nuclear fallout. ^{90}Sr was selected for intensive study in this program because of its high probability for release in certain nuclear accidents and because it represents a typical long-lived beta emitter. ^{90}Sr decays by the emission of a 0.54 Mev (max) beta particle with a physical half-life of 28 years. ^{90}Y is normally in a state of secular equilibrium with its parent, ^{90}Sr ; it has a reported half-life of 64.2 hours and decays by the emission of a 2.28 Mev (max) beta particle to stable zirconium. A study is in progress with a relatively soluble form, $^{90}\text{SrCl}_2$ (this report, pp. 128-136). In the study to be reported here, a more insoluble form, ^{90}Sr entrapped in fused montmorillonite clay, is being studied for comparison with inhaled ^{90}Y , ^{91}Y and ^{144}Ce in fused montmorillonite clay.

As previously described (1970-1971 Annual Report, LF-44, pp. 181-192), research using ^{90}Sr in fused clay is under way in two major areas; (a) evaluation of the radiation dose pattern for inhaled ^{90}Sr fused clay, and (b) a longevity study in which the dose-response relationship is being evaluated for dogs exposed to achieve graded initial lung burdens of ^{90}Sr in fused clay. Dogs in the longevity study were exposed to achieve initial lung burdens of 5 to 80 μCi of ^{90}Sr per kg body weight. To date, 12 blocks of 7 dogs each have been entered into the longevity study, concluding the exposure protocol.

This year's report will emphasize (a) components of lung retention of ^{90}Sr in fused clay particles as determined using data from the radiation dose pattern study, and (b) recent observations on lung neoplasia observed in dogs in the longevity experiment.

Subsequent to deposition of radioactive particles in the pulmonary region of the lung, biological elimination of the material occurs mainly by three routes: (a) muco-ciliary transport, (b) accumulation in lymph nodes, and (c) dissolution.¹⁻³ The solubility of the deposited material will be a major factor in division of biological elimination among these three pathways. For a soluble substance, dissolution will be the major pathway; a slightly soluble material will be influenced by all three methods of biological elimination.

It is of interest to quantitate these three routes of biological elimination of a pulmonary burden of ^{90}Sr in fused clay in terms of their importance at various times post-inhalation exposure. The basis for the model for biological elimination of the pulmonary lung burden, to be discussed in this report, is the ability to describe total lung loss and excreta content of ^{90}Sr in terms of the change in ^{90}Sr content of these compartments in units of percent of lung burden per day.

EXPERIMENTAL PROCEDURES

All of the dogs used were purebred Beagle dogs raised in the program's colony. The 26 dogs used in the Series II radiation dose pattern study were 13 to 16 months old at the time of inhalation exposure and weighed 6.6 to 12.0 kg. The 84 (72 experimental and 12 control) dogs used in the longevity study were 12 to 14 months old at the time of inhalation exposure and weighed 6.6 to 14.2 kg. The size distribution of the ^{90}Sr fused clay aerosols used to expose these dogs was assessed with a cascade impactor and was well represented by log-normal functions. The activity median aerodynamic diameters (AMAD) ranged from 1.4 to 2.8 μm with a σ_g of 1.4 to 2.7 and 1.9 to 2.5 μm with a σ_g of 1.6 to 2.0 for the longevity and Series II dogs, respectively.

Experimental procedures were detailed in last year's annual report (LF-44, pp. 181-192). Additional information will be included where necessary for clarification or for definition of procedures used to update data for this report.

One significant change resulted from a calibration experiment reported elsewhere in this annual report (pp. 107 - 113). Based on these results, new data for initial lung burden (ILB) and radiation dose to the lung were obtained for the 72 longevity dogs that inhaled ^{90}Sr in fused clay. These data are included in Appendix A. Lung burden data, obtained using a rectilinear scanner, for days 3 to 75 post-exposure were fitted to a one-component exponential equation. The time zero intercept was used for estimating the initial lung burden. These values are included in the experimental design in Figure 1, as well as in Appendix A. An equation for gross lung retention of ^{90}Sr fused clay was obtained by fitting all scanner data from day 3 post-exposure through September 30, 1972, to either a one- or two-component exponential equation which was force-fitted to agree with the previously determined initial lung burden. Net lung retention was next determined by subtracting the tracheobronchial lymph node (TBLN) burden of ^{90}Sr from the gross lung burden. Data for TBLN uptake of ^{90}Sr from the Series II experiment fit the equation:

$$\% \text{ ILB} = 8.8 (1 - e^{-0.00065t}) \quad (1)$$

New exponential equations were fitted to the corrected lung retention data to obtain expressions for lung content of ^{90}Sr for each dog as a function of time post-exposure. The net retention equations were used to determine average dose to the lung using the expressions:

$$\text{dose rate (D.R.)} = 51.2 \bar{E} C(t) \text{ rads/day;}$$

$$\bar{E} = \text{average } \beta \text{ energy} = 1.13 \text{ Mev for } ^{90}\text{Sr}-^{90}\text{Y} \text{ in equilibrium;}$$

$$C(t) = \text{activity concentration as a function of time post-exposure } (\mu\text{Ci/gm}).$$

Projected ILB $\mu\text{Ci/Kg}$	BLOCKS												Mean ILB $\mu\text{Ci/Kg}$
	A	B	C	D	E	F	G	H	I	J	K	L	
80	355B 580 62 D-373	355S 540 63 D-342	393A 830 81 D-159	397T 670 78 D-218	403A 570 81 D-386	403U 450 66 D-258	408T 500 58 D-238	417A 1000 94 D-195	415T 810 88 D-220	433A 670 61 E-252	431U 350 49 D-226	500B 600 70 D-255	71
60	357A 530 54 D-341	361T 450 54 D-265	398A 650 57 D-283	398U 460 68 D-329	402D 370 55 D-300	403S 370 53 D-232	405X 540 63 D-243	416B 890 77 D-184	417T 680 72 E-267	435A 840 73 E-231	432T 550 67 D-268	494A 500 53 D-310	62
40	355A 270 25 D-715	354W 220 29 D-477	398D 340 36 E-644	396T 290 34 D-718	402A 400 42 D-340	400S 380 42 D-286	411T 300 39 D-259	411A 630 44 D-201	418S 560 56 D-200	437D 390 47 E-376	433S 350 39 D-279	497B 310 35 A-339	39
20	354A 160 15 A-970	362T 100 15 A-970	398B 220 20 A-788	397S 130 16 A-788	401B 170 19 A-767	399U 140 19 A-767	404S 160 19 A-739	416C 240 22 A-718	414T 160 23 A-718	433C 240 24 A-611	430S 170 22 A-611	495C 140 18 A-339	19
10	367B 68 7.9 A-971	358T 90 10 A-967	393C 66 8.0 A-789	393T 53 8.0 A-789	405A 110 11 A-768	399T 71 8.9 A-768	411S 72 8.9 A-739	413B 160 15 A-719	415V 110 15 A-719	438A 97 9.8 A-612	430V 52 7.2 A-612	494D 60 7.5 A-340	9.8
5	352B 39 4.9 A-967	360T 32 4.1 A-971	393D 44 4.2 A-789	398S 47 4.9 A-789	403B 31 4.2 A-768	399S 42 4.3 A-768	405S 66 6.7 A-739	413C 110 9.0 A-719	413S 62 6.0 A-719	435C 33 3.7 A-612	431T 31 4.7 A-612	494B 46 4.9 A-340	5.1
Control	361B 0 0 A-977	354S 0 0 A-977	399B 0 0 A-792	397U 0 0 A-792	402B 0 0 A-764	401S 0 0 A-764	405W 0 0 A-757	418C 0 0 A-715	413U 0 0 A-715	437A 0 0 A-614	431S 0 0 A-614	497A 0 0 A-340	0
355B = ANIMAL NUMBER 580 = INITIAL LUNG BURDEN (μCi) 62 = INITIAL LUNG BURDEN ($\mu\text{Ci/Kg}$) D-373 = D=DEAD, E=EUTHANIZED, A=ALIVE - DAYS POST-EXPOSURE AT DEATH OR ON 9-30-72													

Figure 1. Experimental design for ^{90}Sr in fused clay longevity study (status as of 9/30/72).

The activity in lung was assumed to be distributed uniformly and all beta energy completely absorbed. The weight of the lung, including circulating blood, was assumed to be 1.4% of the total body weight. Therefore,

$$C(t) = \frac{L_o}{W (TF) 10^3} F(t) \text{ and D.R.} = 51.2 \overline{E} \frac{L_o}{W (TF) 10^3} F(t)$$

where

L_o = initial lung burden (μCi);

W = dog weight in kilograms;

TF = the fraction of body weight which is lung = 0.014;

$F(t)$ = net lung retention of ^{90}Sr in fused clay.

$$\text{Cumulative Dose} = D(t) = 51.2 \overline{E} \int_0^t C(t) dt = \frac{4.13 L_o}{W} \int_0^t F(t) dt$$

The updated values for ILB and dose reported this year are different from those in last year's report (LF-44, pp. 181-192). Differences are slight and in no cases are values radically different from those reported last year. Present numbers reflect our attempt at increased accuracy in reporting lung burden and dose for dogs exposed to ^{90}Sr in fused clay particles in this laboratory.

Beagles in the Series II experiment were assigned to a sacrifice schedule which included days 0 to 2560 post-exposure. Excreta collections were made at frequent intervals for six of the dogs. Data from the Series II experiment will be presented in a model for lung retention of ^{90}Sr fused clay.

The remainder of this year's report will emphasize biological effects observed to date in the ^{90}Sr fused clay longevity dogs which have been necropsied and examined for pathological changes subsequent to aerosol exposure.

RESULTS AND DISCUSSION

A Model for Components of Lung Retention of ^{90}Sr Fused Clay Particles in the Beagle Dog:

Initial lung burdens of ^{90}Sr ranged from 15 to 120 μCi for the Series II dogs. Lung peak data for each animal scheduled for sacrifice more than 499 days post-exposure were normalized to an initial lung burden of 100 μCi and plotted in Figure 2. The resulting plot actually represented the ^{90}Sr present in the lung, including the tracheobronchial lymph nodes (TBLN). Data from animals sacrificed at 0, 16, 64, 128, 256, 499 and 533 days post-aerosol-exposure provided information relevant to the TBLN content of ^{90}Sr . These data are presented as the data points and ranges shown in Figure 3; the 499- and 533-day data were combined and presented as day 516. The lung scanner peak was corrected for the activity present in TBLN to yield a new curve representing the time-dependent lung burden of ^{90}Sr , also indicated in Figure 2. The equation representing the lung burden of ^{90}Sr was differentiated to obtain an expression for the rate of change in the lung burden as a function of time, $0.39e^{-0.01t} + 0.056e^{-0.0009t}$ μCi per day. Division of this equation by the function representing the fraction of the initial lung burden retained as a function of time yielded the percent of the lung burden lost per day.

$$\% \text{ LB/day} = \frac{0.39e^{-0.01t} + 0.056e^{-0.0009t}}{0.376e^{-0.01t} + 0.624e^{-0.0009t}} \quad (2)$$

In an earlier experiment at this laboratory, a group of dogs was administered an aerosol containing $^{85}\text{SrCl}_2$. The loss of ^{85}Sr from these dogs was essentially 50% via urine and 50% by endogenous fecal secretion, at least over the 187-day time period of the excreta collection.

An assumption made in this presentation, based upon the soluble ^{85}Sr data, was that the ratio (urinary ^{90}Sr excretion)/(endogenous fecal secretion of ^{90}Sr) equals 1. The source of ^{90}Sr available for endogenous fecal secretion is dissolution of fused clay particles; the dissolved label is either accumulated in the skeleton (extra-lung body burden) or is lost in urine and feces.

Excreta data, collected for 6 of the dogs exposed to ^{90}Sr -fused clay over a 500-day period post-exposure, are presented in Figure 4 as percent of lung burden of ^{90}Sr per day. A 3-component exponential equation

$$17.5e^{-0.9t} + 0.245e^{-0.0083t} + 0.044e^{-0.000056t} \% \text{ LB/day} \quad (3)$$

represented the total ^{90}Sr present in feces as a function of time. Urinary excretion of ^{90}Sr and/or endogenous fecal secretion of ^{90}Sr , was represented by the equation

Figure 2. Lung retention of ^{90}Sr by Beagle dogs in the Series II experiment. The net lung peak burden was derived by subtracting the theoretical TBLN content of ^{90}Sr from the gross lung peak.

Figure 3. TBLN accumulation of ^{90}Sr by dogs in the Series II experiment. Theoretical values of 0.006 to 0.012% lung burden per day are presented along with mean values observed to date in this sacrifice series.

Figure 4. Fecal and urinary excretion of ^{90}Sr in 6 dogs in the Series II experiment.

$$0.007e^{0.0022t} \% \text{ LB/day} \quad (4)$$

Data from tissue analysis were insufficient to satisfactorily fit TBLN content of ^{90}Sr to a useful equation. The extra-lung body burden was fit to the following equation, as shown in Figure 5:

$$12.3 (1 - e^{-0.0058t}), \% \text{ LB} \quad (5)$$

Some estimate for TBLN uptake of ^{90}Sr was required to pursue this model for loss of ^{90}Sr -fused activity from the dog lung. Mean values and ranges for the percent of lung burden of ^{90}Sr found in TBLN are shown in Figure 3 along with theoretical curves representing transfer from the lung to TBLN of 0.006 to 0.012% LB/day of the lung content of ^{90}Sr . Curve B, representing transfer of 0.01% LB/day to TBLN was a suitable representation of this function and was used in the model. The model is presented in Figure 6. Dissolution of particles in TBLN and GI tract should have values similar to those for particles dissolved in the lung. Omitting these two sources of dissolved ^{90}Sr has little effect on the model due to the relatively small amounts of ^{90}Sr particles retained in both compartments during the 500-day post-exposure period included in this model. Dissolution of ^{90}Sr from TBLN may be significant relative to dissolution of the lung burden after longer times because of accumulation of ^{90}Sr in TBLN; in theory, the lung burden will tend toward zero μCi whereas the percent lung burden accumulated in TBLN will reach a maximum and then decrease. The TBLN will contain ^{90}Sr long after the lung has become devoid.

Rearranging the expression for total lung loss of ^{90}Sr yields

$$\begin{aligned} \text{Dissolution} &= \text{total lung loss} - \text{TBLN uptake} - \text{clearance to GI tract} \\ (\% \text{ LB/day}) & \quad (\% \text{ LB/day}) \quad (\% \text{ LB/day}) \quad (\% \text{ LB/day}) \end{aligned}$$

total lung loss = equation (2); TBLN uptake was assumed constant, 0.01% lung burden per day; clearance to the GI tract = equation (3) - equation (4).

Table 1 includes the results of this model. The ratio of clearance/dissolution was 4.1 at 10 days post-exposure and 0.8 at 500 days post-aerosol-exposure.

This model for total lung loss of ^{90}Sr fused clay particles (AMAD = 2.1; $\sigma_g = 1.8$) includes three main pathways (Fig. 6). One pathway is uptake of ^{90}Sr by the tracheobronchial lymph nodes.

Figure 5. Extra lung body burden of ^{90}Sr for dogs sacrificed to date in the Series II experiment.

Figure 6. Model for components of lung retention of ^{90}Sr - ^{90}Y fused clay particles in the Beagle dog. Primary pathways for movement of ^{90}Sr from the lung to excreta and the extra lung body burden are included.

Table 1
Parameters for Lung Loss of ^{90}Sr Using the Assumption
of a Constant Rate of TBLN Uptake of ^{90}Sr of 0.01% LB/day

Days P. E.*	Total Lung Loss (% LB/day)	Clearance (% LB/day)	Dissolution (% LB/day)	Clearance/Dissolution
10	0.426	0.334	0.082	4.1
20	0.405	0.316	0.079	4.0
40	0.367	0.285	0.072	4.0
60	0.331	0.254	0.067	3.8
80	0.298	0.226	0.062	3.6
100	0.269	0.201	0.058	3.5
150	0.209	0.149	0.050	3.0
200	0.168	0.113	0.045	2.5
300	0.121	0.071	0.040	1.8
400	0.102	0.050	0.042	1.2
500	0.094	0.037	0.047	0.8

* Post-Exposure

A rate of 0.01% LB/day, chosen for presentation here, provides a theoretical curve (Fig. 3) which is a reasonable approximation of what the true function would be, at least over the 500-day time period of this study. Thomas² successfully used this same rate in an interspecies model for lung retention of particulate material. A second pathway during the first few days post-exposure is movement of particles from the nasopharynx to the larynx to be ingested. Material is transferred in this way to the gastrointestinal tract where it is quickly eliminated from the body via feces. Only a very small contribution to the body burden of ^{90}Sr could be made by dissolution of particles in the GI tract and absorption of the ^{90}Sr released during this process.

Loss of ^{90}Sr into urine increased with time post-exposure. The extra-lung burden of ^{90}Sr was increasing due to deposition in skeleton of a portion of the ^{90}Sr released by dissolution. Another factor was that the lung burden was decreasing; an increasing urinary excretion, due to ^{90}Sr released from skeleton and by dissolution of lung particles containing ^{90}Sr , was compared with a

decreasing lung burden to yield an expression for percent lung burden per day. This was, in fact, an expression of convenience to allow correction of values for total fecal ^{90}Sr to expressions representing clearance from the lung of ^{90}Sr associated with particulate matter.

As particles are cleared more quickly from the upper portion of the respiratory tract than the lower, the rate of clearance (% LB/day) decreases with time post-exposure. The model presented in this paper indicates a rate of clearance approximately equal to 0.3% LB/day on day 10 post-exposure which decreased by approximately a factor of 10 by day 500 post-exposure.

The ratio for clearance/dissolution was 4.1 on day 10 and 0.8 on day 500 post-exposure. Both clearance and dissolution showed decreasing rates with time; however, the former decreased faster than the latter. The point in time at which clearance equaled dissolution was approximately 400 days post-exposure. In another experiment using Beagle dogs, Boecker and McClellan⁴ estimated that 0.076% of the insoluble burden of ^{137}Cs in fused clay is solubilized each day, and concluded that clearance rate was approximately equal to dissolution rate. This agrees with the mean value of 1.2 noted at ~ 400 days for the dogs reported in this presentation.

A major source of error in this model could be incomplete collection of excreta samples. Values for urinary and fecal content of ^{90}Sr could therefore be somewhat low, resulting in low values for clearance and high values for dissolution, especially for early time periods post-exposure. Refinements in this model will be made as additional data become available.

It should be emphasized that this model may not pertain to dogs in the longevity study which were exposed to higher levels of ^{90}Sr fused clay. Radiation damage to lung may considerably alter clearance and dissolution processes. In addition, heavy radiation damage to the TBLN results in damage to, or destruction of these tissues. The results could be quite different from those observed in the Series II experiment where dogs were exposed to modest levels of ^{90}Sr in fused clay.

Biological Effects Observed for Dogs in the Longevity Study:

The dose-response relationship observed in the longevity study to date is illustrated in Figure 7. Of the 72 exposed dogs, 35 have died at from 159 to 718 days post-inhalation exposure. Thirty-two dogs, including 6 (433A, 500B, 432T, 494A, 437D and 433S) of the 9 dogs that died during the past year, with ILBs of 29 to 94 $\mu\text{Ci/kg}$ and doses to lung to death of 31,000 to 75,000 rads, died at from 159 to 414 days post-exposure with clinicopathological findings consistent with radiation pneumonitis and/or pulmonary fibrosis. The findings in the 6 dogs that died this year from pulmonary insufficiency were generally similar to those reported previously for the other dogs in this group (1969-1970 Annual Report, LF-43, pp. 188-196; 1970-1971 Annual Report, LF-44, pp. 189-192). The major findings in these 6 dogs were: (1) a severe radiation pneumonitis with increased numbers of alveolar macrophages, hypertrophy and hyperplasia of alveolar lining cells, degeneration and loss of bronchiolar epithelium, occasional foci of acute inflammation congestion, hemorrhage and edema; (2) a generally severe pulmonary fibrosis with septal fibrosis, pleural fibrosis; perivascular fibrosis and large parenchymal scars; (3) mild subintimal proliferation and fibrosis of elastic pulmonary arteries in 3 cases (500B, 432T, 437D) and moderate widespread acute and chronic vasculitis in 2 cases (433S, 494A); (4) bullous emphysemas with formation of large cysts in scarred areas in 2 dogs (500B, 494A); (5) right ventricular dilation and hypertrophy in 3 dogs (500B, 433S, 494A) with congestive heart failure in one (433S) but no right atrial lesions as previously seen (1970-1971 Annual Report, LF-44, pp. 181-192); and (6) severe atrophy and fibrosis of the tracheobronchial lymph nodes in the dogs.

Three dogs (355A, 398D, 396T) with ILBs of 25, 36 and 34 $\mu\text{Ci/kg}$ and doses to lung to death of 40,000, 50,000 and 47,000 rads, respectively, died with pulmonary tumors during the past year at 715, 644 and 718 days post-exposure. Two of the dogs had primary pulmonary hemangiosar-

Figure 7. Relationship between initial lung burden of ^{90}Sr and survival time for dogs that inhaled ^{90}Sr in fused clay.

comas with disseminated metastasis and the third (398D) had a disseminated hemangiosarcoma in which the primary tumor was either the soft tissues of an intercostal space or lung.

Dog 355A, with an ILB of 25 $\mu\text{Ci/kg}$ and a dose to lung to death of 40,000 rads, died at 715 days post-exposure. Four days prior to its death the dog was examined due to a sudden onset of anorexia and depression. At that time the dog's mucous membranes were very pale in color, but external signs of bleeding were not found. The dog's packed cell volume was 12%. Marked anisocytosis, poikilocytosis, polychromasia, hypochromasia and 3 nucleated red blood cells/100 cell differential were present on the blood smear. The serum total bilirubin was not elevated and results of urinalysis were normal. At that time, on thoracic radiographs, the entire left apical region of the lung was occupied by a soft tissue density mass and there were also suggestions of other masses in the lung. Thoracic radiographs taken earlier at 1 year post-exposure were within normal limits.

During the necropsy of dog 355A, four large red friable nodules were found in the lungs, one each in the right apical and cardiac lobes and two large nodules, which were adherent to the parietal pleura, were present in the left apical-cardiac lobe. Many small similar nodules were scattered throughout the parenchyma of the other lobes. Large dense white scars were present on the anterior borders of both diaphragmatic lobes.

The tracheobronchial lymph nodes were very small and dark. Red, friable nodules were found in the spleen, small intestine, esophagus, liver, pancreas, omentum, kidneys, prostate, left adrenal, muscles and subcutis. Several pale dry irregular foci were noted in both the left and right ventricular myocardium.

All the masses seen grossly had a histologic pattern of hemangiosarcoma (Fig. 8). The larger masses invaded and destroyed lung parenchyma, small airways and pleura. The individual neoplastic cells had an oval or oblong nucleus, a small amount of clumped chromatin and one or two large prominent nucleoli. The cytoplasm was scant to moderate in amount and had an indistinct cell boundary. In some locations the cells formed many capillary or sinusoidal spaces filled with blood or soft thrombi. In other areas the neoplastic cells formed swirling masses with numerous mitotic figures. The tumor cells did not stain with PAS or Alcian blue stains indicating they did not produce mucopolysaccharides such as that produced by bronchial epithelium.

Pulmonary fibrosis, in the form of alveolar septal thickening and large parenchymal scars, did occur adjacent to a number of tumor masses. In addition, a moderate radiation pneumonitis was also present.

The tracheobronchial lymph node was practically non-existent. The cortex was completely atrophic and the medulla represented by a clump of fibrous tissue and a few macrophages. The myocardium contained numerous small early infarcts which were associated with small tumor emboli. All of the reddish nodules seen grossly were hemangiosarcomas with a histology similar to those in the lung. In addition, several tumor metastasis were found in the cerbrum.

Dog 396T, with an ILB of 34 μ Ci/kg and a dose to lung at death of 47,000 rads, died at 718 days post-exposure. Two weeks prior to death an episode of hemoptysis was noted. At that time there were at least three masses of various sizes present in the lung field on thoracic radiographs. Thoracic radiographs at 1 year post-exposure were within normal limits. In the two weeks to death there were numerous episodes of hemoptysis. On bronchoscopic examination there was bright red blood present in the trachea and a gelatinous-appearing lesion or clot was present in the right bronchus just distal to the branching of the first or second lobar bronchi. A fluid line was prominent on thoracic radiographs 24 hours prior to death. The dog started having generalized convulsive seizures about 6 hours prior to its death.

At necropsy dog 396T had hemothorax and many red friable masses in the mediastinum, none of which was directly connected with the lung. The lung was enlarged, hemorrhagic and contained numerous dark red foci throughout (Fig. 9). No scarring could be detected grossly but the intermediate and left diaphragmatic lobes were fused. Red, hemorrhagic nodules were present in the heart, left kidney, left adrenal, subcutis and brain. The tracheobronchial lymph nodes were very small and dark.

Histologically, the lung contained abundant hemorrhage, both acute and chronic, and numerous nodules of hemangiosarcoma ranging from microscopic to several centimeters in diameter. Some pulmonary veins were invaded. The right intermediate lobe was nearly replaced by tumor although the external configuration of the lobe was near normal. The tumor pattern ranged from cavernous to solid in form (Fig. 10). Large blood-filled sinusoidal spaces were lined by large anaplastic cells and separated by trabeculae of anaplastic cells and fibrous tissue. The individual cells had a pleomorphic nucleus which had a moderate amount of clumped chromatin, rarely a prominent nucleolus and frequently mitotic figures. The cytoplasm was usually very eosinophilic with indistinct cell borders that formed a syncytium or lined vascular spaces. The reddish masses in the mediastinum had a similar appearance.

The tracheobronchial lymph node was very atrophic and did not contain metastasis. However, microscopic metastasis were found in a sternal lymph node. The red nodules seen grossly in the

Figure 8. Detail of the hemangiosarcoma in the lung of dog 355A. Alcian blue stain. x 515

Figure 9. Ventral view of lung of dog 396T. Note the numerous darkened areas of hemorrhage.

Figure 10. Cavernous pattern of hemangiosarcoma in the lung of dog 396T. Note the anaplastic cells lining the blood filled spaces. Two of these cells are in mitosis (arrows).

other tissues proved histologically to be hemangiosarcoma. In addition, microscopic metastasis was seen in the duodenum.

Dog 398D, with an ILB of 36 $\mu\text{Ci/kg}$ and a dose to lung to death of 50,000 rads, was euthanized when near death at 644 days post-exposure. About 1 week prior to death multiple cutaneous and subcutaneous nodules were found on examination and the dog's respiratory rate was slightly elevated. None of these findings were present on an examination 3 weeks previously. Blood-stained mucus was present in the right main bronchus on bronchoscopic examination at that time. On thoracic radiographs at 6 months and 1 year post-exposure, the lung fields were within normal limits. On retrospective examination of the 1 year post-exposure films there was a 1 cm soft tissue density mass present outside the thoracic cavity in the area of the 5th and 6th ribs. A similar mass was present on 2 series of radiographs taken prior to the animal's death. The mass had enlarged only slightly from the 1 year films. This mass was palpable on examination 1 week prior to death. It differed from the other subcutaneous masses in that it was not freely moveable. Multiple masses were scattered throughout the lung fields and there was a homogenous mass present in the abdomen on radiographs taken 1 week prior to death. The dog was euthanized at 644 days post-exposure due to its rapidly deteriorating condition.

At necropsy of dog 398D, the soft tissue mass of the intercostal space noted on the thoracic radiographs measured 2 x 1 cm and extended through the intercostal muscles and was closely associated with the rib. It was cystic and hemorrhagic when sectioned. Similar masses were noted in the heart, subcutis, kidneys and muscles of the hips, legs and back.

The lungs contained numerous hemorrhagic or gray-white firm masses measuring from 2 mm to 2 cm in diameter. The largest mass was present in the right intermediate lobe.

Histologically, all of the reddish nodules noted at gross necropsy had a pattern of hemangiosarcoma (Fig. 11). The pattern of cellular growth was not well defined but the elements grouped into small or large irregular clusters, some of which formed small capillary-like structures. The individual cells varied from a hyperchromic endothelial cell type to a fusiform cell type resembling fibrosarcoma. Mitotic figures were numerous and invasion of surrounding tissue prominent. The tracheobronchial lymph nodes were very small and did not contain metastasis.

Many tumor nodules were scattered through all lobes of the lung and were accompanied by hemorrhage and necrosis. The neoplasm had invaded vessels and airways. Pulmonary fibrosis was fairly severe in all lung lobes. Some large dense scars were present and septal fibrosis was widespread. Mild radiation pneumonitis was also present.

The remaining 37 exposed dogs with ILBs from 3.7 to 35 $\mu\text{Ci/kg}$ and the 12 control dogs are surviving at from 339 to 977 days post-exposure. Clinicopathological observations will be continued on these dogs over their lifespan with particular attention given to correlating the biological effects with the radiation dose pattern.

CONCLUSIONS

Of the four radionuclides (^{90}Y , ^{91}Y , ^{144}Ce and ^{90}Sr) which have been inhaled by dogs in the fused clay form, ^{90}Sr has the longest physical half-life and thus the longest effective half-life in the lung (~ 400 days). Deaths from radiation pneumonitis and/or pulmonary fibrosis have occurred in all 4 of these studies. The cumulative doses to lung to death in the dogs dying in this study from pulmonary insufficiency have ranged from 31,000 to 75,000 rads. At the dose levels used, the initial dose rate from ^{90}Sr in fused clay has been lower than for the dogs that have died in the ^{90}Y and ^{91}Y in fused clay studies but about the same as for those in the ^{144}Ce in fused clay study. However, as the effective half-life in the lung is longer for ^{90}Sr in fused clay, the dose-rate has decreased more slowly in this study than in the other three studies.

While the primary site of the tumor of dog 396T may have been extrapulmonary, it is of interest that, as has been the most common finding in the ^{144}Ce in fused clay study, all three tumors observed to date in dogs in this study have been hemangiosarcomas. In this respect, the significance of pulmonary hemangiosarcomas has been discussed as well as comparisons made between the dose-response relationships in dogs that inhaled $^{239}\text{PuO}_2$ in another portion of this report (see pp. 157-166). Pulmonary fibrosis has appeared to be more prominent in the lungs of the three ^{90}Sr fused clay dogs with tumors than has been the case in the ^{144}Ce in fused clay dogs with tumors. Also, all three of these dogs died prior to 720 days post-exposure while the mean time to death for the ^{144}Ce fused clay dogs with tumors was 1002 days (range 765 to 1318) even though the ILBs were similar. As in the case in the ^{144}Ce fused clay dose-response study, it will be interesting to see if the pattern of tumor type changes from hemangiosarcoma to carcinoma in the surviving dogs with lower ILBs.

Figure 11. Detail of the hemangiosarcoma in the lung of dog 398D. H + E stain. x 625

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TOXICITY OF INHALED $^{144}\text{CeO}_2$ IN MICE

ABSTRACT

One hundred seventy-eight mice, exposed by inhalation to an aerosol of $^{144}\text{CeO}_2$ particles, were held for lifespan observations and their survival compared with that of 200 non-treated control mice. Approximately 91% of the initial whole-body burden of ^{144}Ce was cleared within six days. The whole-body burden at six days was estimated to be equivalent to the initial lung burden (ILB) and was retained with a long-term effective retention half-time of approximately 145 days as observed in 35 mice that survived 431 days post-inhalation exposure. ^{144}Ce retained in the lungs as percent of body burden at death was determined through day 454 post-inhalation exposure. Decreases in the mean survival time and cumulative percent survival were related to the estimated ILBs. The absorbed beta dose to the lung accumulated to the day of death ranged from 10,000 to 32,000 rads.

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INTRODUCTION

Cerium-144* is a major component of nuclear fission reactors after a sustained period of operation. Studies concerned with retention, distribution in various tissues, and dosimetry of inhaled ^{144}Ce in relatively insoluble forms, simulating what could be an industrial accident type of exposure, have been reported in the Beagle dog,^{1,2} Syrian hamster (1970-1971 Annual Report, LF-44, pp. 90-95), rat,³ and mouse⁴ (this report, pp. 77 - 84). Long-term effects of inhaled ^{144}Ce in relatively insoluble forms have been studied only in the Beagle dog^{1,2} and laboratory rat.³ As part of a study on the effect of inhaled ^{144}Ce in relatively insoluble forms on the course of influenza virus infection in mice (1969-1970 Annual Report, LF-43, pp. 254-262), a lifespan study of the toxic effects of inhaled $^{144}\text{CeO}_2$ was conducted with a special view to developing dose-response information that could be compared to that obtained in the dog.

MATERIALS AND METHODS

Seven hundred fifteen 8- to 10-week-old female mice (strain C57 BL/6J, Jackson Laboratory) were given single inhalation exposures to an aerosol of $^{144}\text{CeO}_2$ produced by heat treatment at 1100°C of an aerosol generated from a suspension of ^{144}Ce hydroxide stabilized with 25 molar percent diethylenetriamine pentaacetic acid. The $^{144}\text{CeO}_2$ particle size distributions could be approximated by log-normal functions characterized by activity median aerodynamic diameters of 1.35 to 1.42 μm and σ_g of 1.7 to 1.9 for 11 different groups of 65 mice each that were exposed by inhalation. The density of the $^{144}\text{CeO}_2$ particles was approximately 4.5 gm/cc. After passing through the heating column, the aerosol was mixed with dry, cool diluting air and directed into an essentially nose-only inhalation chamber in which a plenum was used to direct the air flow to the unanesthetized mice. The concentration of ^{144}Ce in the aerosols varied from 106 to 157 $\mu\text{Ci/liter}$ of air in the 11 exposures. All exposures were 20 minutes long. The size distribution of the aerosol was characterized with a cascade impactor and visualized by electron micrography of electrostatic precipitator samples of the aerosol. All mice were housed five per stainless steel cage (7" x 8" x 9-1/2" with 3/8" wire mesh front and bottom) and given food (Wayne Lab Blox) and water ad libitum prior to and after inhalation exposure.

* ^{144}Ce refers to ^{144}Ce in equilibrium with its daughter, ^{144}Pr .

Whole-body and relative lung retention of ^{144}Ce with time post-inhalation exposure was determined by whole-body counting in a well-type scintillation counter. Ten mice were sacrificed and dissected at 60 days post-inhalation exposure, 14 at 105 days and 10 at 154 days. The lungs and carcasses of these mice were analyzed for ^{144}Ce in the same counters used for whole-body counting. In addition, the lungs and carcass of eight mice that died between 218 and 405 days post-inhalation exposure were similarly analyzed for ^{144}Ce .

Average retention kinetics and each animal's estimated initial lung burden (ILB) were used to calculate individual cumulative beta radiation doses to the lung to the day of death. The six-day body burden was assumed to be representative of the ILB. The beta radiation dose absorbed by the mouse lungs was calculated using the following formula:

$$\text{Rad dose beta radiation absorbed} = 73.77 \bar{E} C_o T_{\text{eff}} (1 - e^{-\lambda t}) f$$

where

73.77 = standard value for calculating absorbed beta dose

$\bar{E}$ = average energy for ^{144}Ce - ^{144}Pr in equilibrium (1.29 Mev)

C_o = estimated initial lung burden of ^{144}Ce in the lungs ($\mu\text{Ci/gm}$), using an average lung weight of 0.25 gm for all mice

T_{eff} = effective half-time of ^{144}Ce in the lungs

λ = (decay constant) $0.693/T_{\text{eff}}$

t = time in days after the inhalation of ^{144}Ce

f = fractional energy absorption (0.35)⁵

Of the 715 mice exposed by inhalation to $^{144}\text{CeO}_2$, 178 were held for lifespan observation; the remaining were utilized in other studies. Two hundred non-treated control mice were housed in the same type of cages and in the same room as the mice that had inhaled $^{144}\text{CeO}_2$. All mice were observed daily and the cumulative percent survival was analyzed.⁶

RESULTS

Three mice, selected at random from each of the 11 groups of mice exposed by inhalation to $^{144}\text{CeO}_2$, were whole-body counted on day 0 and periodically thereafter through day 56 (Fig. 1). Because all but one of these mice died between days 56 and 181, the long-term effective retention of ^{144}Ce was not calculated. These mice had a mean initial whole-body burden (BB) of 74 μCi ^{144}Ce with a range of 21 to 106 μCi and a mean six-day BB of 6.5 μCi with a range of 2.8 to 9.9 μCi . The retention of ^{144}Ce in the lungs was determined for a group of 35 mice that survived through day 431 post-inhalation exposure (Fig. 2). These mice were whole-body counted on day 6 post-inhalation exposure and approximately monthly thereafter. The mean six-day body burden of these mice was 3.8 μCi with a range of 1.8 to 6.8 μCi ^{144}Ce . A three-component curve (Fig. 2) was fit to the lung retention data:

$$\text{LB}(t) = 0.32e^{-0.099t} + 0.49e^{-0.0248t} + 0.19e^{-0.0048t}$$

The intermediate and longest term components correspond to effective ^{144}Ce retention half-times for lung of approximately 28 and 145 days, respectively. Analysis of ^{144}Ce retention data for mice with various ranges of estimated ILB indicated no significant difference between those with the higher and those with the lower ILBs.

Clearance of ^{144}Ce was rapid between days 0 and 6 (Fig. 1). This early clearance was presumably from external contamination and the upper respiratory tract. Clearance after day 6 was primarily from ^{144}Ce originally deposited in the lower respiratory tract. This observation is consistent with the clearance of other relatively insoluble particles of similar AMAD containing a radioisotope.⁴ In addition to the loss of ^{144}Ce from the body (Fig. 2), there was a gradual loss of

^{144}Ce from the lung to the carcass, presumably to the liver and bone (Fig. 3). The cumulative radiation dose to lung as a function of time post-inhalation of ^{144}Ce is shown in Figure 4. The infinite beta radiation dose to the lung per μCi of estimated ILB was approximately 5,700 rads and was reached in about 800 days.

The mice were grouped according to their estimated ILBs for analysis of the lifespan and radiation exposure data (Table I). The higher the estimated ILB, the shorter the mean survival time up to 6.0 to 7.0 μCi ^{144}Ce . Above this level the mean survival times were relatively similar. At the highest ILBs, with death generally occurring in less than 200 days, the cumulative radiation doses to lung to death ranged upward from 20,000 rad. At lower ILBs with longer survival times, the cumulative radiation doses to lung to death were somewhat lower, however, it should be noted that the dose was accumulated over a longer time period.

Figure 1. Effective whole-body retention of ^{144}Ce in mice that inhaled $^{144}\text{CeO}_2$ particles.

Figure 2. Effective lung retention of ^{144}Ce in mice that inhaled $^{144}\text{CeO}_2$ particles.

Figure 3. ^{144}Ce in lungs and carcass at death in mice that inhaled $^{144}\text{CeO}_2$ particles.

Figure 4. Cumulative beta radiation dose to the lungs of mice that inhaled $^{144}\text{CeO}_2$ particles.

Table 1
Lifespan Observations in Mice that Inhaled $^{144}\text{CeO}_2$

Estimated ILB* $\mu\text{Ci } ^{144}\text{Ce}$	Number Of Mice	Estimated Mean ILB $\mu\text{Ci } ^{144}\text{Ce/kg}$ Body Weight	Rads To Lungs To Death		Survival in Days**		Mean Percent Post ^{144}Ce Inhalation Lifespan Shortening**
			Mean $\pm$ 1 S.D.	Range	Mean $\pm$ 1 S.D.	Range	
Controls	200	---	---	---	754 166	242-1064	---
1.0- 2.0	1	52	10,000	10,000	791	791	- 5
2.0- 3.0	6	99	14,300 2,700	11,400-16,800	769 138	520-948	- 2
3.0- 4.0	23	143	18,600 2,000	14,100-21,200	569 194	162-885	24
4.0- 5.0	19	173	18,500 3,400	13,400-26,300	283 183	91-639	62
5.0- 6.0	23	218	20,500 3,300	16,800-29,200	173 144	86-621	77
6.0- 7.0	28	257	22,700 2,600	19,500-31,600	133 61	87-405	82
7.0- 8.0	34	297	24,500 2,500	20,100-34,700	109 41	77-325	86
8.0- 9.0	26	334	26,200 1,900	22,800-30,200	96 16	73-127	87
9.0-10.0	13	374	27,400 2,200	24,500-31,800	86 12	69-115	89
10.0-11.0	3	417	33,300 2,200	30,800-34,800	103 12	89-112	86
11.0-12.0	2	446	31,400 1,200	30,600-32,300	78 5	75- 82	90

* ILB - Initial Lung Burden

** Post-inhalation exposure (8-10 weeks of age at exposure)

The cumulative percent survival of the mice is illustrated in Figure 5. The curves for all groups of mice, including the controls, tended to follow a typical sigmoid survival curve. The nature of this type of curve is not as apparent in mice with estimated ILBs above 7.0 μCi since the slopes of the curves are so steep in this illustration. The exact day of death of 36 control mice that died between 350 and 600 days was not known; thus this portion of the survival curve of the control mice was estimated from available data. The relationship between ILB and lifespan shortening is illustrated in Figure 6.

DISCUSSION

The rapidearly clearance of a large percentage of the IBB of $^{144}\text{CeO}_2$ observed in the present study is similar to the early clearance patterns in other laboratory mice,⁴ laboratory rats⁷ and Syrian hamsters (1970-1971 Annual Report, LF-44, pp. 90-95) after they had inhaled ^{144}Ce in relatively insoluble forms such as $^{144}\text{CeO}_2$ or in fused clay. The effective retention half-time ($T_{1/2}$) for the longest term component of lung retention was approximately 145 days. Somewhat similar observations have been reported for other laboratory rodents: Berkley-Pacific Laboratory's Swiss mice $T_{1/2} \approx 192$ days and the rat $T_{1/2} \approx 200$ days.⁷ A $T_{1/2}$ of inhaled CeO_2 particles¹ and ^{144}Ce in fused clay particles in the order of 200 days (this report, pp. 157-166) in Beagle dogs was similar to that observed in the Syrian hamsters and rats and longer than that observed in mice. The longer effective retention of inhaled ^{144}Ce containing particles in the Beagle dog is apparently related to the AMAD of the particles initially deposited in the lungs.⁸

The concentration of ^{144}Ce as percent of the terminal body burden (sacrifice or at death) of $^{144}\text{CeO}_2$ (Fig. 3) is similar to that observed in mice that had inhaled ^{144}Ce in fused clay particles and in Syrian hamsters that had inhaled $^{144}\text{CeO}_2$ particles.

Assuming that the control mice observed in this study were approximately 63 days of age when entered into this experiment, the mean total lifespan of 817 days with a range of 307 to 1127 days is similar to observations reported elsewhere.⁹ Mean lifespans of female C57 BL/6J mice of 653, 692, and 695 days have been reported for mice maintained under conventional conditions. A median lifespan of 890 days for mice of both sexes has been reported for animals maintained in a barrier-type of facility and a maximum lifespan of 1200 days for mice maintained under specific pathogen-free conditions.

Figure 5. Cumulative percent survival of mice that had inhaled $^{144}\text{CeO}_2$ particles.

Figure 6. Lifespan shortening in mice that had inhaled $^{144}\text{CeO}_2$ particles.

It is of interest to compare the present dose response data for mice to those being developed in dogs that inhaled ^{144}Ce fused clay. Figure 7 includes both the dog and mouse survival data related to initial lung burden presented as $\mu\text{Ci/kg}$ body weight, a relative measure of dose. The data may be normalized to absorbed rad dose by shifting the mouse data downward by a factor of 0.35, the value assumed for fractional absorption of β -energy in the mouse lung. When this is done, the mouse data points vary only slightly from those for the dogs. At the highest levels the mice die earlier than do the dogs. This may be a real species difference or it may be related to the fact that the mice receive a significant portion of their radiation exposure from the 49% of the lung burden cleared with an effective half-time of 28 days. From the ^{90}Y , ^{91}Y , ^{144}Ce and ^{90}Sr fused clay dog studies, it is apparent that the time of early deaths is related to the effective half-time of retention of the radionuclide; the ^{90}Y dogs dying at the earliest time interval with lower doses than the ^{144}Ce or ^{90}Sr dogs with the ^{91}Y dogs being intermediate in time and dose (1970-1971 Annual Report, LF-44, p. 195). At lower ILBs the mice die at later times in a relationship that is qualitatively similar to that for the dogs, although it should be emphasized that at the lowest data point shown (6 mice - mean ILB of $99 \mu\text{Ci/kg}$) the mean survival time (769 days) is not distinguishable from that of the controls (754 days). This is of special interest since the displaced point ($35 \mu\text{Ci/}$

kg) represents an ILB level in the dog where a number of primary lung neoplasms have been observed. It is tempting to speculate, as others have, on the relationship between lifespan and induction of neoplasms and suggest that the lifespan may not be sufficiently long to allow development of a high incidence of lung neoplasms induced by deposited radionuclides. The lungs of mice that died in this study are currently being evaluated histopathologically and may shed some light on this speculation.

Figure 7. Survival of Beagle dogs following inhalation of ^{144}Ce fused clay and mice following inhalation of $^{144}\text{CeO}_2$.

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A COMPARISON OF THE PULMONARY FUNCTION OF IMMATURE, YOUNG ADULT, AND AGED BEAGLE DOGS

ABSTRACT

Pulmonary function measurements were performed on immature young adult, and aged Beagle dogs. The results of those measurements are compared and apparently age-related differences are noted.

PRINCIPAL INVESTIGATOR

J.L. Mauderly

INTRODUCTION

In experiments involving the observation of subjects over a major portion of their lifespan, the normal effects of aging on the systems studied must be differentiated from effects related to the experimental treatment. The investigation of the changes in the pulmonary function of Beagle dogs resulting from the inhalation of radionuclides is such a situation. With the exception of a small number of measurements on older Beagles included in an earlier report¹ from this laboratory, there is no literature available on age-related differences in pulmonary function of the dog. Measurements performed on immature and aged dogs prior to their inclusion in studies of the inhalation toxicity of ¹⁴⁴Ce in fused clay (see this report, pp. 167 - 171, and 172 - 176) provided an opportunity to compare data from those age levels with data previously obtained from young, adult Beagles.

METHODS

The pulmonary function of resting unanesthetized Beagle dogs at three age levels was measured using the techniques and apparatus previously described¹ (1969-1970 Annual Report, LF-43, pp. 205-211 and 1970-1971 Annual Report, LF-44, pp. 227-236). Duplicate measurements were performed on 10 dogs at 3 months of age and on 36 dogs at 8 to 10.5 years of age. Single measurements were performed on 60-100 dogs at 12 to 14 months of age. None of the dogs exhibited outward signs of respiratory distress when presented for testing. Parameters measured included body weight, respiratory frequency, tidal volume, and minute volume breathing 100% O₂, dynamic pulmonary compliance and resistance, functional residual capacity, lung clearance index, steady-state CO diffusing capacity, fractional uptake of CO, alveolar and arterial tensions of O₂ and CO₂, alveolar arterial gradients for O₂ and CO₂, O₂ saturation of hemoglobin, arterial pH, O₂ uptake, CO₂ output, respiratory exchange ratio, specific ventilation, and percent alveolar, effective, and alveolar deadspace ventilation. The immature dogs would not stand quietly with the esophageal catheter in place, therefore measurements using intraesophageal pressures were not obtained. Measurements were performed at an altitude of 1680 meters where the inspired P_{O₂} was approximately 120 mmHg.

RESULTS AND DISCUSSION

Means and ranges for each parameter at the three age levels are listed in Table 1. Values for parameters related to body size (minute volume, functional residual capacity, diffusing capacity, and O₂ uptake) were much smaller for the immature dogs than for the adults. When those values were divided by the body weight, however (Table 2), it was noted that the young dogs had a higher ventilation, a larger resting lung volume, and consumed more O₂ per kilogram than the adults. Only diffusing capacity remained lower for the younger dogs when calculated on a weight basis. The higher ventilation and O₂ uptake probably reflected the higher metabolic rate known to exist in young

Table 1
Values for Pulmonary Function of Beagle Dogs at 3 Age Levels

Age		3 Months		12-14 Months		8-10.5 Years	
No. of Dogs		10		60-100		36	
Parameters Measured	Units	Mean	Range	Mean	Range	Mean	Range
Body weight	kg	2.6	2.2-4.0	9.1	6.6-13.6	10.9	6.6-18.3
Respiratory frequency	breaths/minute	37	31-45	20	10-31	19	10-36
Tidal volume	ml BTPS*	65	49-90	186	99-350	170	125-248
Minute volume	liters BTPS/ min	2.40	2.04-3.54	3.72	2.29-6.02	3.23	1.69-4.45
Dynamic Pulmonary Compliance	ml/cm H ₂ O	**		73	36-125	53	25-82
Dynamic Pulmonary Resistance	cm H ₂ O/liter/sec	**		10	1-40	13	3-34
Functional Residual Capacity	ml BTPS	175	99-257	434	277-670	549	347-782
Lung Clearance Index	liters expired/ liters FRC	36	25-47	23	15-36	29	14-46
CO Diffusing Capacity	ml/min/mmHg	1.6	1.2-2.5	9.0	2.9-16.6	7.3	3.1-13.8
Fractional Uptake of CO	decimal fraction	0.373	0.321-0.421	0.480	0.194-0.749	0.471	0.350-0.654
Alveolar O ₂ tension	mmHg	81	77-85	83	71-97	84	71-89
Arterial O ₂ tension	mmHg	74	69-80	75	64-90	73	57-85
Alveolar-arterial O ₂ gradient	mmHg	7	0-15	8	1-15	9	2-20
Arterial CO ₂ tension	mmHg	43	36-49	41	31-50	41	34-49
Alveolar CO ₂ tension	mmHg	36	34-40	37	27-45	35	29-40
Arterial-alveolar CO ₂ gradient	mmHg	7	0-13	4	0-7	6	3-11
O ₂ saturation of arterial hemoglobin	%	91	90-93	91	86-94	87	80-93
Arterial pH	pH units	7.40	7.37-7.43	7.38	7.31-7.45	7.37	7.33-7.43
O ₂ uptake	ml STPD***/ min	50	26-76	90	54-169	94	61-181
CO ₂ output	ml STPD/ min	48	36-64	85	50-155	84	54-161
Respiratory exchange ratio	O ₂ uptake/ CO ₂ output	0.96	0.65-1.62	0.95	0.78-1.16	0.89	0.53-1.40
Specific ventilation	minute volume/ O ₂ uptake	25	11-36	40	28-57	41	29-65
Alveolar ventilation	% of minute volume	80	65-89	57	45-71	54	44-67
Effective ventilation	% of minute volume	68	48-88	51	40-66	45	36-57
Alveolar deadspace ventilation	% of minute volume	12	1-18	6	2-9	9	6-18

* BTPS = Body temperature and pressure, saturated

** Measurements using transpulmonary pressures not obtained on immature dogs

*** STPD = Standard Temperature and Pressure, dry

Table 2
Mean Values (from Table 1) for Selected
Pulmonary Function Parameters of Beagle
Dogs at 3 Age Levels Divided by Body Weight

Age	<u>3 mo.</u>	<u>12-14 mo.</u>	<u>8-10.5 yrs.</u>
Minute volume (liters BTPS/ min)/kg.	0.92	0.41	0.30
O ₂ Uptake (ml STPD/ min)/kg	19	10	9
Functional residual capacity (ml/BTPS)/kg	67	48	50
Diffusing capacity (ml/min/ mmHg)/kg	0.62	0.99	0.67

growing animals than in adults.² The immature dogs were more efficient than the adults in extracting O₂ from inspired air, as demonstrated by their lower specific ventilation (Table 1). The immature and young adult groups had very similar values for arterial O₂ and CO₂ tensions, O₂ saturation of hemoglobin, and respiratory exchange ratio.

Differences between values obtained from young and aged adult dogs (Table 1) indicate certain age-related changes in pulmonary function similar to those reported for man. A slightly lower diffusing capacity was observed in the older dogs. Donevan *et al.*,³ using a steady-state CO method, reported a lower mean diffusing capacity in persons over 35 than in those under 35. Other authors using various methods reported similar findings.^{4, 5, 6} The finding of a significantly lower pulmonary compliance in the older dogs presents a contrast to changes in the compliance of human lungs with aging. The elastic recoil of the lungs is lower in elderly persons than in younger subjects, resulting in an increase in pulmonary compliance with age.^{7, 8} This apparent species difference is unexplained at this time. A reduction in the O₂ saturation of hemoglobin has also been observed in older human subjects,⁹ as has a progressive increase in functional residual capacity.¹⁰

The characterization of the normal pulmonary function of dogs of various ages will aid in the interpretation of experimental pulmonary function results. The similarities observed in the age-related changes of certain function parameters of Beagle dogs and humans further indicate the usefulness of the Beagle dog as a model for studying a variety of research problems in the fields of pulmonary toxicology, lung aging and lung physiology.

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NORMAL RANGES OF SOME CHEMICAL CONSTITUENTS IN THE BLOOD OF BEAGLE DOGS OF VARIOUS AGES

ABSTRACT

The evaluation of the health status of Beagle dogs prior to and after inhalation of radionuclides has created a need for "normal" (expected) ranges of blood constituents from dogs of both sexes and at various ages. Expected ranges for 21 blood constituents were developed for Beagle dogs from 2 months to 10 years of age. Cholesterol concentration and gamma globulin percentage increased as a function of increasing age from 1 to 6 years in females, but not in males. Total serum protein concentration also increased as a function of increasing age, in both sexes, for dogs 2 months to 6 years of age. Serum inorganic phosphorous decreased as a function of increasing age in both sexes for dogs 2 months to 6 years of age. Serum alkaline phosphatase was higher in 8- to 10-year-old females and 2- to 3-month dogs than in animals 11 to 14 months of age. An appreciable portion of the population variability was present as "within animal" variability.

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INTRODUCTION

Normal ranges for serum chemical constituents have proven useful for a variety of toxicity studies using different species. In our laboratories, the evaluation of the health status of Beagle dogs prior to and for long periods after inhalation of radionuclides has created a need for "normal" (expected) ranges of blood constituents from dogs of various ages. A common method for deriving expected ranges is to use the means $\pm$ multiples of the standard deviation for a group of data. This method however is valid only if the data are approximately normally distributed (Gaussian distribution) as in Figure 1. Occasionally, when the distribution of a chemical constituent is neither normal, nor capable of being normalized by routine transformations of the data, an estimate of a reliable confidence interval necessitates the use of a nonparametric method which makes no, or few, assumptions concerning the data. The percentile method of Herrera¹ meets this criterion, and was used to find the expected ranges (normal ranges) for various constituents of serum from Beagle dogs 2 months to 10 years old. Concentration differences with respect to sex and age were noted in some constituents.

MATERIALS AND METHODS

Blood samples were obtained by venipuncture, allowed to clot, centrifuged for approximately 10,000 to 15,000 g-minutes and the supernatant serum decanted. The serum was frozen at -5 to -10°C, stored, and subsequently analyzed. Analysis was performed using a Sequential Multiple Analyzer (SMA) 12/30, a flame photometer, a chloridometer and an electrophoresis apparatus. Twenty-one chemical constituents of the blood of Beagle dogs of both sexes and various ages and with no observed clinical abnormalities were selected for evaluation (Table 1).

Blood constituent values for both sexes were initially divided into nonoverlapping age groups ranging from 1 to 6 years. Specific age groups were as follows: 11-14, 14-18, 18-30, 30-42, 42-54, 54-66, and 66-78 months of age. Values exceeding analytical limits of the SMA 12/30 analyzer were excluded from any statistical analyses. These were usually LDH values and only a relatively small fraction of such values. The study included values from several years, thus one animal

Figure 1. Frequency of occurrence of values from Beagle dogs as a function of (left) concentration of total serum protein (gm/100 ml) (normal distribution) and (right) concentration of alkaline phosphatase (KAU) (skewed distribution).

could be included in more than one age group but only once in a given age group. The large number of samples obtained in our regular evaluation of all animals before and control animals after placement on long-term toxicity studies were used to initially develop normal ranges from these animals. Later, groups of 30 females, 8 to 10 years old and 29 dogs of both sexes, 2 to 3 months old, were also evaluated in similar studies.

In a separate study to evaluate the mechanism causing an increase in alkaline phosphatase quantities in 8- to 10-year-old female dogs, 12 dogs, 6 with normal and 6 with elevated alkaline phosphatase quantities, were intravenously injected with 5 mg/kg of sulfobromophthalein (BSP) dye. The percent retention of this dye was used as an assessment of liver function. Correlations with heart or spinal disc lesions or tumors suggested by clinical, electrocardiographic (EKG) and radiographic analysis were also attempted.

Clinical, EKG and radiographic workups were also routinely performed on the dogs of various age groups. The day-to-day variability of some chemical constituents for 4 animals was compared with the 11 to 18 month population variability to more completely define the reliability of an individual animal's value.

RESULTS

Selected groups of values obtained from 2-month-old to 10-year-old dogs are shown in Table 1. Age-related differences in dogs one to six years old were noted in four parameters: cholesterol, inorganic phosphorus, total serum protein, and gamma globulin (Fig. 2).

In females, serum cholesterol concentrations had an appreciably greater range than in males. There was a consistent increase in mean concentration with age that persisted through five years of age (Fig. 2a).

The serum inorganic phosphorus concentration decreased as a function of increasing age from 2 months through 4 years of age. The concentration then remained constant; no sex-related differences were observed (Fig. 2c). By contrast, the 8- to 10-year-old female dogs had serum inorganic phosphorus concentrations similar to 1-year-old dogs.

Table 1

Means and 2.5-97.5 Percentile Ranges for Various Chemical Constituents in the Blood of Beagle Dogs

Constituent	Age											
	2-3 Months		11-14 Months		18-30 Months		42-54 Months		66-78 Months		8-10 Years	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Cholesterol (mg/100 ml) (M)	181 (19)*	128-254	172 (311)	124-237	160 (23)	122-286	154 (17)	134-191	198 (16)	118-377	—	—
(F)	165 (10)	120-195	183 (321)	129-273	178 (25)	121-253	217 (14)	128-340	216 (14)	125-295	192 (30)	135-319
Calcium (mg/100 ml)	11.0 (29)	10.2-11.9	11.0 (635)	10.3-11.7	10.8 (48)	9.9-11.4	10.6 (34)	9.6-11.8	10.8 (31)	10.3-11.6	10.8 (30)	9.7-11.7
Inorganic Phosphorus (mg/100 ml)	7.8 (29)	6.8-9.1	4.4 (632)	3.4-5.5	3.6 (48)	2.8-4.7	3.4 (36)	2.4-4.4	3.4 (30)	2.4-4.3	3.9 (30)	3.1-5.2
Total Bilirubin (mg/100 ml)	0.3 (29)	0.26-0.34	0.2 (636)	0.1-0.3	0.2 (45)	0.1-0.3	0.2 (34)	0.1-0.3	0.3 (30)	0.2-0.5	0.2 (30)	0.2-0.3
Albumin (g/100 ml)	1.5 (29)	0.9-1.9	2.2 (636)	1.6-2.9	2.4 (48)	1.8-3.1	2.3 (35)	1.9-2.9	2.4 (31)	1.8-3.0	2.3 (30)	1.9-3.2
Total Protein (g/100 ml)	4.6 (29)	4.0-5.1	6.0 (633)	5.4-6.7	6.1 (48)	5.7-6.6	6.3 (34)	5.9-7.2	6.5 (31)	5.8-7.9	6.4 (30)	5.7-7.3
Uric Acid (mg/100 ml)	0.7 (29)	0.5-1.2	0.7 (633)	0.4-1.1	0.6 (48)	0.4-1.0	0.6 (32)	0.4-1.2	0.6 (31)	0.4-0.9	0.6 (30)	0.4-0.8
BUN (mg/100 ml)	10.0 (29)	7.9-13.9	12.9 (636)	8.4-19.1	13.3 (48)	9.6-19.3	12.0 (36)	7.2-20.5	11.5 (31)	7.8-17.7	10.7 (30)	5.4-20.5
Glucose (mg/100 ml)	101 (29)	80-118	87 (625)	66-106	88 (48)	67-104	87 (36)	64-105	89 (31)	66-104	90 (30)	67-118
LDH (Technicon Units) (M)	184 (15)	68-344	122 (309)	37-246	102 (23)	42-219	112 (19)	53-200	133 (16)	41-270	—	—
(F)	212 (9)	117-300	140 (321)	38-288	123 (25)	48-268	141 (17)	42-255	98 (15)	44-162	98 (30)	17-290
Alkaline Phosphatase (King & Armstrong Units) (M)	21.1 (19)	16.9-28.5	7.9 (311)	5.2-13.9	7.2 (23)	5.0-12.4	8.3 (19)	4.5-17.4	7.0 (16)	5.2-11.5	—	—
(F)	20.0 (10)	16.6-26.7	8 (325)	5.2-13.1	8.5 (25)	5.5-14.1	8.2 (17)	5.2-14.7	8.0 (15)	4.6-14.8	13.8 (30)	6.9-45.0
SGOT (Karmen Units)	42 (29)	29-62	33 (633)	23-47	38 (48)	29-52	35 (28)	25-57	32 (31)	20-48	29 (30)	21-49
Sodium (mEq/l)	—	—	148 (787)	143-153	148 (44)	143-153	148 (28)	144-153	—	—	146 (30)	143-150
Potassium (mEq/l)	—	—	4.8 (787)	4.2-5.3	4.5 (44)	4.0-5.2	4.7 (28)	4.2-5.6	—	—	4.7 (30)	3.9-6.4
Chloride (mEq/l)	—	—	111 (788)	105-117	111 (44)	105-115	112 (28)	105-116	—	—	106 (30)	103-111
Albumin (%)	—	—	55.8 (837)	45.3-64.3	56.5 (65)	45.0-64.9	55.8 (47)	45.9-64.9	55.7 (26)	47.5-61.2	52.6 (30)	44.3-61.8
α_1 Globulin (%)	—	—	8.5 (837)	3.1-13.3	8.9 (65)	3.6-14.1	7.3 (47)	2.2-11.8	7.2 (26)	1.9-12.7	9.9 (30)	8.3-12.3
α_2 Globulin (%)	—	—	9.3 (837)	5.6-15.6	8.7 (65)	5.3-17.9	9.4 (47)	3.5-17.8	9.7 (26)	6.5-15.0	7.9 (30)	5.0-10.5
β_1 Globulin (%)	—	—	8.3 (837)	5.2-12.0	8.1 (65)	5.4-12.7	8.2 (47)	5.8-11.0	8.4 (26)	5.1-10.8	9.3 (30)	6.4-13.3
β_2 Globulin (%)	—	—	9.0 (837)	6.5-12.1	9.0 (65)	6.8-12.1	9.7 (47)	6.6-14.8	9.7 (26)	7.7-14.4	10.8 (30)	8.7-13.4
γ Globulin (%) (M)	—	—	9.4 (415)	6.3-13.4	9.0 (31)	6.1-14.4	9.7 (25)	6.6-13.2	9.0 (13)	7.6-12.3	—	—
(F)	—	—	9.0 (422)	5.8-12.9	8.4 (34)	4.3-12.2	9.5 (22)	6.3-19.8	9.5 (13)	6.2-22.8	9.4 (30)	6.3-13.1

* () Number of Animals

a.

b.

c.

d.

Figure 2. (a) Cholesterol concentration in the female Beagle dog; (b) gamma globulin percentage in the female Beagle dog; (c) inorganic phosphorus concentration in the Beagle dog; and (d) total serum protein concentration in the Beagle dog. All values are for the mean, and upper and lower limits of the 2.5-97.5 percentile range as a function of increasing age. (Number of animals in each group is shown in parentheses).

The total serum protein concentration increased with increasing age in both sexes and persisted through 54 to 66 months of age (Fig. 2d). Total serum protein in 8- to 10-year-old females was intermediate between the concentration observed in 5-year-old dogs and in the 1-year-old dogs.

The relative percentage of serum gamma globulin fraction seemed to increase with age in females but not in males. Percentile ranges through two years of age were similar. Between three to six years of age the range increased irregularly (Fig. 2b). In 8- to 10-year-old females, however, the concentration was indistinguishable from 1-year-old animals.

Serum LDH did not change as a function of increasing age, however a sex-related difference was noted (Table 1). Values from females were somewhat higher than those observed in males. Serum alkaline phosphatase was elevated in dogs 2 to 3 months and 8 to 10 years old compared to dogs 11 to 14 months of age. In female dogs 8 to 10 years of age, concentrations of the remainder of the chemical constituents were similar to dogs 11 to 14 months of age. In these animals, no consistent correlation of heart, spinal disc or neoplastic lesions or elevated BSP retention to increasing alkaline phosphatase retention was shown (Table 2). The variations of chemical constituent values of four dogs observed over several months as compared to normal population variability are shown in Table 3. These variations represented a substantial portion of the population variations.

DISCUSSION

The ranges of parameters in which concentration had no relationship to increasing age were similar in most cases to those reported by McKelvie² and Brunden et al.³ A finding by Berliner et al.⁴ of a small age-related decrease in serum albumin was not observed. The increase in cholesterol concentration as a function of increasing age in females differs from that of McKelvie and Brunden et al. because their dogs were not partitioned into separate age or sex groups for this

TABLE 2
Quantity of Alkaline Phosphatase (Alp;KAU) and Sulfobromophthalein (BSP) Retention
Compared to Diagnosis of Heart or Disc Lesions or Tumors

Animal Number	1st Alk-P	2nd Alk-P	BSP Retention (%)	Heart ^a	Spinal Disc ^b	Mammary Tumor ^c	Other Tumor ^c
FD 145	38.3	21.9	7.5	-	-	+	-
FD 104	30.5	21.3	8.8	-	-	-	-
FD 4	23.2	20.0	5.4	+	-	-	-
FD 6	17.5	19.9	4.3	+	+	+	-
FD 7	15.0	14.3	4.5	+	-	-	-
FD 150	8.6	10.0	11.4	-	+	-	-
FD 147	6.9	8.7	5.8	+	+	-	-
FD 12	6.9	8.1	11.5	+	-	-	-
FD 101	7.4	7.7	8.0	-	-	-	+
FD 118	8.2	7.4	6.8	-	-	-	-
FD 154	8.4	7.3	5.6	-	-	-	-
FD 117	11.0	7.0	6.2	-	-	+	+

(a) Diagnosis made by radiographic or electrocardiographic evaluation. Changes were generally mild and of little immediate clinical significance.

(b) Diagnosis made by radiographic analysis (cervical pain on manipulation).

(c) Diagnosis made by physical examination, some removed and examined histologically.

Table 3
A Comparison of the Ranges of Some Chemical Constituents Within Each
of Four Animals and of the Normal Population

		Animal No. 1*	Animal No. 2*	Animal No. 3*	Animal No. 4*	11-18 Mo. Range**
Cholesterol (mg/100 ml)	M	---	158-180	137-156	---	121-250
	F	197-318	---	---	163-225	123-273
Calcium (mg/100 ml)		10.8-11.3	11.3-12.4	10.8-11.3	10.0-11.6	10.0-11.7
Inorg. Phosphorus (mg/100 ml)		2.8-4.4	3.3-8.1	2.8-4.5	2.7-4.4	2.6-5.5
Total Bilirubin (mg/100 ml)		0.19-0.22	0.16-0.23	0.18-0.21	0.18-0.21	0.13-0.33
Albumin (g/100 ml)		2.0-2.3	2.3-2.7	1.7-2.6	1.8-2.4	1.6-2.9
Total Protein (g/100 ml)		5.7-5.9	6.2-6.6	5.8-6.2	5.4-6.0	5.3-6.7
Uric Acid (mg/100 ml)		0.6-1.0	0.47-0.52	0.4-0.9	0.4-0.9	0.4-1.3
BUN (mg/100 ml)		12.1-24.4	11.4-27.5	10.0-12.2	7.9-15.5	8.4-20.2
Glucose (mg/100 ml)		79-98	72-101	70-92	51-102	64-106
LDH (Technicon Units)	M	---	42-135	32-81	---	26-246
	F	50-162	---	---	22-152	19-288
Alkaline Phosphatase (KAU)	M	---	7.4-15.0	6.3-9.2	---	3.3-13.9
	F	6.1-8.6	---	---	5.8-9.7	5.2-13.9
SGOT (Karmen U)		25-34	23-41	19-35	21-33	23-55
Albumin %		42.8-47.2	51.1-57.6	54.1-58.0	56.5-59.4	44.9-66.7
α ₁ %		13.1-13.3	6.5-11.7	6.7-10.0	8.7-11.6	3.1-14.7
α ₂ %		7.1-12.5	8.0-9.7	4.8-9.9	6.4-9.5	5.3-17.2
β ₁ %		7.0-10.5	8.4-8.5	7.9-9.0	4.7-8.0	5.2-13.3
β ₂ %		10.1-11.1	7.7-9.4	7.9-9.4	7.9-9.0	6.2-12.1
γ %	M	---	8.7-11.2	9.5-11.8	---	6.3-13.4
	F	10.4-12.7	---	---	7.8-10.7	4.2-15.2

* 4-6 samples are represented on each S.M.A. 12/30 parameter for each animal and 3 samples are represented on each electrophoresis parameter for each animal.

** This range was obtained by using the extreme limits of the 2.5-97.5 percentile ranges for 11-14 and 14-18 month old dogs.

parameter. The absence of a trend toward increase in 2- to 3- or 66- to 78-month-old dogs and 8- to 10-year-old females suggests that the biochemical conditions which produced this age-related increase were not present at the time. In humans, cholesterol increases as a function of age but somewhat more in males than in females.⁵

The persistent fall in inorganic phosphorus concentration as a function of increasing age, observed by McKelvie in dogs 4 to 12 months of age is in agreement with our findings in both older and younger (2- to 3-month-old) dogs. The trend persists through 4 years of age with the major changes occurring between 2 months and 2 years of age; no changes were observed in 8- to 10-year-old females. Berliner et al.⁴ report no such trend; however their younger dogs were apparently ≥ 17 months old. The age-related decrease in inorganic phosphorus also is in agreement with the findings in man.⁵ The age-related increase in serum protein concentration reported by McKelvie² and for dogs ≥ 17 months of age by Berliner et al.⁴ was similar to the increase observed in our dogs 2 to 3 months old and 1 to 6 years old. The finding that serum protein concentration of 8- to 10-year-old females was intermediate between that in animals 11 to 14 months old and 5 to 6 years of age suggests a partial reversal of the metabolic conditions which produced this age-related increase. The finding that the relative fraction of gamma globulin increased sporadically from 1 to 6 years of age but returned to a level similar to 1-year-old dogs in 8- to 10-year-old females suggests that the metabolic conditions underlying this constituent may be somewhat unstable. Although the serum phosphorus concentration decrease with increasing age may be related to growth, there is no clear explanation for the increase in serum cholesterol concentration, protein concentration and gamma globulin relative fraction with increasing age to 6 years.

The increase of alkaline phosphatase in 2- to 3-month-old animals was probably related to bone growth, but a similar increase observed in 8- to 10-year-old females was surprising, since no consistent age-related trend in this parameter had been observed between 1 and 6 years of age. The finding, however, agrees in part with Berliner et al.⁴ who report that age in animals produced a slight increase in the level of this enzyme. Elevated serum alkaline phosphatase is observed in a variety of disease processes including hepatocellular and obstructive liver disease as well as primary and secondary bone disorders. However, there have been no consistent clinical manifestation of such disorders or of cardiac or neoplastic lesions in our aging population of dogs nor any correlation of this enzyme activity with percent BSP retention. The etiology of this elevated alkaline phosphatase concentration is unclear. Since elevated serum alkaline phosphatase levels have been roughly correlated with increased BSP retention⁶ in the presence of liver disease, the meaning of the lack of elevated BSP retention in our apparently healthy dogs is also unclear. These findings indicated that observed concentrations of this enzyme must be interpreted in older dogs, using either our or other pertinent normal ranges.

An appreciable portion of the population variability may be attributed to variation within a given animal (Table 3). When one combines the observation of considerable individual animal variability (Table 3) with the use of completely reliable normal ranges (distribution of population = distribution of sample) for 21 parameters, only about 34% of the animals will have normal values for all parameters⁷ and the conclusion that guidelines must be used in the interpretation of animal profiles seems obvious. For example, when one observes an altered value, the question arises whether this alteration is repeatable or merely random, and how the alteration compares with the "clinical picture." Each range of constituents is diagnostically useful in considering the health status of the animal and is partitioned for this reason. Such ranges increase the ability to distinguish diseased from non-diseased animals, when one uses ranges derived from the changes in concentration of serum constituents with age.

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SURVIVAL DATA OF LITTERS 1-621 MAINTAINED AT THE FISSION PRODUCT
INHALATION LABORATORY

ABSTRACT

Survival data of Beagle dog litters 1-621 from the Fission Product Inhalation Laboratory were analyzed, utilizing the life table method of Cutler and Ederer. The highest percent of death, (18.5%), during any one-month interval occurred from 0 to 1 month of age. The cumulative survival rate at 12 months of age, when dogs for longevity studies are assigned, was 79.4%. This rate dropped to 74.9% by the end of 84 months of age for a decrease in cumulative survival rate of 4.5% during the first 6 years. A comparison of the cumulative survival rate for litters 1-621 has been made with an adjusted cumulative survival rate of control dogs maintained on lifespan studies at the University of California at Davis.

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INTRODUCTION

Survival data on control Beagle dogs on lifespan experiments and other unexposed dogs born and maintained in the same animal facilities are necessary for the evaluation of the effects of the inhalation of radionuclides on experimental animals. This report summarizes data on the first 621 litters that were born and raised at the Fission Product Inhalation Laboratory. Litters 1-293 were produced under the old breeding system. Litters 294-621 were produced from a breeding program which was designed to provide for the production of dogs from a stable gene pool over an extended period of time. These dogs, scheduled for entry into a series of sequentially and inter-related experiments,¹ were all raised and maintained in accordance with previously published procedures.²

RESULTS AND DISCUSSION

Survival data of litters 1-621 were analyzed, utilizing the life table method of Cutler and Ederer.³ These data are complete through April 19, 1972 (72110). The data used in the calculation of the cumulative survival rate in the Cutler and Ederer method is as follows: the first column is the age in months, each of which is 30 days in length; the l_x are the total numbers alive at the beginning of the interval; the d_x are the number who have died during the interval; the u_x are the number lost to follow-up during the interval; the w_x are the withdrawn alive during the interval; the l'_x are the effective number exposed to the risk of dying; the q_x are the percent dying; the p_x are the percent surviving; the P_x are the cumulative percent surviving through the end of the interval; the $1-P_x$ are the cumulative percent dying through the interval; and the s_x is the associated standard error. For purposes of analyses, the animals were classified into several categories, as follows: to determine whether to list them in the d_x , u_x , or w_x :

1. B = Breeding stock (presently assigned as breeding stock or reserve breeding stock).
2. E = Exposed (all animals exposed, less control animals).
3. C = Control (all control animals, unassigned animals, animals assigned to aged and epileptic studies).
4. O = Other (animals assigned to studies other than noted above and who had no record of being exposed to radioactive material).

Categories B and C were grouped as d_x (died or euthanized), u_x (removed, sacrificed, or withdrawn alive), and w_x (alive on 72110) according to the appropriate age. Category E was grouped as u_x (as of the age at exposure). Category O was grouped as u_x (as of most recent assignment date). The date interval used in the study are based on a 30-day month starting from birth.

Table 1 shows the analysis of data on litters 1-621 for 84 months (7 years), using the life table method of Cutler and Ederer. It is shown on a monthly basis for the first 6 months. The next year is shown on a quarterly basis and the final 5-1/2 years are shown on a semi-annual basis. The number of dogs decreases from 3,268 present during the first-month interval to 117 present at 84 months. As can be seen from the q_x column of this table, the highest percent dying was 18.51 during the time interval 0 to one month of age. The majority of these deaths occurred during the first 72 hours and a detailed analysis of litters 1-293 have been published² and litters 294-526 appear in the 1970-1971 Annual Report, LF-44, pp. 219-222. As can be seen in the d_x column, this represents a loss of 600 animals. Fourteen animals had died at the 1- to 2-month interval, representing 0.54% deaths. Twenty-two animals, between 2 to 3 months of age, died for an 0.86% death during this time period. These two time intervals had the second and third highest percentage of deaths. As can be seen in the P_x column, the cumulative survival rate reduced from 81.49% at the end of 1 month of age to 74.92% at the end of 84 months of age. A majority of the lifespan studies on the effect of the inhalation of radionuclides in this laboratory begin on dogs between the ages of 12 to 14 months. It is interesting to note that the cumulative survival rate at 12 months of age is 79.41%. This is reduced only to 74.92% by the end of 84 months of age; this being a decrease in survival of 4.49% during the first 6 years post-exposure. This compares favorably with previously reported cumulative survival data on control Beagle dogs, maintained for their lifespan, as reported by Andersen and Rosenblatt⁴ for the University of California X-irradiation study controls. Their data contained 57 control dogs maintained for their normal lifespan observations. The animals were placed on experiment between the ages of 10 and 12 months and their percent

Table 1
Analysis of the Survival Data of Litters 1-621
of the Lovelace Colony by the Life Table Method of Cutler and Ederer

Intervals in Months	l_x	d_x	u_x	w_x	l'_x	q_x	p_x	P_x	$1-P_x$	s_x
0-1	3268*	600*	36	16	3242.0	18.51	81.49	81.49	18.51	0.68
1-2	2616	14	3	26	2601.5	0.54	99.46	81.05	18.95	0.69
2-3	2573	22	1	22	2561.5	0.86	99.14	80.36	19.64	0.70
3-4	2528	6	1	21	2517.0	0.24	99.76	80.17	19.83	0.70
4-5	2500	10	1	18	2490.5	0.40	99.60	79.84	20.16	0.71
5-6	2471	3	0	19	2561.5	0.12	99.88	79.75	20.25	0.71
6-9	2449	5	2	67	2414.5	0.21	99.79	79.58	20.42	0.71
9-12	2375	5	14	43	2346.5	0.21	99.79	79.41	20.59	0.71
12-15	2313	2	921	149	1778.0	0.11	99.89	79.32	20.68	0.71
15-18	1241	3	281	44	1078.5	0.28	99.72	79.10	20.90	0.72
18-24	913	3	181	87	779.0	0.39	99.61	78.80	21.20	0.74
24-30	642	1	91	37	578.0	0.17	99.83	78.66	21.34	0.75
30-36	513	2	93	22	455.5	0.44	99.56	78.32	21.68	0.79
36-42	396	0	20	45	363.5	0.00	100.00	78.32	21.68	0.79
42-48	331	0	23	10	314.5	0.00	100.00	78.32	21.68	0.79
48-54	298	1	36	12	274.0	0.36	99.64	78.03	21.97	0.83
54-60	249	2	26	7	232.5	0.86	99.14	77.36	22.64	0.95
60-66	214	2	4	21	201.5	0.99	99.01	76.59	23.41	1.09
66-72	187	2	9	24	170.5	1.17	98.83	75.69	24.31	1.25
72-78	152	0	2	33	134.5	0.00	100.00	75.69	24.31	1.25
78-84	117	1	0	38	98.0	1.02	98.98	74.92	25.08	1.45

* Includes 9 dead unsexed pups

cumulative survival rate for 1 year of age was 100%. With the assumption that the survival rate of these dogs to 1 year of age was identical to that of dogs raised within the Lovelace breeding colony, the cumulative survival rate was adjusted to 79.4% at 1 year of age and the cumulative survival rate for each year of age calculated from their published data.

The cumulative survival for the controls of the ^{90}Sr study and ^{226}Ra study from the University of California has been reported.⁵ There were 79 dogs maintained as controls on the ^{90}Sr study and 81 dogs assigned as controls on the ^{226}Ra study. Their survival data begins at 0.5 years of age. With the assumption that the percent cumulative survival rate for their dogs were the same as the Lovelace controls at 0.5 years of age, the cumulative survival rate was adjusted to 79.8% at this age and the cumulative survival rate at 6 months intervals to two years of age and annually to 7 years of age calculated from their published data. A plot of the cumulative survival rate for the Lovelace controls and the adjusted cumulative survival rate for the controls of the University of California X-irradiation, ^{90}Sr , and ^{226}Ra studies is shown in Figure 1. The cumulative survival rate for the Lovelace colony at 7 years of age is higher than the X-irradiation study, however, lower than the ^{90}Sr and ^{226}Ra studies. These data are of value in predicting lifespan for control and low-level radiation exposure experimental dogs and the associated housing and manpower requirements for their maintenance.

Figure 1. The cumulative survival rate for litters 1-621 from the Lovelace colony and the adjusted cumulative survival rate of the control dogs maintained on three lifespan studies at the University of California at Davis.

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SECTION IV

PATHOGENESIS OF RADIATION-INDUCED DISEASE

TOXICITY OF INHALED ^{144}Ce FUSED CLAY IN BEAGLE DOGS -

PATHOGENESIS STUDIES

ABSTRACT

A group of 77 Beagle dogs have been exposed to ^{144}Ce fused clay and stable cerium fused clay aerosols and are being studied to elucidate the pathogenesis of the lesions induced by inhaled insoluble forms of ^{144}Ce . Three separate but related studies have been initiated: (1) a serial sacrifice study to follow the histology of the induced lesions, (2) a cardiopulmonary function study with sacrifice animals to relate functional and structural changes, and (3) a bronchial cytology study to correlate the cytology findings with pulmonary lesions. The current status of these studies is reported.

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INTRODUCTION

Over the past four years a series of dose response studies in which dogs have inhaled aerosols of beta-emitting radionuclides (^{90}Y , ^{91}Y , ^{144}Ce and ^{90}Sr) in the fused clay form have been initiated in this laboratory.¹ The philosophy of these dose-response studies has been that the animals should be subjected to clinical procedures which carry a minimal risk to the animal, and that the dog be studied over its total lifespan. Thus, while considerable information relating to the dose-response relationships following chronic irradiation of the lung has been, and will be, gained from these studies, it is not possible to subject the dogs to certain clinical procedures or to study the pathogenesis of the disease processes by serial sacrifice of the animals.

During the past year a study has been initiated in which dogs have been exposed to aerosols of ^{144}Ce in fused clay and aerosols of stable cerium in fused clay. These animals are to be serially sacrificed and subjected to various clinical procedures over the next several years in order to study the pathogenesis of pulmonary injury following chronic irradiation of the lung. ^{144}Ce was the radionuclide chosen for the initial study due to its relatively long half-life and our present knowledge relating to its biological effects.

The dogs have been divided into three groups termed ^{144}Ce in fused clay Sacrifice Study Series II, III and IV. This report briefly summarizes the experimental design of the study and reports the results obtained to date. All investigators within the laboratory have been encouraged to utilize samples from the dogs in their studies.

EXPERIMENTAL PROCEDURES

Seventy-seven Beagle dogs (39 males and 38 females) raised in this colony were exposed to aerosols of either ^{144}Ce in fused clay or to stable cerium in fused clay. At the time of exposure they were 12 to 14 months old.

The dogs were exposed nose-only to the experimental aerosols for varying periods of time so as to achieve three levels of initial lung burdens (ILB) approximately 45, 25 and 12.5 $\mu\text{Ci/kg}$. The particle size distributions of the ^{144}Ce fused clay aerosols were log normal with AMAD approximately 1.4 to 2.7 μm with a geometric standard deviation of approximately 1.5 to 2.3. The de-

tailed technique of aerosol generation and exposure have been previously described (1970-1971 Annual Report, LF-44, pp. 164-180).

After the aerosol exposure the dogs were whole-body counted and the data for the first 14 days post-inhalation exposure used to graphically determine the initial lung burdens. Practically all of the values were slightly higher than the desired ILBs. In Appendix A, all dogs in these experiments are listed and ranked according to their initial lung burdens expressed as μCi of ^{144}Ce per kg of body weight. These ILB values ranged from 14 to 71 $\mu\text{Ci/kg}$. Absorbed beta radiation doses to lung have been calculated for the dogs that have been sacrificed to date. Calculation of these doses was based on the whole-body counting data for each dog plus an expression describing lung as a fraction of total body burden as a function of time post-exposure. This method was described in detail previously (1968-1969 Annual Report, LF-41, pp. 19-35 and 1969-1970 Annual Report, LF-43, pp. 184-186).

From the ILB values obtained, a sacrifice schedule for a morphological pathogenesis study utilizing 50 dogs (26 males and 24 females) was determined (Table 1) and designated ^{144}Ce fused clay Series II. Several points were considered especially pertinent in setting this schedule. All dogs in the ^{144}Ce fused clay longevity series with ILBs ranging from 48 to 71 $\mu\text{Ci/kg}$ have died with radiation pneumonitis from 180 to 410 days post-inhalation exposure. Thus, the higher level dogs are scheduled for sacrifice at comparatively early time periods. To achieve a better distribution of ILB values among the 4 sacrifice groups scheduled for the 12 highest dogs, the following scheme was used. The top 12 dogs were divided into 3 groups of 4 on the basis of their ILBs, i. e., the dogs with highest ILBs were the first group and so on down the list. Within each of these 3 groups, dogs were chosen randomly for sacrifice at 80, 128, 180 and 256 days. In selecting sacrifice times at later times consideration was given to sacrifice times of previous radiation dose pattern studies and to times at which dogs have died from pulmonary neoplasms. These times may need to be re-evaluated in the future in light of new knowledge.

The schedule for the 25 $\mu\text{Ci/kg}$ ILB dogs is based on the intent of finding early neoplastic lesions, thus most of the dogs are scheduled for later periods. The control dogs are scheduled for sacrifice at times corresponding to sacrifice times used in Radiation Dose Pattern studies with an emphasis on the later time periods when normal aging processes will need to be recognized.

A group of 18 dogs (including 6 controls) designated ^{144}Ce fused clay Sacrifice Series III, was retained to study the correlation of cardiopulmonary function and the development of cardiopulmonary lesions (Table 2). These dogs will be tested monthly with cardiopulmonary function tests (1969-1970 Annual Report, LF-43, pp. 205-211 and 1970-1971 Annual Report, LF-44, pp. 227-236). Six of the dogs (including 2 controls) have been treadmill trained to assess the effects of exertion on cardiopulmonary function. Dogs will be sacrificed in groups of 6 at the following times: (1) when the first signs of cardiopulmonary dysfunction appear, (2) when moderate cardiopulmonary dysfunction is present, i. e., the changes observed are compensated, and (3) when severe cardiopulmonary dysfunction is present, i. e., the changes observed are no longer compensated.

A group of 9 dogs (including 3 controls) designed ^{144}Ce fused clay Sacrifice Series IV was retained to study the cytology of bronchial washings (Table 3). These dogs have ILB values (29-41 $\mu\text{Ci/kg}$) which have resulted in pulmonary neoplasms in earlier studies. The bronchial washing cytology will be used as a method of early neoplasia detection. Dogs will be sacrificed at the first indication of pulmonary neoplasia using this technique or any technique that may be developed in the coming years.

After exposure, each dog in all series is observed daily and given a detailed physical examination and thoracic radiography every 6 months through one and one-half years and every 3 months

thereafter. EKG recordings are obtained at six-month intervals. Samples for hematology are obtained weekly for the first 28 days, monthly to 180 days, every 90 days to 1 year and semi-annually thereafter. In addition, samples for clinical chemistry are obtained every 6 months. Pulmonary function profiles were obtained on all the dogs in Series II, III and IV prior to exposure and are to be repeated just prior to sacrifice on the Series II animals. Bronchial washing samples during bronchoscopy are to be obtained on all dogs just prior to sacrifice.

RESULTS

To date, two sacrifice times in Sacrifice Series II, 80 and 128 days post-exposure, have passed. No dogs have been sacrificed on Series III or IV. No dogs have died spontaneously. The sacrificed dogs are listed in Table 4. The clinical and necropsy findings from sacrificed dogs will be discussed.

Table 1
Sacrifice Schedule for ^{144}Ce Fused Clay
Sacrifice Series II Dogs (Morphological
Pathogenesis Study)

Sacrifice Times Post-Exposure	Initial Lung Burden $\mu\text{Ci/kg}$ Body Weight			
	39-71	24-35	14-16	0
80 days	3*	-	-	-
128	3	2	-	1
180	3	-	-	-
256	3	2	-	1
512	2	2	-	-
730	1	2	2	2
2 1/2 years	-	2	-	-
3	-	2	-	2
3 1/2	-	2	-	2
4	-	2	2	2
5	-	2	-	2
Unscheduled**				

* Number of dogs scheduled for sacrifice

** One dog (ILB = 31 $\mu\text{Ci/kg}$) unscheduled and to be used as a replacement if necessary

Table 2
Experimental Protocol for ^{144}Ce Fused Clay
Sacrifice Series III Dogs
(Cardiopulmonary Function-Structure)

Dog Number	ILB $\mu\text{Ci/kg}$	Treadmill Trained
538A	69	
540U	59	
535T	50	
535B	50	x
540S	50	x
539B	48	
542C	48	
547C	35	
535A	34	x
547T	32	
544T	32	x
530T	29	
6 Control Dogs	0	2

Table 4
 ^{144}Ce Fused Clay Sacrifice Series II
(Dogs Sacrificed to September 30, 1972)

Table 3
Experimental Protocol for ^{144}Ce Fused Clay
Sacrifice Series IV Dogs (Bronchial Cytology)

Dog Number	ILB $\mu\text{Ci/kg}$
539T	41
543A	33
541V	33
542B	32
543B	31
543S	29
3 Controls	0

Days Post Exposure	Dog Number	ILB $\mu\text{Ci/kg}$	Cumulative Rads To Death
81	526S	51	15,000
81	526A	52	15,000
80	530B	60	16,000
127	533A	0	0
130	519T	28	12,000
128	522U	33	14,000
131	525T	48	22,000
128	521T	52	23,000
128	541S	72	30,000

The most apparent clinical finding has been a lymphopenia similar to that seen in earlier studies with ^{90}Y , ^{91}Y , ^{144}Ce and ^{90}Sr in fused clay. This effect is generally dose related. The decreased absolute lymphocyte values are first seen about 2-3 weeks after exposure and, in the dogs with ILB values 52 $\mu\text{Ci/kg}$ or higher, these continued to decrease until sacrifice time. The most severe lymphopenia occurred in dog 521T which had an absolute lymphocyte count of 980 per cmm at 126 days post-exposure.

Dogs with ILB values of 48 $\mu\text{Ci/kg}$ or lower all had at least a moderate lymphopenia but the lymphocyte numbers returned to normal at sacrifice time of 128 days.

Thoracic radiographs revealed a mild increased density of the lung fields of two dogs (521T and 525T) immediately prior to sacrifice at 128 days. No other clinical abnormalities have been noted in any of the dogs. Gross and microscopic lesions were found in all dogs at sacrifice.

80 Days - (Dogs 526S, 530B, 526A) Gross Lesions - The lungs collapsed normally when the thorax was opened. Pale yellow moderately firm nodules varying in size from 1/2 cm to 2 cm were found in the apical and cardiac lobes and on the anterior portions of the diaphragmatic lobes (Fig. 1). The remainder of the lung was pink or purple and compliant. The tracheobronchial lymph nodes were moderate in size (ranging from .85 to 1.1 gms) and uniformly gray-white. No significant gross abnormalities were noted in other major organs.

Microscopic Lesions - Lesions of radiation pneumonitis were present in all lobes examined (right apical, right cardiac and left diaphragmatic) from all three dogs. The lesions were generally mild, focal, widespread and more severe in the right apical than in the other two lobes. Highly vacuolated alveolar macrophages were present throughout the lung but were more numerous adjacent to other pulmonary lesions (Fig. 2). There was a hemorrhage or edema and a very scant amount of hemosiderin present in the lung. In scattered foci, hypertrophy and hyperplasia of alveolar lining cells occurred. These foci were more prominent in areas of fibrosis. Fibrosis

Figure 1. Representative gross photograph of lungs from dog 526A sacrificed at 81 days post-inhalation exposure. Note the nodule in the left apical lobe.

Figure 2. Increased number of vacuolated alveolar macrophages in the lung of dog 530B sacrificed 80 days post-inhalation exposure. H + E stain. x 310

occurred as a fibrillar septal thickening in a few large foci in two of the dogs (Fig. 3). There was little tendency for sclerosis or large scars and no pleural fibrosis. The bronchiolar epithelium was generally vacuolated. In some foci degeneration and erosion of the epithelium occurred (Fig. 4). Small accumulations of acute inflammatory cells accompanied a few of these degenerative changes. In one dog (530B) there was a focal mucoid degeneration of the media of elastic pulmonary arteries.

Very subtle lesions were present in the right atrium of one dog (530B). They consisted of a sparse macrophage and neutrophil infiltration under the epicardium and in the epicardial surface of the myocardium. Some of the macrophages contained hemosiderin. No unequivocal evidence of myocardial degeneration could be found.

Tracheobronchial lymph node changes were present in all three dogs. In the cortex the number and size of the germinal centers were reduced (Fig. 5). Necrotic cells could be seen in the centers. A small mantle of small lymphocytes surrounded the remnants of the germinal centers. In two dogs a reticuloendothelial cell proliferation filled much of the paracortical areas of the node. The medullary cords were generally small and contained moderate numbers of lymphocytes, plasma cells and foci of neutrophils. The medullary sinuses were dilated and filled with reticuloendothelial cells. Some contained red blood cells or hemosiderin. A striking feature in two of the dogs was the adherence of red blood cells to the reticuloendothelial cells creating a rosette appearance (Fig. 6). No significant microscopic lesions were found in other tissues.

128 Days (Dogs 533A, 522U, 521T, 541S, 519T, 525T) Gross Lesions - No lesions were found in the control dog. The lungs of the two dogs with the highest lung burdens (541S, 521T) did not collapse fully when the thorax was opened due to moderate pulmonary edema. The lung of the other dogs collapsed normally. A few yellow or gray foci 1/2 to 2 1/2 cm at widest dimension were scattered in the apical, cardiac and anterior portions of the diaphragmatic lobes. In all dogs these were more prominent where lung lobes touched one another.

Figure 3. Septal fibrosis in the lung of dog 526A sacrificed at 81 days post-inhalation exposure. EM stain. x 310

Figure 4. Degeneration of bronchiolar epithelium in dog 530B sacrificed at 80 days post-inhalation exposure. H + E stain. x 185

Figure 5. Tracheobronchial lymph node in a dog sacrificed at 80 days post-inhalation exposure. Note the absence of germinal centers and proliferation of reticuloendothelial cells in the paracortical areas. H + E stain. x 625

Figure 6. Adherence of red blood cells to a macrophage in the sinusoid of the tracheobronchial lymph node of a dog sacrificed 80 days post-inhalation exposure. H + E stain. x 625

The tracheobronchial lymph nodes were generally slightly small and had a pale gray cortex and tan medulla. In one dog (521T) there was hemorrhage in the wall of the right atrium. No other significant lesions were seen.

Microscopic Lesions—No microscopic lesions were seen in the control dog. Lesions of radiation pneumonitis, similar to those seen in the 80-day sacrifice dogs, were present in all 5 dogs. The lesions were generally more severe than those present in the lungs of the dogs sacrificed at 80 days in all dogs except dog 519T which had the lowest ILB. In this dog the lesions were generally less severe than those of the 80-day sacrifice dogs. Septal fibrosis was present in the four most severely affected dogs but sclerosis and pleural fibrosis were not yet prominent. Changes in large elastic pulmonary arteries were present in two dogs (541S, 522U).

Severe heart lesions were present in dog 521T. These included degeneration and necrosis of myocardial fibers in the right atrium, along with hemorrhage, edema and fibrosis. Hemosiderin laden macrophages were also present along the epicardial surface. A very mild lesion was present in the right atrial myocardium of dog 525T which consisted of a small amount of interstitial hemosiderin.

The microscopic lesions in the tracheobronchial lymph nodes were generally similar to those in the 80-day sacrifice dogs but were more severe. In the dog with the highest ILB (540S) the germinal centers were gone and the cortex was nearly filled with reticuloendothelial cells. The medullary cords were thin and contained only pigment laden macrophages. The tracheobronchial lymph nodes from the other dogs had a few germinal centers but were very similar in other respects. No other significant lesions were found in other tissues.

DISCUSSION

While this study is in its early stages, several interesting observations and comparisons with results from the ^{144}Ce in fused clay dose-response studies can be made at this time. It is apparent that even though clinical evidence of pulmonary insufficiency has not been observed in the dose-response study prior to 4-5 months post-exposure, there are microscopic lesions of radiation pneumonitis present by 80 days post-exposure. Also, there is evidence of radiation pneumonitis and/or pulmonary fibrosis in dogs with ILBs as low as 28 $\mu\text{Ci/kg}$. In the dose-response study, dogs in this dose range have not generally had clinical evidence of pulmonary insufficiency and have died at much later times from pulmonary neoplasms. Right atrial lesions were seen at 128 days indicating that the heart lesions previously reported (1967-1968 Annual Report, LF-39, pp. 33-53) may well be a primary effect of irradiation of the heart and not secondary to pulmonary fibrosis as has previously been speculated.

The atrophy of the tracheobronchial lymph nodes occurs initially in the germinal centers and then with the paracortical and medullary cord lymphocytes. The cortex is replaced by proliferating reticuloendothelial cells. These changes are under way by 80 days post-exposure and are continuing at 128 days post-exposure.

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HEMATOLOGICAL EFFECTS OF INHALED ^{90}Y , ^{91}Y , ^{144}Ce AND ^{90}Sr FUSED CLAY IN BEAGLE DOGS

ABSTRACT

An attempt has been made to shed light on the etiology of the lymphopenia observed in dogs following inhalation of relatively insoluble forms of beta-emitting radionuclides. The lymphocyte response of dogs that inhaled graded initial lung burdens of either ^{90}Y , ^{91}Y , ^{144}Ce or ^{90}Sr in fused clay was analyzed over a period of one year post-inhalation exposure. The magnitude of lymphopenia was found to be related to the level of inhaled activity whereas the time at which depression and recovery occurred was found to correlate well with changes in dose rate associated with the four distinctive patterns of radiation exposure to the lung. This suggests that lymphopenia is due to direct radiation damage to circulating lymphocytes as they traverse through the pulmonary vasculature.

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INTRODUCTION

With the increased use of a variety of nuclear materials in science and industry, concern has developed over their toxicity when deposited in the human body. A number of laboratories around the country, therefore have been investigating the effects of various radionuclides deposited in the body of animals by injection,¹ ingestion,² or inhalation.³⁻⁵ Many such studies have been of a long-term nature with serial clinical evaluations being conducted over the total lifespan of the experimental subject. Beagle dogs have proven to be the most popular of large animals for these experiments; in part, because of the ease with which such clinical studies can be conducted, and in part, because of their fairly long median lifespan, approximately 13 years.

Hematologic studies have been an integral part of most of these experiments and it has come as no surprise that bone-seeking alpha- or beta-emitting radionuclides produce a profound effect on circulating elements that are derived from bone marrow precursors. The observed reduction in circulating lymphocytes, however, has been somewhat of a surprise in lieu of the fact that the major lymphoid structures of the body receive very little radiation exposure. Of perhaps even greater interest have been the reports by Clarke⁶ at Battelle-Northwest and Yuile⁷ at the University of Rochester, that sustained lymphopenia is also seen in dogs that have inhaled quite insoluble ^{239}Pu and ^{238}Pu dioxide particulates. Under these circumstances, the radiation dose is more or less restricted to lung and tracheobronchial lymph nodes with little dose to either marrow or large lymphoid structures at relatively early times. Although a number of mechanisms have been proposed to explain this curious phenomenon, none have been entirely satisfactory. A series of life-span studies being conducted with Beagle dogs exposed by inhalation to radionuclides in a relatively insoluble form at the Lovelace Foundation has provided an opportunity to investigate the manner in which both cumulative dose and dose rate to lungs influence the number of circulating blood corpuscular elements and to possibly shed some new light on this peculiar effect on lymphocytes.

MATERIALS AND METHODS

Data utilized in this study were taken from the lifespan and radiation dose pattern experiments in which Beagle dogs received a single nose-only exposure to graded levels of either ^{90}Y , ^{91}Y ,

^{144}Ce or ^{90}Sr fused clay. In all instances, the particle size distribution of the fused clay aerosol was polydisperse and best characterized by a log normal distribution with activity median aerodynamic diameters ranging from 0.8 to 2.8 μm and real diameters of less than 1 μm . The dogs were exposed at approximately 13 months of age, were then held singly for varying periods of time in metabolism cages and were subsequently maintained in pairs in a kennel. Whole-body counting and scans were performed to determine the initial lung burdens (ILB) for each animal.

One to three baseline hematological determinations were performed on each dog. These included RBC, hemoglobin, hematocrit, WBC, WBC differential, platelet count, sedimentation rate and calculations for absolute numbers of specific circulating WBC. During the first year post-inhalation exposure, samples were taken at 4, 8, 12, 16, 26, 39 and 52 weeks in all studies and additional weekly samples were taken from dogs in the ^{90}Y study during the first four weeks.

The post-inhalation exposure hematological values for each animal were compared with its own pre-exposure values in order to compute the percent of pre-exposure values for each time period. Mean values were then calculated for each group of living dogs at the various activity levels in all four studies over the first year of observation.

RESULTS

Figure 1 shows the hematologic response of a group of dogs which received the highest ILB of ^{144}Ce fused clay. This mean burden of 47 $\mu\text{Ci/kg}$ resulted in the death of 8 of 12 dogs by one year post-inhalation exposure. These data typify the response seen at other exposure levels in this and the other three studies. However, the magnitude and temporal occurrence of change were somewhat different, depending on the ILB achieved and the radionuclide inhaled.

In general, there was little change in RBC parameters and the reduction in platelets was observed only in groups of animals where death occurred prior to one year post-inhalation exposure. Granulocytes also were generally unaffected at lower activity levels in each study but increased to above normal values in groups where either pulmonary dysfunction or death occurred prior to one year. The broad line shows the most dramatic change, that is, a reduction in circulating lymphocytes. Generally the severity of lymphopenia appeared to be dependent on ILB and the sequence relative to depression and recovery related to radionuclide inhaled. The former is illustrated in Figure 2 which depicts changes in lymphocyte values occurring in five groups of dogs that received graded ILBs of ^{144}Ce in fused clay, with an effective half-life of retention in the lung on the order of 200 days. The control group is also plotted as a reference. Greater depression appears with the higher ILB, while the time at which maximum depression occurs, approximately 26 weeks, is relatively constant at the three highest levels.

Figure 1. Hematological response of dogs receiving a mean lung burden of 47 $\mu\text{Ci/kg}$ ^{144}Ce in fused clay.

Figure 2. Lymphocyte response of dogs exposed to ^{144}Ce in fused clay.

Figure 3. Lymphocyte response of dogs exposed to ^{90}Y in fused clay.

By contrast, dogs that received ^{90}Y in fused clay, effective half-life of retention in lung of 2.6 days, showed a somewhat different pattern of lymphocyte response (Fig. 3). Here the maximum depression occurred at 14 days post-inhalation exposure, and with but one exception, the values were approaching pre-exposure levels at 39 to 52 weeks post-exposure. The divergent point represents one surviving dog from the highest exposure group.

Figures 4 and 5 show similar data on animals that received either ^{91}Y or ^{90}Sr in fused clay, respectively. Dogs that received ^{91}Y , effective half-life for lung retention of 50 days, displayed a somewhat less dose-related depression and the maximum lymphopenia was observed to occur at from 12 to 39 weeks post-inhalation exposure, an intermediate time between that observed for dogs receiving ^{90}Y and ^{144}Ce in fused clay. Further, in all but one instance, recovery toward pre-exposure values was evident at 52 weeks. Dogs that received ^{90}Sr in fused clay, effective half-life for lung retention of over 400 days, demonstrated the most severe and protracted lymphopenia which again appeared to be dose-related. Further, there was little evidence of recovery at 52 weeks.

DISCUSSION

In general, dogs with the highest ILB in each study showed the greatest lymphopenia; however, convincing evidence for a clear dose response relationship was only apparent in those dogs receiving ^{144}Ce or ^{90}Sr in fused clay. Perhaps this is due to the limited number of animals available at each sample point or due to the spectrum of ILBs represented in each identified group. Unfortunately, with inhalation experiments, one cannot be as precise in delivering a desired dose as is the case of IV injection studies.

Of greater interest is the appearance of distinctive patterns of lymphocyte depression and recovery for each of the radionuclides under study. Several possibilities can be suggested to explain

Figure 4. Lymphocyte response of dogs exposed to ^{91}Y in fused clay.

the occurrence and pattern of lymphopenia under the experimental conditions existing in these four radionuclide toxicity studies: (1) lymphopenia may be one manifestation of a general stress response associated with radiation pneumonitis and developing pulmonary fibrosis; (2) depression may be due to radiation exposure to lymphocytes passing through the thoracic duct or to those in intrathoracic lymphoid structures; and (3) lymphocytes are damaged and destroyed due to direct radiation exposure occurring as they traverse through the capillaries of the lung.

Several bits of evidence tend to dispel the assertion that stress plays a significant role in this response: (1) If stress and an associated release of corticosteroids were responsible for lymphopenia one would consistently expect to find maximal depression at times when pulmonary fibrosis or radiation pneumonitis had so compromised respiratory function so as to lead to death of the animal. That such is not the case is illustrated in Figure 6. This graph compares the lymphocyte response of dogs that received 1800 rads to the thorax at a single exposure⁷ to

Figure 5. Lymphocyte response of dogs exposed to ^{90}Sr in fused clay.

Figure 6. Comparative lymphocyte response of dogs receiving external upper body x-irradiation to dogs inhaling ^{144}Ce in fused clay.

that of dogs that received the highest activity from a single inhalation exposure to ^{144}Ce in fused clay. In both instances, animals died as a result of severe pulmonary fibrosis and the gross and histologic pulmonary changes at death were quite similar. In the case of animals exposed to X-irradiation, the lymphocytes fell at early times and then rose and remained well above pre-exposure values at the time of death. However, with ^{144}Ce in fused clay there was a progressive fall and the lymphocyte counts remained depressed at the time of death from severe respiratory insufficiency. (2) Dougherty⁸ reports that profound sustained lymphopenia was observed in two dogs that had been adrenalectomized prior to administration of $2.8 \mu\text{Ci/kg}$ of ^{239}Pu .

Both of these observations lend support to the theory that radiation exposure to lymphocytes is the predominant factor in producing the observed lymphopenia. Although the magnitude of depression observed in the four studies with radionuclides inhaled in fused clay particles appears to be related to cumulative dose since the greater effect was found in animals that inhaled the higher activity levels for each radionuclide, the pattern of depression and recovery appears to be more dependent on radiation dose pattern or changes in dose rate. Since ^{90}Y , effective half-life in lung of 2.6 days, decays so rapidly, essentially all of the dose is delivered to the lung during the first 30 days and no radiation exposure is occurring at one year. ^{91}Y with its somewhat longer effective half-life in lung of 59 days results in somewhat more protracted exposure but at one year only 1% of the dose remains to be delivered.

By contrast, the percent of the infinite dose to be delivered after one year is 28% and 53%, respectively, for the longer lived radionuclides, ^{144}Ce and ^{90}Sr in fused clay. Thus, the patterns of lymphocyte depression seem to correlate well with the radiation dose patterns described for each of the four radionuclides. ^{90}Y in fused clay results in high early dose rate and early lymphocyte depression followed by recovery during the period when the lung is receiving an insignificant radiation dose. At the other end of the spectrum, ^{90}Sr in fused clay results in a slowly declining, fairly uniform dose rate over the first year and the lymphocytes follow a similar pattern with minimal evidence of recovery to that time. Further, the lymphocyte response pattern in animals exposed to the other two radionuclides (^{91}Y and ^{144}Ce) appeared as predicted on the basis of their radiation exposure patterns.

It is suggested that lymphocyte depression is due to direct radiation effects on circulating lymphocytes as they pass through or adjacent to intrathoracic structures. Since the number of cells at risk in pulmonary capillaries is greater than those in the thoracic duct, it appears that damage to blood-borne lymphocytes is largely responsible for the lymphopenia.

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THE RESPONSE OF CANINE LYMPHOCYTES TO MITOGENIC STIMULATION IN VITRO

ABSTRACT

Peripheral lymphopenia has been observed in dogs that inhaled relatively insoluble forms of radionuclides. It has been postulated that this phenomenon is due to direct exposure to circulating lymphocytes as they pass through the lung. Studies have been undertaken in order to establish whether one lymphoid cell population is selectively affected. Relative responses of thymus dependent and bursa dependent lymphocytes from canine peripheral blood have been evaluated by in vitro culture and stimulation with phytohemagglutinin and pokeweed mitogen. Results have been variable both between different dogs and within a single dog sampled on different days, although significant stimulation of canine lymphocytes with both compounds has been consistently observed.

PRINCIPAL INVESTIGATORS

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INTRODUCTION

Beagle dogs, which have been exposed by inhalation to a variety of alpha- and beta-emitting radionuclides, have been found to develop lymphopenia (see this report, pp. 216-220).^{1,2} The cause of this depression is not firmly established although it has been proposed that it is due to direct irradiation of lymphocytes circulating through the lung. One important question is whether the total lymphocyte population is depressed or whether a specific sub-population is selectively reduced. There is some evidence to suggest that the thymic-dependent lymphocyte population is selectively reduced after irradiation.³

Recently, it has been reported that functional differences between thymus-dependent (T cells) and bursa-dependent lymphocytes (B cells) may be reflected in their response to various stimulatory agents in vitro.⁴ Among these agents are two commonly used plant mitogens, phytohemagglutinin (PHA) and pokeweed mitogen (PWM). Recent work has demonstrated that PHA selectively stimulates T cells while PWM stimulates B cells predominantly but also a small proportion of T cells. B cells appear to be selectively stimulated by specific anti-globulin serum since they carry IgG determinants on their surface. Using the selective stimulatory capacities of these materials it is hoped to derive information bearing on the effect of irradiation from insoluble radionuclides in the lung on the two lymphocyte populations.

This paper summarizes the early results of stimulation of lymphocytes from normal dogs with PHA and PWM.

MATERIALS AND METHODS

The techniques described have been selected as optimal after an extensive series of pilot experiments which involved variations in amounts of chemicals and cells, times of harvesting, and methods.

Since peripheral blood is the necessary source of lymphocytes, several methods of lymphocyte separation were evaluated. The method giving the most consistent results was as follows: Heparinized whole blood was collected and mixed with 6% Dextran in saline at one-half the volume of the blood and allowed to stand for 60 minutes at 37°C. The suspension was then centrifuged for 20 minutes at 430 RPM and the leukocyte-rich plasma removed. It usually contained from 60 to 90%

lymphocytes of over 90% viability and 2 to 6% monocytes. This cell suspension was used directly for preparation of cultures. Lymphocytes in unseparated whole blood and lymphocytes purified by passing through a glass wool and glass bead column were also utilized for comparative purposes. All the blood utilized was drawn from healthy, adult (1-2 year old) Beagle dogs from the Lovelace colony.

Eagle's minimal essential medium was used for all cultures and supplemented with 100 units penicillin G/ml, 100 µg streptomycin sulfate/ml, and 10% homologous pooled, inactivated Beagle dog serum. The pH was adjusted to 7.3 ± 1.0 with NaHCO_3 .

Phytohemagglutinin-m (DIFCO Laboratories) and pokeweed mitogen (Grand Island Biological Co.) were the two plant mitogens utilized. Anti-dog IgG serum (Colorado Serum Co.) was also utilized. Both PHA-m and PWM were used at a concentration of 10 µl of stock solution, prepared as directed, per ml of culture medium. The anti-IgG serum was used at 10 and 50 µl per ml of medium. Saline in an equivalent amount was added to the control cultures.

Each culture consisted of 2 ml of medium containing 1×10^6 viable lymphocytes in unsealed screw cap culture tubes. After addition of the proper amount of mitogen the cultures were incubated at 37°C under a humidified 5% CO_2 atmosphere.

Twenty-four hours prior to termination of incubation, at 72 hours of culture time, 2 µCi ^3H -thymidine ($^3\text{H-T}$) was added to each tube. After 24 hours, at 96 hours of culture time, 8.7 µM cold thymidine was added to each tube and allowed to stand for 10 minutes. Each culture was then decanted into a Millipore filter apparatus. The cells were washed three times with Hank's balanced salt solution and then two times with cold 0.2 N aqueous perchloric acid. The filters were then transferred to a vial containing 2 ml of 1.0 N aqueous perchloric acid. This was incubated in a water bath for 20 minutes after attaining a temperature of 70°C. After cooling, a 1 ml sample was placed in 10 ml of toluene-based scintillation cocktail and analyzed in a liquid scintillation counter.

RESULTS

An extensive series of interrelated experiments was performed in order to determine optimal conditions for lymphocyte transformation. These are the conditions outlined in the previous section. Several methods of lymphocyte separation and DNA extraction were also used. Briefly, it was found that lymphocytes from sedimented plasma reacted more strongly to mitogenic substances than lymphocytes in whole blood without separation. It would appear that the high concentration of other cell types (mainly granulocytes and erythrocytes) interfered with the lymphocyte response. It was also found that high concentrations of mitogen (50 µl/ml) had an inhibitory effect on transformation. Under the optimal conditions, DNA synthesis in lymphocytes as measured by $^3\text{H-T}$ uptake, reached a consistent peak between 72 and 96 hours of culture. Before and after this time the response was somewhat more variable, especially in the latter case where the lymphocytes were thought to be undergoing secondary waves of division.

A series of experiments were run under optimal conditions to establish normal baseline values for 10 dogs, 5 male and 5 female. These dogs were sampled and cultured on four different occasions. The basic culture conditions were kept constant but three different DNA extraction methods were tried. Table 1 shows representative results in two of these dogs. The other 8 dogs had similar responses. In this particular experiment the dogs were cultured twice at a 3-day interval. All conditions were kept constant and the filter extraction method as outlined in the previous section was used. As can be readily seen there is a tremendous range within the five replicate cultures for any particular treatment although they were prepared from the same dog at the same time.

There also tends to be a great variation between the responses of the same dog on different days as well as between different dogs on the same day. The different extraction procedures did not appreciably alter these results. Cultures did not respond to anti-canine IgG serum in our tests.

An analysis of variance was done on the data for all 10 dogs from the experiment shown partially in Table 1. The data were viewed as a two-stage nested or hierarchic design for PHA, PWM, and controls, separately. Essentially, the two days were nested inside of dogs and separate analysis of variance was done for PHA, PWM and controls in order to test the two day's values against one another. Because of the nature of the variability in the data they were transformed with the natural logarithm to alleviate the non-normality before analysis of variance was performed. Significant changes at the 99% confidence level were found with the F-test in all cases except between the control cultures for different dogs. That is, there were statistically significant differences between stimulated cultures between different dogs on the same day and between the same dogs on different days. To show the differences between the stimulated and control cultures the data were analyzed as a three-stage nested design with treatments within days within dogs. This showed a significant difference at the 99% level for both PHA and PWM.

Table 1
DNA Synthesis in Canine Lymphocytes After Mitogenic Stimulation:
Representative Results on 2 of 10 Dogs

Dog No.	Treatment	Day I [†]		Day II [†]	
		CPM- ³ H (mean and range) *	Stim. Index**	CPM- ³ H (mean and range) *	Stim. Index**
482A (Male)	PHA	8580 (3720 - 18600)	22	17200 (15200 - 22000)	114
	PWM	3840 (1170 - 10800)	10	9160 (5190 - 16600)	61
	Control	394 (138 - 1170)	--	151 (115 - 179)	--
494S (Female)	PHA	612 (129 - 2220)	2	4190 (444 - 17400)	36
	PWM	1921 (134 - 6110)	7	2020 (474 - 6910)	18
	Control	286 (144 - 626)	--	115 (77 - 134)	--

† Replicate experiments run on each dog at a 3-day interval.

* Mean and range are of 5 replicate cultures for each treatment.

** Stimulation Index = $\frac{\text{mean of treated cultures}}{\text{mean of control cultures}}$;

Stim. Index for all 10 dogs on both days for PHA ranged from 1-141 and for PWM from 1-134

DISCUSSION

The preliminary study reported here was designed to evaluate the normal response to mitogenic substances of peripheral blood lymphocytes from the Beagle dog. The ability to evaluate such data quantitatively is important in interpreting lymphocyte response after irradiation. As is evident, quantitative evaluation of the data reported would not be meaningful for our purposes. Such erratic results are not unique and have been reported by others working with lymphocyte transformation studies.⁵ On the other hand, there is consistently significant stimulation by the plant mitogens used. Such data could be useful if this was considered as an all-or-none phenomenon and the magnitude of response was ignored.

Further attempts are under way to reduce the variability within this system by altering both culture and extraction procedures. Furthermore, other tests such as rosette formation,³ are being evaluated as possible alternate methods to derive the desired information.

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HEMATOLOGIC CHANGES IN DOGS WITH RADIATION-INDUCED HEMANGIOSARCOMAS

ABSTRACT

In dogs exposed to $^{90}\text{SrCl}_2$, $^{144}\text{CeCl}_3$, ^{90}Sr fused clay and ^{144}Ce fused clay, the most common neoplasms found have been hemangiosarcomas. These have been bone-related in the $^{90}\text{SrCl}_2$ study, liver-related in the $^{144}\text{CeCl}_3$ study, and mostly lung-related in the ^{90}Sr and ^{144}Ce fused clay studies. Most of these dogs, (20/23) have shown an anemia at least terminally. The genesis of this anemia is discussed. Although there are significant differences between groups with respect to radiation dose and dose pattern to bone marrow, there do not appear to be any differences in the ability of the marrow to respond to hematopoietic stimuli. Microangiopathic hemolytic mechanisms might play a role in causing the anemias but firm evidence for these occurring is lacking. Most of the dogs with anemia had either large or multiple vascular neoplasms or severe hemorrhage or a combination of these. Hemorrhage or sequestration of blood in the tumors could account for most of the anemic changes seen.

PRINCIPAL INVESTIGATORS

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INTRODUCTION

Malignant neoplasms of endothelial origin have been the most common tumors found in our colony after inhalation exposure of Beagle dogs to the soluble $^{90}\text{SrCl}_2$ and $^{144}\text{CeCl}_3$ and relatively insoluble ^{90}Sr and ^{144}Ce in fused clay (1966 through 1971 Annual Reports, LF-38, LF-39, LF-41, LF-43 and LF-44). Most of these dogs (20 of 23) with hemangiosarcomas have shown an anemia at least in the terminal stages. Since the radionuclides used resulted in a variety of radiation doses and dose patterns to bone and bone marrow, it was of interest to evaluate the possible genesis of anemia and the associated response of the animal's hematopoietic tissue.

Three main factors must be considered as possibly playing a role in causing the anemia: (1) radiation damage to the bone marrow resulting in decreased capacity for regenerative response, (2) severe hemorrhage and/or sequestration of blood associated with the vascular tumors, and (3) the occurrence of microangiopathic hemolytic anemia associated with the neoplasm itself.

MATERIALS AND METHODS

In the studies discussed here, dogs were exposed to graded levels of $^{90}\text{SrCl}_2$, $^{144}\text{CeCl}_3$, ^{90}Sr fused clay, or ^{144}Ce fused clay and maintained for lifespan observation. (See this report, pp. 128 - 136, pp. 137 - 139, pp. 177 - 188 and pp. 157 - 166.) Serial observations of body burden and excretion of the radionuclide, clinical condition, serum chemical, hematologic and microbiological parameters have been made and are being continued on all survivors. The hematologic parameters have been sampled on a quarterly basis, except in animals that are clinically ill, in which case samples are taken more frequently as needed. Complete necropsies have been performed on all dogs that were sacrificed, died or were euthanized. Samples of femoral shaft marrow, femoral head, vertebra and ribs were fixed in either Zenker's solution or 10% neutral buffered formalin. Fresh marrow smears were made from these bones and stained with Wright-Leishman's stain. The fixed marrow samples were decalcified if necessary, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin. For bone marrow evaluation, undecalcified femoral shaft sections were used wherever available. In several cases vertebral marrow was used due to lack

of an adequate femoral shaft sample. The marrows were evaluated as to the presence or absence of hyperplasia, the degree of hyperplasia, and whether this was predominantly erythrocytic, granulocytic or both. Differential counts on the bone marrow smears were used to supplement the histopathologic findings. Only those dogs having hemangiosarcomas were evaluated, although some of these dogs had other neoplasms as well.

RESULTS

Table 1 is a summary of the pertinent findings in all of the dogs which had hemangiosarcomas in these studies. Following inhalation of $^{90}\text{SrCl}_2$ all the hemangiosarcomas seen arose within, or in close relation to, bone. All four hemangiosarcomas in the $^{144}\text{CeCl}_3$ study were hepatic in origin, and all of these dogs died because of intra-abdominal hemorrhage from the tumors. In the cases of ^{90}Sr and ^{144}Ce in fused clay, 8 of the 9 vascular neoplasms appeared to arise in the lung and one, which was disseminated in nature, may have originated in lung or have been bone-related, arising in the ribs. In a number of instances there was another neoplasm as well as the hemangiosarcoma. In two cases noted in Table 1, the hemangiosarcomas were small and insignificant with respect to the overall clinical picture.

The differing radiation doses to skeleton between the groups are shown in Table 1. The radiation dose patterns between the four groups also differ significantly. The animals exposed to $^{90}\text{SrCl}_2$ have the highest skeletal radiation doses and, due to the long effective half-life, this dose is still being delivered at the time of death or euthanasia. In the case of $^{144}\text{CeCl}_3$, the skeletal doses are lower, but still quite significant, and the potential infinite radiation dose has been delivered by the time of death. In the animals exposed to ^{90}Sr or ^{144}Ce in fused clay, the total radiation doses to skeleton overall are low, about 1/20th of the $^{144}\text{CeCl}_3$ doses, although the doses to the ribs overlying the lung may be quite significant (over 5000 rads locally).

Table 1
Hematologic Findings in Dogs with Radiation-Induced Hemangiosarcomas

Isotope	Dog No.	Other Neoplasms	Death Days Post-Exposure	Rads to Skeleton	Bone Marrow Hyperplasia			Terminal Hematology		
					Severity	Granulocytic	Erythrocytic	Hematocrit/%	Platelets/mm ³	WBC/mm ³
$^{90}\text{SrCl}_2$	7B	-	928	15000	++++	+	+	15	10,000	38,000
	9D	-	1316	13000	++++	+	+	19	50,000	11,600
	10B	Osteosarcoma*	2034	22000	++++	+	-	20	145,000	30,600
	12E	Osteosarcoma	2380	14000	+	+	+	22	25,000	7,200
	12F	-	1046	10000	++	+	+	29	85,000	12,600
	22A	-	2247	13000	+++	+	-	21	125,000	61,300
	26F	Osteosarcoma*	1938	18000	-	-	-	46	485,000	5,700
	158E	-	927	17000	+++	+	-	54	165,000	11,300
	160B	Fibrosarcoma	864	17000	+++	+	+	30	250,000	22,700
	160C	-	1142	9900	++	-	+	14	50,000	12,000
$^{144}\text{CeCl}_3$	62E	-	1735	4600	++	-	+	22	100,000	18,600
	199E	-	1808	7500	++++	-	+	No Terminal Sample**		
	200E	-	1783	4700	++++	+	-	18	50,000	36,000
	201C	-	1760	6400	++++	+	-	16	50,000	7,200
^{90}Sr Fused Clay	355A	-	715	220	++++	+	+	13	300,000	28,500
	396T	-	718	290	++	-	+	40	190,000	29,900
	398D	-	644	270	++++	+	-	31	80,000	62,000
^{144}Ce Fused Clay	211D	-	1185	330	+	+	-	35	100,000	8,800
	228A	-	1318	440	++	+	+	41	345,000	11,700
	297S	Fibrosarcoma	750	440	+++	+	-	30	240,000	57,800
	310T	-	765	250	++++	-	+	23	320,000	19,500
	315A	Bronchiolo-Alveolar Ca	916	390	++	+	-	60	280,000	16,900
	330U	-	1077	440	++	+	-	43	405,000	11,400

* Major neoplasm in this case

** This dog is assumed to have had an anemia since there was severe intraabdominal hemorrhage ante-mortem.

Table 1 outlines the bone marrow changes for each dog as well as showing the hematocrit, thrombocyte count and total leukocyte count for each just prior to death or euthanasia. All of the dogs except one show some degree of hyperplasia of the bone marrow. It is readily seen that there appears to be no correlation between the radiation dose to the bone marrow and the severity of the bone marrow response. There also appears to be no correlation between the degree of anemia seen terminally and the predominant cell type in the bone marrow. It should be remembered that in most of these animals the bone marrow hyperplasia was generalized and the table only indicates which cell series was increased beyond normal proportions. All of the animals with anemia did have some degree of erythroid hyperplasia. The only area where there appears to be some difference between groups is in the thrombocyte counts. The dogs exposed to the soluble radionuclides show a definite tendency toward severe thrombocytopenia with 11 of 13 animals having counts well below the normal range. Of the animals exposed to the relatively insoluble radionuclides only 3 of 9 show any degree of thrombocyte reduction and this is not as severe as in the former group. Histologically, megakaryocytes were present in adequate numbers in all of these animals.

Two of the three animals that did not show anemia had relatively small tumors. All but one of the remaining animals, all of which had anemia, had either large vascular neoplasms, multiple metastatic lesions, severe hemorrhage or a combination of these findings. Thrombosis was common within some of these tumors.

DISCUSSION

Of the two soluble radionuclides utilized, $^{90}\text{SrCl}_2$ is translocated rapidly to bone after inhalation and thereafter has an effective half-life in the skeleton of more than 5 years. Cerium-144 is translocated from the lung at a somewhat slower rate with the majority of the radionuclide going to the liver (50%) and less to the skeleton (40%) where it is retained with an effective half-life approaching the physical half-life of 285 days. Thus, after inhalation of $^{90}\text{SrCl}_2$ the skeleton receives virtually the entire radiation dose and after inhalation of $^{144}\text{CeCl}_3$, lung, liver and skeleton all receive significant radiation doses. The same radionuclides, ^{90}Sr and ^{144}Ce , when contained in fused clay particles, are relatively insoluble in the lung after inhalation exposure. A small percentage of the ^{90}Sr and ^{144}Ce are dissolved and translocated to skeleton or liver and skeleton, respectively, resulting in a relatively small radiation dose to these organs.

One main question which must be addressed is whether the levels of chronic bone marrow irradiation in the $^{90}\text{SrCl}_2$ or $^{144}\text{CeCl}_3$ dogs has in any way compromised or changed the ability of the marrow to respond to stress. That there is some effect of the radiation on the bone marrow is known since in both these studies, there have been animals dying within 2 months post-exposure with bone marrow aplasia. These dogs received high dose rates to bone marrow during this early period. Radiation damage to bone marrow has been reflected at later times post-exposure by development of two cases of myeloproliferative disorder at about 1 1/2 years and 7 years post-exposure in the $^{90}\text{SrCl}_2$ study and 3 cases of myeloproliferative disorder at about 5 years post-exposure in the $^{144}\text{CeCl}_3$ study. The skeletal doses in the animals with myeloproliferative disorders were comparable to, or lower than, those in the dogs developing hemangiosarcomas in these studies. In the groups exposed to ^{90}Sr or ^{144}Ce in fused clay there has been no evidence to date of any effect on the bone marrow as a whole, nor would this be expected from the doses involved.

With this background, it might be expected that the marrow in the dogs exposed to $^{90}\text{SrCl}_2$ or $^{144}\text{CeCl}_3$ could be damaged and not able to respond normally. If this was the case there should be significant differences between the animals exposed to soluble and "insoluble" radionuclide forms. With respect to the severity of overall hyperplastic response and the type of response found, the two groups appear no different. This would seem to indicate that the levels of irradiation of the

bone marrow in the dogs exposed to relatively soluble radionuclides has not affected the ability of the marrow to respond to either erythropoietic or leukopoietic stimuli. In this light, the tendency of these dogs to develop thrombocytopenia terminally is puzzling. It is possible that this is due to irradiation and although megakaryocytes did not appear decreased in the marrow in these cases the functional capacity of the megakaryocytes present was not assessed. In the animals which had severe hemorrhage at terminus, such as the $^{144}\text{CeCl}_3$ dogs, the thrombocytopenia can be explained on that basis alone. From the foregoing it would seem that inability of the bone marrow to respond to hematopoietic stimuli is not the cause of, and probably does not contribute to, the anemia seen in association with hemangiosarcomas.

As was noted previously, all but one of the animals showing anemia did have either a significant degree of hemorrhage and/or large or multiple vascular neoplasms. Blood loss from the hemorrhage and sequestration of blood within the large volume of tumor tissue must be considered a major possibility in the etiology of the anemias. Conversely, one animal did have a severe anemia in the presence of only a small hemangiomatous lesion and no significant hemorrhage. In this case, some other mechanism must have been at work since there was a good erythroid response in the marrow.

The third mechanism which must be considered is occurrence of microangiopathic hemolytic anemia in these dogs. There is a well-established association between benign and malignant hemangiomatous neoplasms and hematological disorders, including anemia and thrombocytopenia.^{1,2} One current theory is that there may be mechanical damage to the red blood cells occurring during circulation through vascular channels in the tumor. This may be associated with circulation through a fibrin network. That there is increased intravascular coagulation, at least locally in the tumors, is suggested by the association of thrombocytopenia with the anemia in many reported cases. The occurrence of thrombocytopenia and intra-tumor vascular thrombi in some of these dogs might support this theory. The contribution, particularly at earlier stages of tumor growth, of a microangiopathic hemolytic process to the overall anemia cannot be stated at this time, however, it does not appear necessary to invoke this mechanism to account for the terminal hematologic changes in the majority of our cases. Hemorrhage and sequestration of blood in the large vascular tumors could account for these findings.

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EFFECT OF AGING ON THE FREQUENCY OF METAPHASE CHROMOSOME ABERRATIONS IN THE LIVER OF THE CHINESE HAMSTER

ABSTRACT

One hundred and forty-eight Chinese hamsters, ranging in age from 70 to 1100 days of age, were partially hepatectomized and the frequency of metaphase chromosome aberrations in the liver determined. When the percent of cells with abnormal chromosomes was related to age, there was a linear increase according to the equation $Y = 0.44 + 0.016 X$; where Y is the percent of cells abnormal, X is age in days, 0.44 is the intercept of the line and 0.016 is the aberration coefficient. These results confirm previously reported data in the literature which indicate an increase in aberration frequency with time. The aberration frequency does not seem to be inversely related to life span, however, as the slope of our age-response data for hamsters is equal to that observed for long-lived guinea pigs.

PRINCIPAL INVESTIGATORS

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INTRODUCTION

The somatic mutation theory of aging states that the number of unrepaired somatic mutations increases in the cells of the body with time. This increase in mutation frequency compromises the cell function by producing abnormal proteins and other abnormal cell products. These mutations are thought to be the underlying cause of aging, a theory that has gained some impetus through the work of Curtis¹ showing a positive correlation between the life span of several species and the rate at which the animals accumulated chromosome aberrations in the liver. Chromosome aberrations are thought to be an indicator of somatic mutations. The aberrations in Curtis' studies were measured in anaphase-telophase cells of the liver following stimulation for cell division by treatment with CCl_4 . Curtis² also demonstrated that the amount of radiation-induced life-shortening from whole-body radiation exposure was related to dose rate, dose pattern and type of exposure in the same way as the frequency of chromosome aberrations was related to these same dose parameters. This evidence has been used to suggest a cause-effect relationship between aging and the observed frequency of chromosome aberrations and thus somatic mutations.

Alexander³ points out two observations which seem to disagree with a direct relationship between somatic mutations and agings as follows: (1) the phenomenon of radiation-induced life-shortening is substantially different from normal aging, and (2) mutagenic chemicals which are not cytotoxic do not shorten life span.

The present study was designed to measure aging effects on metaphase rather than anaphase-telophase chromosomes in the Chinese hamster which has a life span⁴ slightly longer than the mice used by Curtis. These data will be useful in long-term radiation studies to establish a baseline for the change in aberration frequency for control animals as they age.

METHODS

The study was performed with 73 previously unassigned Chinese hamsters and 75 which had served as controls in other long-term radiation studies. These animals were sacrificed at ages ranging from 74 to 1100 days. Two days prior to sacrifice, the animals were partially-hepatectomized to stimulate cell division. At fifty hours post-partial-hepatectomy, they were injected

with 0.3 ml of 1% colchicine and sacrificed four hours later. The methods employed in cell preparation and scoring the chromosomes have been previously described.⁵ All slides were coded and scored without a knowledge of the age of the animals.

RESULTS AND DISCUSSION

Table 1 summarizes the data on relationship between age and chromosome aberration frequency. Additional data are needed at 1100 days where only two animals and 74 cells were scored. Data are usually minimal at this age since it is approximately the maximum life span of the Chinese hamster.

The most common types of aberrations scored were dicentrics and isochromatid deletions, both of which are scoreable in anaphase-telophase cells. Dicentrics are recorded as bridges and the isochromatid deletions as fragments. The efficiency of scoring the two aberration types in metaphase is thought to be higher than it would be in the later stages of the cell cycle since about 50% of the time the centromeres in dicentrics separate properly and do not form bridges. Isochromatid deletions, which do not have a centromere, occasionally will not form visible fragments at anaphase. The aberration types which can be recorded only at metaphase and not in anaphase-telophase cells are symmetrical chromosome exchanges. These did not occur at a high enough frequency to influence the shape or slope of the dose response curve.

The percent abnormal cells are plotted against age in Figure 1 and compared with data from Curtis.¹ The figure shows that the aberration frequency in young Chinese hamsters is less than that seen in mice, dogs or guinea pigs. This low value was thought to be related to the method of scoring. Metaphase aberration scoring seems to have less "noise" or "background" than anaphase-telophase scoring. The comparison of interest is the rate of increase of the chromosome damage with age. The slope of the Chinese hamster age-response line is less than observed in mice, greater than that for dogs, and almost equal to that seen in the guinea pigs. Since these data were not collected at the same stage of the cell cycle as those of Curtis, it may not be possible to relate them directly. If, however, the rate of aging and life span are directly correlated with the observed chromosome damage, regardless of the stage of the cell cycle where the damage is recorded, the slopes of the aberration frequency, plotted against age would decrease in the following order: mice, Chinese hamsters, guinea pigs and dogs. This order was observed with one exception, the relationship of the hamster to the guinea pig. The guinea pig has a mean survival time of six years¹ as compared to the 3-year life span of hamsters.⁴ If the spontaneous aberration rate is inversely proportioned to the life span,¹ the slope of the age-aberration curve for the hamster should be twice that seen for guinea pigs.

Although these data seem to point out a possible exception to the somatic mutation theory of aging, they do indicate that aberration frequency increases with increasing age regardless of the stage of the cell cycle stage utilized. In addition, the data provide a control baseline for comparison when long-term radiation or other studies are carried out in which age may also influence the aberration frequency measured.

Table 1

The Effect of Age on the Frequency and Types of Metaphase Chromosome Aberrations in the Liver of the Chinese Hamster

<u>Age</u>	<u>Number of Animals</u>	<u>Cells Total</u>	<u>Normal</u>	<u>Abnormal</u>	<u>ICTD</u> ¹	<u>Dic</u> ²	<u>Ring</u>	<u>Cs Exch</u> ³	<u>CTD</u> ⁴	<u>Other</u>	<u>Total Aberrations</u>	<u>Aberrations/Cell</u>	<u>Abnormal Cells/Total Cells</u>
74	2	85	83	2	2						2	0.02	0.02
95	2	39	39									0	0
100	2	96	95									0	0
106	22	821	802	17	8	5		1	3		17	0.02	0.02
115	19	635	611	20	15	3		1	4		23	0.04	0.03
120	5	181	173	4	4						4	0.02	0.02
142	20	762	754	7	4	2		1			7	0.009	0.009
220	8	370	358	12	8	3		1			12	0.03	0.03
300	11	240	223	8	3	4			1		8	0.03	0.03
400	10	260	240	12	5	3		3	1		12	0.05	0.05
460	7	296	279	13	5	7			2		14	0.05	0.04
500	5	138	122	13	6	2	1	2	2		13	0.09	0.09
600	4	134	120	12	3	5	1	1	1	1 CT Exch ⁵	12	0.09	0.09
700	8	254	212	33	15	17		3	2		37	0.15	0.15
800	5	164	145	19	8	7		3	2		20	0.12	0.12
820	4	158	128	27	15	9	2	3		1 CT Exch	30	0.19	0.17
900	5	207	101	20	16	7		2	1		26	0.13	0.10
1000	7	194	158	31	14	16		4			34	0.18	0.16
1100	2	74	59	12	9	3	1	1			14	0.19	0.16

¹Isochromatid deletion; ²Dicentric; ³Symmetrical chromosome exchange; ⁴Chromatid deletion; ⁵Chromatid exchange.

Figure 1. The effect of age on the frequency of metaphase chromosome aberrations in the Chinese hamster liver.

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THE EFFECT OF $^{239}\text{PuO}_2$ PARTICLE SIZE ON THE FREQUENCY
OF CHROMOSOME ABERRATIONS IN THE LIVER CELLS
OF THE CHINESE HAMSTER

ABSTRACT

Fifty-four Chinese hamsters received intravenous injections of $10^{-3} \mu\text{Ci } ^{239}\text{Pu/gm}$ body weight as monodisperse particles of three sizes: 0.84, 0.44 and 0.12 μm . Eighteen additional animals were injected with saline as controls. The animals were partially-hepatectomized in three groups of 24 each at 4, 13 and 40 days to stimulate cell division to permit the recording of metaphase chromosome damage in the liver. Particle size had no effect on the average number of aberrations produced per cell when related to average dose. The aberration frequency increased as a function of dose and time with an aberration coefficient of 1.6×10^{-3} aberrations/cell/rad and an intercept of 0.05 aberrations/cell. This slope was about one-fourth that observed following ^{241}Am citrate injection. When related to local dose, the efficiency of producing aberrations decreased as particle size increased. There was no significant difference in the distribution of aberrations among cells for the different particle sizes. However, at each sampling time, animals injected with the largest particles had cells with the largest number of aberrations. The distribution of aberrations from $^{239}\text{PuO}_2$ showed a higher proportion of cells in the normal class and more cells with extreme damage as compared to ^{241}Am citrate.

PRINCIPAL INVESTIGATORS

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INTRODUCTION

One of the potential hazards associated with nuclear industry operations is the release of alpha-emitting radionuclides in an airborne form with subsequent inhalation. These particles can be deposited in the deep lung and result in intense localized radiation to a small volume of tissue surrounding the particle. Thus, risk estimates from such exposures must consider the local dose to cells around the particle as well as average dose to the whole organ.

The current experiment was designed, using the chromosome aberration frequency in the liver as a model system, to measure the biological damage following exposure to monodisperse $^{239}\text{PuO}_2$ particles of three different sizes and a constant total activity. The liver system makes it possible to expose cells at various times and record the damage done to the chromosomes with little interference from cell division or particle redistribution. In addition, the distribution of damage within the cell population may be determined.

METHODS

The experimental design for this report is shown in Table 1. Seventy-two Chinese hamsters of both sexes and 90 to 120 days old received intravenous injections of monodisperse $^{239}\text{PuO}_2$ particles or saline. The particles were 0.84, 0.44 and 0.12 μm real size with a geometric standard deviation of about 1.09. The production and characterization of these particles have been reported

(see this report, pp. 21 -28). All animals were projected to receive 1×10^{-3} μCi $^{239}\text{PuO}_2$ /gm body weight and were partially-hepatectomized in three groups of 24 each at 4, 13 and 40 days post-injection.

Because of the time required for the partial-hepatectomy and injection procedures, the experiment was divided into two equal blocks. Thus, only 36 animals were injected at one time and 12 were partially-hepatectomized on a given day.

A modified method of Holland *et al.*¹ was used for the intravenous injection of $^{239}\text{PuO}_2$. A Luer stub adapter was attached to a 1 cc syringe and an 18-inch 0.023 I.D. polyethylene tube. A 23-gauge needle was cut off at the hub and inserted into the other end of the tubing. The animals were anesthetized with methoxyflurane and a small longitudinal cut made to expose the right jugular sinus. The needle was inserted through the leading edge of the pectoralis muscle and into the jugular vein. The solution was injected, the needle withdrawn, and local pressure applied to prevent bleeding. The incision was then closed with a suture.

Our success rate on this operation was about 85%; bleeding and improper needle placement were the major causes of failure. When an animal was not successfully injected, it was replaced.

At the time of hepatectomy and at sacrifice, liver samples were removed for radioanalysis, autoradiography and pathological examination. At sacrifice, an additional sample was taken for cytogenetic scoring. Methods for preparing and scoring the chromosomes have been previously described.²

The alpha activity was determined on weighed liver samples. Samples were dissolved in 5 ml of 7 M HNO_3 over low heat, 250°C , for 20 minutes. The solution was then evaporated to dryness and 30% H_2O_2 added a few drops at a time to bleach the sample. This was repeated, if necessary, until the material was colorless or faint yellow. The material was redissolved in 3 ml of (3M) HNO_3 and 13 ml of aquasol added. A Packard Tri-Carb Liquid Scintillation Counter was used to detect the activity present. This activity in $\mu\text{Ci/gm}$ was used in subsequent average dose calculations.

RESULTS AND DISCUSSION

The whole-body and liver retention of the particles have been reported (see this report, pp. 61 - 64). Whole-body retention decreased to about 95% of the initial body burden by 40 days and the liver contained almost a constant 90% of the sacrifice body burden at all sacrifice times. The average dose to the liver was calculated by measuring the specific activity in $\mu\text{Ci/gm}$ at sacrifice and extrapolated to time of injection assuming a uniform retention. The specific activity was assumed to decrease linearly as the liver enlarged following partial-hepatectomy and the dose between partial-hepatectomy and sacrifice calculated accordingly.

In addition to the average doses calculated from the liver retention data, micro doses for a sphere of tissue around each particle were also calculated. These doses involved the following assumptions:

Table 1
Experimental Design for Determining the Frequency and Distribution of Chromosome Damage in the Liver Following Injection of Monodispersed $^{239}\text{PuO}_2$

Particle Size (μm) *	Time of Hepatectomy		
	4	13	40
Control	6**	6	6
0.12	6	6	6
0.44	6	6	6
0.84	6	6	6

* All treated animals injected with 1×10^{-3} μCi $^{239}\text{PuO}_2$ /gm body weight.

** Number of animals to be divided into two blocks.

1. There was no overlap of fields between particles.
2. The range of the alpha particles in liver tissue is 40 μm .
3. The dose rate is uniform in the volume of tissue around each particle.
4. There was no change in activity/particle.
5. The specific activity of the particles was $5.4 \times 10^{-2} \mu\text{Ci/gm}$ and the density 10.5 gm/cm^2 .

Dose calculation parameters shown in Table 2 were derived using the general dose equation:

$$\text{Dose rate} = 51.2 (\bar{E}_{\text{Mev}}) (\mu\text{Ci/gm}) (f_A)$$

where

$$\bar{E} = 5.13 \text{ Mev}$$

$$f_A \text{ (fraction of energy absorbed)} = 100\%$$

$\mu\text{Ci/gm}$ = specific activity derived by dividing the weight of the tissue irradiated by the activity of the individual particle for local dose and the total activity divided by total liver weight for average dose

Table 2 indicates that with the largest particle size, only 2.5% of the liver cells are exposed to a very high (190 rads/day) dose rate whereas it is assumed that the smallest particles irradiate the total liver and local dose rate and total dose rate are considered to be equal. The cumulative local dose to 42 days varied by a factor of four while the average dose was constant.

Autoradiographs of the liver indicate that, in addition to the radiation overlap, biological processes further complicated the system. By counting the number of tracks originating from each star, several particles were found in a single location. This could have been caused by a single Kupffer cell taking up multiple particles or by particle aggregation prior to or following injection. This tendency seemed marked with the smaller particles while the two larger-sized particles showing more uniform number of tracks per star. Examples of the types of stars seen are shown in Figure 1a, b and c. The marked difference in the number of tracks per star noted in Figure 1a was characteristic of the 0.12 μm particle size. At the longer time interval, 40 days, the star track distribution seemed to be a little less uniform than seen in the four-day samples.

The average aberration frequency in animals injected with all three particle sizes increased as a function of time and average dose (Fig. 2). No change was noted as a function of particle size. There were some large between-animal differences noted in the aberration frequency for animals

Table 2
Dose Parameters Following Deposition of Monodispersed $^{239}\text{PuO}_2$ Particles
in the Liver of the Chinese Hamster

Particle Size (μm)	Activity/Particle (μCi)	Number of Particles/Liver	Volume of Liver Irradiated	% of Liver Irradiated	Local Dose Rate (rad/day)	Local Dose (rads)	42-day Exposure Average Dose (rads)
0.84	1.93×10^{-7}	1.40×10^5	$3.76 \times 10^{-2} \text{ cm}^3$	2.5	190.0	7790	190
0.44	2.77×10^{-8}	9.75×10^5	$2.61 \times 10^{-1} \text{ cm}^3$	17.4	27.0	1107	190
0.12	5.62×10^{-10}	4.77×10^7	1.5 cm^3 *	100*	4.7*	190*	190

* Significant overlap of radiation fields would occur with the large number of particles resulting in an assumed irradiation of total liver; the local dose was calculated assuming homogeneous irradiation.

Figure 1. Autoradiographs of Chinese hamster liver following intravenous injection of monodisperse $^{239}\text{PuO}_2$ particles: (a) $0.12\ \mu\text{m}$ particles of $^{239}\text{PuO}_2$ 40 days post-injection with a 3-day autoradiographic exposure; (b) $0.44\ \mu\text{m}$ particles of $^{239}\text{PuO}_2$ 40 days post-injection with a 3 day autoradiographic exposure; and (c) $0.84\ \mu\text{m}$ particles of $^{239}\text{PuO}_2$ 40 days post-injection with a 2 day autoradiographic exposure.

injected with the same particle size and activity and exposed over the same time. The basis for these differences was not found in the doses as they were relatively constant for a given treatment. The average dose and response was greatest for the largest particle size but could be fit to the same dose response curve. A line was fit to the data by a linear least squares method and had a slope of 1.6×10^{-3} aberrations/cell/rad and an intercept of 0.05 aberrations/cell. This slope is about one-fourth that seen following injection with ^{241}Am citrate.³ There are several potential explanations for this difference. The radiation dose delivered very close to the particle may be wasted, as far as aberration scoring is concerned, by producing cells that cannot divide and be scored. Also, the exposed cells may be delayed in progressing through the cell cycle and result in a population of more normal cells for scoring at 54 hours post-partial-hepatectomy. This differential delay combined with differential response to the stimulus of partial-hepatectomy could explain some of the between-animal variability noted. Finally, the particles were taken up by the Kupffer or littoral cells which have been shown to divide after the peak of division in the parenchymal cells.⁴ All of these explanations suggest that the use of a single sampling time may be suspect in deriving an index of damage which is representative of the liver as a whole. The extent of these problems is being determined with additional experimentation, using multiple sampling intervals.

Figure 2. The frequency of metaphase chromosome aberrations in the Chinese hamster liver following intravenous injection of $^{239}\text{PuO}_2$ particles as a function of average radiation dose.

Of more interest than the mean aberration frequency related to mean dose should be the localized dose related to the aberration frequency (Fig. 3). When theoretical local doses are used, the larger the particle size, the less effective is the treatment in the production of aberrations per rad of local dose. This observation again emphasizes the need for additional experiments to determine other endpoints such as cell death, differential mitotic lag and localized liver cell hyperplasia.

The distribution of aberrations among cells (the percent of the cells that have 0, 1, 2, etc. aberrations at 42 days post-injection) is shown in Figure 4. For comparative purposes, the distribution of aberrations following ^{241}Am citrate injection over the same time interval is also included. There was no significant difference in the distribution of aberrations as a function of particle size. Therefore, all particle sizes were grouped in the figure. However, at each sampling time the animals injected with the largest particle size contained a few cells with the largest number of aberrations. The real differences were seen when the distribution of aberrations following ^{241}Am citrate was compared with that following $^{239}\text{PuO}_2$ particle injections. The two ^{241}Am groups were selected first for a comparable level of injected activity and dose and the second group because of a comparable aberration frequency. When the dose was comparable, the average aberration frequency in ^{241}Am citrate treated animals was 1.0 aberration/cell and that in the $^{239}\text{PuO}_2$ was only 0.30. In spite of the lower total aberration frequency, however, the $^{239}\text{PuO}_2$ treated animals had liver cells with as high as nine abnormalities in a single cell while the ^{241}Am treated liver cells had only as high as four aberrations. When comparing animals treated with ^{241}Am citrate which had the same average aberration/cell frequency as those treated with the $^{239}\text{PuO}_2$ particles, no cell contained more than two aberrations. This can again be related to the nine aberrations seen in the $^{239}\text{PuO}_2$ treated cells. These differences in the distribution of aberrations among cells may be explained on the basis of micro dose distribution. The ^{241}Am exposed the liver cells to a rather uniform alpha dose whereas the $^{239}\text{PuO}_2$ particles had cells around them that received rather large doses while most of the tissue received no dose. If a representative

Figure 3. The frequency of metaphase aberrations in the Chinese hamster liver following intravenous injection of $^{239}\text{PuO}_2$ particles related to the calculated local dose delivered to a sphere of tissue 40 μm in radius.

Figure 4. The distribution of chromosome aberrations among the cells of the Chinese hamster liver 42 days after injection with ^{241}Am citrate or $^{239}\text{PuO}_2$ particles.

irradiated cell population could be scored, then it would be possible to relate this population to the local dose and determine whether the aberration/cell/rad value obtained is comparable for different particle sizes. This will not be possible without additional cell scoring to characterize a larger population of damaged cells.

This experiment demonstrates biologically the effect of microdose distribution using chromosome aberrations as an endpoint. The differences observed in absolute amount of damage per rad of exposure require further study on the effect of localized irradiation on interphase cell death, mitotic inhibition, mitotic delay, and hyperplasia stimulated by damage before a true representation of damage can be related to micro dose distribution. Further work on the effect of particle size and number also needs to be done using other biological endpoints and other tissues, especially the lung which has the major risk of particle deposition.

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THE EFFECT OF ^{144}Ce - ^{144}Pr AND PARTIAL-HEPATECTOMY
ON THE PRODUCTION OF LIVER TUMORS IN THE CHINESE HAMSTER

ABSTRACT

Six hundred and fifty (650) Chinese hamsters of similar age and genetic background have been introduced into an experiment to determine the combined effect of partial-hepatectomy and intraperitoneal injection of ^{144}Ce - ^{144}Pr citrate on the production of liver tumors. These animals were given a variety of treatments with activity levels from 0.036 to 0.25 μCi ^{144}Ce /gm body weight and had either no further treatments or were partially-hepatectomized at either 20 days before or 20 or 200 days after the start of the experiment. Controls were subjected to the same surgical schedule. To date, 50 animals have been sacrificed at 600 days and 92 animals at 700 days post-injection. The dose to the liver at sacrifice has ranged from 1,600 to 15,000 rads and produced from 0.50 to 4.70 chromosome aberrations/cell. The criteria for classification of hyperplastic changes in the liver and hepatocellular neoplasms is discussed in detail. In the animals sacrificed at 600 days, only one hepatocellular neoplasm has been found. Animals allowed to live another 100 days developed nine hepatocellular neoplasms and 2 fibrosarcomas of the liver. Of the hepatocellular neoplasms, 6 developed in hepatectomized and 3 in non-hepatectomized hamsters. The number of animals available for study, to date, does not allow conclusions as to the effectiveness of partial hepatectomy as a tumor-inducing treatment.

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INTRODUCTION

Many physical and chemical factors which alone are ineffective or only mildly effective in producing tumors greatly augment the production of tumors when combined with a known carcinogen.

The classical explanation for this observation has been that certain substances or phenomena are tumor "initiators" and others are "promoters", implying that cancer production is a multi-step process. To bring these terms into a cellular framework, Cole and Nowell¹ defined "initiator" substances or phenomena as those that produce genetic change and "promoters" as those that allow the cells to express these alterations through cell division. Finally, a process of clone formation and cellular selection acts and the malignancy is produced. Curtis *et al.*² tested this principle by combining the hepatotoxic agent, CCl_4 , with acute neutron or gamma irradiation and showed an increase in the number of liver tumors induced by combining the treatments over that number observed following either treatment administered alone.

In the present study the work of Curtis² which involved acute radiation exposures has been extended by using ^{144}Ce as a source of protracted exposure to the liver with the hope that the results could be more easily related to protracted environmental radiation exposures. Further, in the present study partial-hepatectomy was used to stimulate the liver cells to divide rather than CCl_4 since CCl_4 has been shown to cause cell death, necrosis and cancer which are not produced by the surgical procedure. This research should provide a further test of the hypothesis that cell division is an important prerequisite in the development of radiation-induced neoplasia.

METHODS

A total of six hundred and fifty (650) Chinese hamsters raised in our laboratory with known genetic background and 12 to 16 weeks old were divided into eleven experimental groups (Table 1).

Table 1
Experimental Design for Determining the Interaction
Between ^{144}Ce - ^{144}Pr and Partial-Hepatectomy in Producing Liver Tumors

Group	Number of Animals	Activity ^{144}Ce - ^{144}Pr $\mu\text{Ci/gm}$	Surgical Schedule	Sacrifice Time*
1	90	0	0	700 days
2	60	0	30 animals partial-hepatectomy at 3 months and 30 animals partial-hepatectomy at 9 months of age	700 days
3	60	0.036	None	700 days
4	60	0.10	None	700 days
5	40	0.25	None	700 days
6	60	0.036	Partial-hepatectomy 20 days pre-injection	700 days
7	60	0.05	Partial-hepatectomy	700 days
8	60	0.10	20 days post-injection	700 days
9	60	0.04	Partial-hepatectomy	700 days
10	60	0.12	200 days post-injection	700 days
11	40	0.25		

* The first block of 50 animals was sacrificed at 600 days.

The number of animals in each group is shown on the table and the experimental treatments are as follows: Group 1 were intraperitoneally injected with citrate and served as controls. Group 2 animals were partial-hepatectomy controls with half undergoing the surgery at 20 days and half at 200 days post-citrate injection. Groups 3, 4 and 5 were ^{144}Ce -injected only at activities at 0.036, 0.10 and 0.25 $\mu\text{Ci } ^{144}\text{Ce/gm}$ of body weight, respectively. Group 6 had the partial-hepatectomy 20 days prior to injection with 0.036 $\mu\text{Ci } ^{144}\text{Ce/gm}$ body weight. Groups 7 and 8 received 0.05 and 0.10 μCi of $^{144}\text{Ce/gm}$ and were partially-hepatectomized 20 days post-injection. Groups 9, 10 and 11 were injected with 0.04, 0.12 and 0.25 $\mu\text{Ci/gm}$, respectively, and underwent the surgical procedure 200 days post-injection.

In order to have enough animals of similar background available for the experiment and because of time factors involving hepatectomy and sacrifice procedures the experiment was divided into 12 blocks. These blocks were introduced into the experiment over a three-year time span thus animals from only the first three blocks are available for analysis at the present time. Animals in the first block were sacrificed at 600 days post-injection and found to have a low tumor incidence. All subsequent animals were scheduled for sacrifice at 700 days post-injection. No animals injected with 0.1 or 0.12 $\mu\text{Ci } ^{144}\text{Ce/gm}$ body weight have as yet been sacrificed. To date, 50 animals sacrificed at 600 days and 92 animals sacrificed at 700 days are available for study and are included in this report.

A sample of liver was taken at hepatectomy and another at sacrifice to determine the ^{144}Ce content for use in combination with retention data³ to derive an estimate of the radiation dose to the liver of each animal.

In order to evaluate the frequency of chromosome aberrations 5 additional animals were added to each treatment group. In groups which received ^{144}Ce and did not have a hepatectomy as

part of the treatment, chromosome aberrations were scored by doing a partial-hepatectomy two days prior to sacrifice (1967-1968 Annual Report, LF-39, pp. 174-178.) In each treatment group which did include hepatectomy, five additional animals were hepatectomized at 20 or 200 days and chromosomes scored. In these groups another 5 animals were hepatectomized at 20 or 200 days, and then subjected to a second hepatectomy two days prior to sacrifice, after which chromosomes were scored. In the controls 5 animals were used for chromosome scoring at 20 days, 5 at 200 days, and 5 at the time of sacrifice.

Complete necropsies were performed on all animals that were sacrificed or died. Gross evaluation was made as to the number, size and appearance of lesions in the liver. Any abnormalities in other tissues were also recorded. The entire livers and samples of other major organs including heart, lung, spleen, kidney, adrenal, ovary, uterus, testes, sternum, vertebra and femur were fixed in 10% neutral buffered formalin. After fixation, the liver lobes were separated and cut into 1 to 2 mm sections. Any nodules found were processed for histopathological evaluation. In some livers, nodules were so numerous that only a representative sample could be sectioned as it would have been necessary to serial section the entire liver to see all the nodules. Samples of lung, spleen, kidney, adrenal and bone were also routinely processed for histopathology. Any other major lesions noted grossly were also processed. Tissues were embedded in paraffin, sectioned at 5 μ m and routinely stained with hematoxylin and eosin (H & E). Special stains were utilized as required.

RESULTS AND DISCUSSION

Survival:

The survival data for the Chinese hamsters, when death due to surgical procedures is eliminated, is shown in Figure 1. There is little difference between the control and the ^{144}Ce -treated animals with the exception of those injected with 0.25 $\mu\text{Ci/gm}$ where the median survival is 560 days for animals with no partial-hepatectomy and 650 days if the animals had the surgery. The lower level animals had the same survival with or without the surgery. The control and low level animals had survival times that were not significantly different.

Figure 1. Cumulative survival of Chinese hamsters following ^{144}Ce - ^{144}Pr injection and partial hepatectomy.

Pathology - Gross:

Many of the livers examined at necropsy were found to contain grossly visible nodules. These lesions were more frequent and larger in the irradiated than in the unirradiated animals. The nodules in a single liver were often too numerous to count. In the control and lower level irradiated groups (0.036, 0.04 and 0.05 $\mu\text{Ci/gm}$), there were usually nodular lesions of varying size in a relatively normal-looking liver. In the high level animals (0.25 $\mu\text{Ci/gm}$), the nodular lesions were often found along with evidence of severe hepatic destruction. The gross appearance of the nodules varied considerably. The majority ranged in size from less than 1 mm to several cm in diameter, were soft, pink to tan and bulging, and bore a close resemblance to normal liver parenchyma. A smaller number of lesions were just as variable in size, but were firmer, gray to white, and sharply demarcated from the surrounding liver. All gradations between these two types of lesions were found, in all treatments and in the controls. The livers from the high level animals had greatly varied appearance. Hemorrhage and necrosis with architectural distortion were prominent. Two specific abnormalities were occasionally found in this group. One was a cystic, hemorrhagic lesion of the parenchyma, which in several instances led to the death of the animal from intra-abdominal hemorrhage. The other was also a cystic, nodular lesion, but in this case the cysts were thin walled and filled with a green, serous, bile-like fluid.

The only other gross lesions commonly found were multiple, gray to tan, nodular, irregular foci in the lungs and small, pitted kidneys. In a few instances, the focal lung lesions tended to coalesce involving most or all of a lung lobe.

Pathology - Microscopic:

Hyperplastic and degenerative lesions were seen in most of the livers of animals in this study, including the controls. As a general rule, these changes were much more pronounced in the irradiated than in the control groups. The degenerative changes in the controls and lower level groups were generally mild and consisted of foci of hydropic and fatty degeneration. Occasionally foci of frank necrosis were found but these were not frequent or prominent. The degenerative changes were, by far, most pronounced in the 0.25 $\mu\text{Ci/gm}$ animals. Here there was often frank necrosis or evidence of pre-existing necrosis leading to hepatic fibrosis or telangiectasis. Hemorrhagic cysts were occasionally found, as noted grossly. These were usually in areas of severe degeneration and were lined by large, atypical cells of undetermined origin. Fibroblasts in the fibrotic areas were often atypical, large and sometimes bizarre in appearance. The atypical changes seen in both cell types were presumed to be radiation-induced, but they were not felt to be neoplastic.

The serous cysts noted grossly had the general appearance of bile ducts microscopically. These lesions consisted of from a few to many tubular structures, often having a papillary configuration, and lined by a single layer of uniform epithelial cells. These cells ranged from cuboidal in the small ducts to flattened and attenuated in the large cystic lesions. The cells themselves usually had the appearance of normal bile duct epithelium, but in some areas were indistinguishable from hepatic parenchymal cells. There appeared to be transitional forms between the two cell types. Although these lesions were often indistinguishable from bile duct adenomas histologically, it was not felt that they represented actual neoplastic transformation. A more likely possibility is that these lesions represent a regenerative response to injury. This is supported by the fact that they always occurred in livers with significant degenerative and fibrotic changes and seemed to arise in association with portal triads. All stages of lesions from just a few dilated bile ducts, through early, small, obviously proliferative foci, to large, cystic adenoma-like nodules were found. The above findings suggested that these lesions may be the result of blockage of an interlobular bile duct, with subsequent dilation of ductules and sinusoids with bile,

and finally metaplasia of hepatic parenchymal cells toward bile duct epithelium. There is considerable evidence that hepatic cells are capable of differentiating into cells resembling bile duct epithelium.⁴ Besides these nodular lesions bile duct hyperplasia was common in the irradiated livers.

The solid nodular lesions seen in the livers of these animals were unquestionably of hepatic parenchymal cell origin. These nodules had as varied a histological appearance as they did a gross appearance. There was no correlation between the gross appearance and the ultimate histopathological classification. These lesions ranged from very benign appearing nodules of hyperplasia up through very bizarre, anaplastic cellular masses, including one unequivocal hepatocellular carcinoma. They were divided into two categories, nodular hyperplasia and hepatocellular neoplasia, for evaluation of the effects of irradiation and hepatectomy. It is recognized that this classification is artificial as there was no clear dividing line between the two groups. It is impossible to determine when the hyperplastic lesions actually become neoplastic on a histopathological basis. The appearance of these lesions and the criteria used to separate the two categories are described below.

At one end of the scale the nodules consisted of normal appearing liver cells which were often somewhat larger than usual. These nodules usually retained a normal architectural pattern of sinusoids and central vein and often involved only one lobule. These very benign appearing lesions did, at times, coalesce to form larger masses grossly resembling neoplasms. They remained, however, histologically benign and were composed of typical liver cells. Portal triads were characteristic findings in these lesions. Frequently, there would be atypical cellular changes in the hyperplastic nodules. In these the hepatic parenchymal cells would often be greatly enlarged, 10 or more times normal size. Most commonly the majority of this increased cell size was accounted for by cytoplasmic enlargement. The cytoplasm was usually quite eosinophilic and often vacuolated. The nuclei were also enlarged but generally had a benign appearance with a single nucleolus and a smooth, round nuclear membrane. Sometimes, whole nodules were composed of these atypical hyperplastic cells but the basic hepatic architecture was evident.

Some of these atypical nodules contained cells which were quite bizarre. These cells had a great variation in both cytoplasmic and nuclear size and appearance. In these cells the nuclei often occupied more than 50% of the cell volume and were often irregular and hyperchromatic with clumped chromatin and multiple or abnormally prominent nucleoli. Although many of these cells were quite poorly differentiated they were still recognizable as hepatic in origin. The nodules composed entirely of the latter cell types fit all the usual cytologic criteria for neoplasia (Figs. 2 and 3). At the far end of the scale there was one lesion that fit all the histopathological criteria for an hepatocellular carcinoma of the trabecular type (Fig. 4). The cells were quite bizarre with deeply eosinophilic cytoplasm and a high nuclear to cytoplasmic ratio. The nuclei were pleomorphic, hyperchromatic and often contained multiple, large nucleoli. The cells themselves were fairly uniform, however, compared to those seen in the atypical nodules described. Vascular invasion and pulmonary metastases were found.

Separation of hyperplastic and neoplastic lesions was performed on the basis of the following criteria. In order to be classified as a neoplasm the nodules must have had no normal architecture, that is, there had to be a disorganized growth pattern. Along with this, nuclear rather than cytoplasmic changes had to predominate in the cytologic picture. The majority of the cells had to show nuclear pleomorphism, have high nuclear to cytoplasmic ratios, and show hyperchromatism and multiple or abnormal nucleoli. If both of these changes, abnormal growth pattern and predominantly nuclear atypism, were not present, the mass was termed hyperplastic. If a particular nodule was borderline in its characteristics, a conservative route was chosen and it was classed

Figure 2. Relatively benign appearing hepatocellular neoplasm in an animal injected with 0.25 μ Ci $^{144}\text{Ce/gm}$ as well as nuclear variation and no discernible architectural organization. H + E stain. X 335

Figure 3. Hepatocellular neoplasm with a greater degree of nuclear atypism and relatively less cytoplasmic variation. This animal was injected with 0.4 μ Ci $^{144}\text{Ce/gm}$. This lesion had cytologic features consistent with malignancy, but did not metastasize. H + E stain. X 335

Figure 4. Hepatocellular carcinoma in an animal injected with 0.04 μ Ci $^{144}\text{Ce/gm}$. The tumor had a characteristic trabecular growth pattern and, although nuclear pleomorphism was marked, the cell sizes tended to be relatively uniform when compared to the other hepatocellular neoplasms. H + E stain. X 335

as hyperplastic rather than neoplastic. Size was not considered a pertinent factor in this judgment. Except for the hepatocellular carcinoma, metastases were not demonstrated in any of the other neoplasms. For this reason, they have been termed hepatocellular neoplasms since proof of their malignancy is lacking.

Aside from the hepatocellular neoplasms, two other neoplasms were found in the liver. Two 0.25 $\mu\text{Ci/gm}$ animals had locally invasive, well-differentiated fibrosarcomas of the liver. Metastases were not found in either case.

No other lesions were found that definitely correlated with the irradiation exposure. The lung lesions were basically focal granulomas which, on occasion, were so severe as to be diffuse granulomatous pneumonias. The kidney lesions were found to be nephrosclerosis. A number of other neoplasms were identified including a myelogenous leukemia, a reticulum cell sarcoma, 2 uterine adenocarcinomas, a uterine leiomyosarcoma, a fibrosarcoma of the thoracic wall, 2 adrenal cortical adenomas and a hemangiosarcoma of the maxillary region. These neoplasms were found in both control and treated animals.

General Discussion:

A summary of the results for animals that were sacrificed at 700 days post-injection is seen in Table 2. The table includes the treatments, number of animals, radiation dose to the liver at partial-hepatectomy and sacrifice, calculated chromosome aberration frequency at the time of hepatectomy and sacrifice, and the number and types of liver tumors observed. The animals sacrificed at 600 days are not included in the table since there was only one hepatocellular neoplasm in the fifty animals examined. This tumor was found in a hepatectomized animal that was injected with 0.25 μCi $^{144}\text{Ce/gm}$ and died at 585 days post-injection.

The doses recorded in the table at hepatectomy and sacrifice were calculated by measuring the activity/gm of liver and extrapolating this value back to the day of injection using a pre-determined retention curve for ^{144}Ce in the liver of the Chinese hamster.³ This results in an integrated activity per gram. Using this value and assuming that 60% of the energy released from the beta particle(s) was absorbed in the liver⁵ and that the average energy of the ^{144}Ce - ^{144}Pr was 1.29 Mev, the dose to the liver of each animal was calculated. The dose in the table is the average of the individual calculated doses.

In animals injected with 0.25 $\mu\text{Ci/gm}$, the dose calculated at sacrifice may be low since there were many deaths in this group which may have acted as a selective force against animals with higher body burdens. Survival was high in all other groups and dose calculations should represent the real dose except for cases with nodular hyperplasia or tumors. Both hyperplastic and neoplastic conditions dilute the concentration of ^{144}Ce in the tissue and will make the dose estimated lower than that received.

The aberration/cell values recorded on the table were calculated using the previously derived slope of 3.1×10^{-4} aberrations/cell/rad times the dose in rads. This slope has been checked on a total of 29 animals which were included for this purpose as outlined and found to be representative of the data. The two possible exceptions were found with animals that were partially-hepatectomized twice or received 0.25 μCi $^{144}\text{Ce/gm}$. In both cases the observed aberration frequency was lower than the calculated frequency. Cell division was thought to play a role in both of these cases. On one hand the partial-hepatectomy creates massive mitotic activity which selects against damaged mitotic cells. On the other hand, the dose delivered by the ^{144}Ce at the 0.25 μCi level causes cell death, necrosis and subsequent hyperplasia, again selecting against damaged cells.

Table 2
The Effect of ^{144}Ce - ^{144}Pr and Partial Hepatectomy on the Production
of Liver Tumors in the Chinese Hamster at 700 Days Post-Injection

Activity Injected	Hepatectomy Time (days)	Number of Animals	Dose at Partial- Hepatectomy (rads)	Dose at Sacrifice (rads)	Aberrations/cell at Partial- Hepatectomy	Aberrations/cell at Sacrifice	Liver Tumors	
							Hepatocellular	Fibrosarcoma
0.25	+200	3	9,000	14,700	2.80	4.60	1	0
0.25	-	2	-	15,000	-	4.70	0	2
0.05	+ 20	8	370	1,600	0.12	0.50	1	0
0.04	+200	12	2,600	3,300	0.80	1.02	4	0
0.036	-	15	-	2,900	-	0.90	3	0
0.036	- 20	18	-	2,500	-	0.78	0	0
Control	+ 20	7	-	-	-	-	0	0
Control	+200	8	-	-	-	-	0	0
Control	-	19	-	-	-	-	0	0

These data illustrate that at the time of sacrifice all of the ^{144}Ce -treated animals had aberration frequencies greater than 0.50 aberrations/cell with the highest two treatments showing more than 4.0 aberrations/cell. In spite of this severe chromosome damage, the liver tumor incidence remained relatively low. The liver tumor incidence does not seem to be directly related to the aberration frequency or the radiation dose. The highest tumor frequency occurred in animals which had aberration frequency of 1.0 and an average dose of about 3,000 rads. This lack of a direct correlation could be related to an overkill effect both at the cellular and whole organism level. The higher doses did result in a large proportion of the animals dying at early times before they could develop liver tumors. It is of interest to note that in animals which had partial-hepatectomy after ^{144}Ce injection, there were six hepatocellular tumors in 23 animals while those that had ^{144}Ce only had three hepatocellular tumors in 17 animals. This difference is not significant considering the number of animals involved but may be indicative of a trend. An additional comparison can be made between animals partially-hepatectomized at 20 and 200 days post-injection and injected with 0.5 and 0.4 μCi ^{144}Ce , respectively. In the first group there is one tumor in eight animals while the second group has 4 tumors in 12 animals. This would suggest that the 200-day partial-hepatectomy was more effective in producing liver tumors than the 20-day operation. However, the dose received by the animals partially-hepatectomized at 20 days was about one-half seen in the animals that had surgery at 200 days.

The tumor incidence is related to the ^{144}Ce radiation exposure as there have been 11 liver-related tumors in the 58 animals injected with ^{144}Ce and no tumors found in 34 control animals, regardless of surgical history. This tumor incidence (19%) is lower than observed in mice by Curtis² following treatment with CCl_4 and 500 rads of x-rays or 250 rads of fission neutrons where he noted that 48% of the animals in either treatment had liver tumors. Those doses were much lower than the 1,600 to 15,000 rads delivered in the present study. This dose difference may imply that protracted irradiation is less effective in producing tumors than acute exposures, however, a species difference, or a difference in the method of scoring tumors may also be involved. Furthermore, CCl_4 acts as a necrotizing and inflammatory-inducing agent by itself and rather than simply stimulating cell division it might act as a co-carcinogen.

As the additional animals and treatment levels are evaluated it will be possible to make more definite statements about the relationships which exist among the variables; chromosome aberrations, radiation dose and partial-hepatectomy, and tumor incidence. To date, the results indicate that protracted ^{144}Ce exposures which produce about 1.0 aberration per cell and deliver over 1600 rads cause an increase in liver tumor frequency over that observed in the controls.

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THE UPTAKE OF RADIOACTIVE LABELED PROLINE IN SYRIAN HAMSTERS

ABSTRACT

The uptake of radioactive labeled proline was assessed as a means of evaluating collagen biosynthesis in lung tissue. Radioactive proline was injected intraperitoneally into Syrian hamsters which were sacrificed at various times post-injection. Radioactive uptake into pulmonary tissue, the radioactive hydroxyproline content of this tissue, and radioactive uptake into various connective tissue fractions were determined. Carbon-14 whole-body retention and $^{14}\text{CO}_2$ exhalation were also determined in some animals. No appreciable incorporation was observed in the crude acid mucopolysaccharide or the insoluble elastin fractions. Both soluble and insoluble collagen fractions had appreciable amounts of radioactivity incorporated into them. Little difference in proline incorporation into either soluble or insoluble collagen was noted between day 1 and day 2 post-injection; however, by day 7, appreciable decreases in the radioactivity of the soluble collagen uptake were noted. Radioactive proline incorporation into pulmonary tissue paralleled to a degree the incorporation into the soluble and insoluble collagen fraction. A gradual fall observed in radioactive proline concentration in pulmonary tissue to day 3, suggests that proline was incorporated into proteins with relatively short biological half-lives. Very little radioactive hydroxyproline was present in pulmonary hamster tissue. These data lend confidence to the use of this procedure to monitor collagen biosynthesis and to more adequately describe the pathogenesis of pulmonary fibrosis.

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INTRODUCTION

Pulmonary fibrosis has been shown to be one of the more important debilitating sequelae of pulmonary radiation exposure. This appears to be the case whether the radiation dose is delivered from external sources or from inhaled radionuclides. Although sequential changes can be demonstrated histologically, such studies do not provide the type of information required to define the mechanisms responsible for the development of pulmonary fibrosis nor do they provide other than a qualitative appraisal of the degree of total lung involvement.

Radiation-induced pulmonary fibrosis could result from either an increased rate of collagen biosynthesis, a decreased rate of collagen breakdown or a combination of both. However, the long biological half-life of some tissue collagens suggests that increased biosynthesis may be the more important mechanism. Therefore, this study was initiated to determine if autoradiographic and biochemical estimations of radioactive proline uptake into Syrian hamster pulmonary tissue could be used to describe normal collagen biosynthesis and to determine the minimal injection activity levels of radiolabeled proline required to obtain useful results. In addition, the time of maximum uptake into pulmonary tissue of labeled proline was determined.

MATERIALS AND METHODS

Clinically normal Syrian hamsters, ranging in age from 160 to 170 days and weighing 100 to 150 grams, were utilized for this study. They were intraperitoneally injected with either 40 μCi (1/2 ml) of 3, 4-tritiated-L-proline (N), specific activity 4.8 Ci/mMol, or with 10 μCi (1/2 ml) uniformly labeled ^{14}C -L-proline, 265 mCi/mMol. Stock solutions were diluted to these total activities using normal saline. Thirty-two animals (8 per group) were injected with either solutions which contained 12×10^6 or 20×10^6 dpm ^{14}C proline or 77×10^6 or 78×10^6 dpm ^3H proline. Ra-

radioactive counting standards were obtained by delivering 1/2 ml injectate from a syringe into a volumetric flask and diluting to 50 ml with 2% ethanol in normal saline to minimize radiolysis.

Following injection of ^{14}C proline into animals, the extent of oxidation to $^{14}\text{CO}_2$ was determined in glass metabolism cages by trapping expired CO_2 in columns containing 200 ml of 1 M ethanolamine.¹ Ethanolamine aliquots (1 ml) were taken for radioactive analyses by liquid scintillation at 30-minute intervals for approximately 3 to 5 hours post-injection or until a level plateau was reached. In addition, a 24-hour urine collection was taken on these animals, and aliquots were counted by liquid scintillation.

At specified times, the animals were intraperitoneally anesthetized with approximately 0.2 ml/100 gm body weight of Nembutal and the abdominal aorta was severed to attain an adequate bleed-out. Equal numbers of hamsters were processed for connective tissue and histopathology measurements. The lung, liver, spleen, kidney, bladder and skin were saved for histopathological evaluation. Samples of lung, liver, kidney, spleen, bladder and skin were fixed in 10% buffered formalin. They were embedded in paraffin and sectioned at 6 micra. The slides were prepared for autoradiography by dipping in NTB3 (Kodak) nuclear tract emulsion and were exposed for 30, 60 and 90 days. The lungs, liver and a skin sample were taken from animals utilized for connective tissue biochemical studies. A blood sample and the whole-body remains were saved to determine, at a later date, the whole-body retention of ^{14}C and ^3H -proline in the respective animals. All tissues were stored at -20 or -75°C pending analysis. Tissues were minced, frozen in a mortar immersed in an approximate -20°C dry ice-Dowanol (1-methoxy-2-propanol) bath, pulverized to a fine powder and lyophilized. Subsequent to lyophilization, either hydrolysis or extraction was performed on the lyophilized tissue. Acid hydrolysis was performed on tissue in a manner previously described, with slight modification.² After hydrolysis, vacuum evaporation and dilution to 5 mg/ml concentration, differential extraction of radioactive proline and hydroxyproline was effected by previously reported methods.³

Recovery of proline and hydroxyproline was determined by adding radioactive proline or hydroxyproline to lyophilized hamster pulmonary tissue. The mixture was hydrolyzed and extraction recovery determined as a fraction of hydrolysate count. Calculated recovery percentages were compared in samples corrected for quenching effect and in the same samples not corrected. Counting efficiencies, when performed, were determined by the addition of either ^{14}C or ^3H toluene as an added internal standard.

Products of this extraction procedure were counted by liquid scintillation. Two scintillation cocktail formulae were utilized. The first cocktail was composed of 33% Triton X-100, 67% toluene, 5.5 gPPO/l, and 0.1 gPOPOP/l. This cocktail (15 ml) was utilized to emulsify hydrolysates and injectate counting standards. The second cocktail was designed to count toluene extract from the radioactive proline-hydroxyproline extraction procedure. This cocktail was composed of 50% Triton X-100, 50% toluene, 8.25 gPPO/l and 0.15 gPOPOP/l. Ten ml of this cocktail was then added to 5 ml of a toluene extract. Water volume added to either cocktail brought total aqueous material to 1.5 ml in each sample. Thus, more uniform counting efficiencies in this system could be expected.

A modification of a reported method (1970-1971 Annual Report, LF-44, pp. 263-271) was used to fractionate samples of lyophilized pulmonary tissue into the following connective tissue constituents: soluble and insoluble collagen, insoluble elastin, and crude acid mucopolysaccharides. Residues of these fractions were either suspended in dilute acid, distilled deionized water or were hydrolyzed. Fractions were counted by liquid scintillation and the radioactive quantity in each fraction noted.

RESULTS

Recovery of ^{14}C proline added to hamster pulmonary tissue was consistent (37-46%). No effect of count rate upon recovery was seen over a tenfold cpm range. Recovery percentages of ^{14}C hydroxyproline added to hamster tissue varied (14-35%). The recoveries determined as controls for this study were somewhat more uniform (25-35%); the count rate over the same tenfold difference did not effect recovery of ^{14}C hydroxyproline either. Recovery of ^{14}C proline and ^{14}C hydroxyproline (in some different samples) were similar whether corrected or not corrected for counting efficiency (^{14}C proline 38.9% and 38.9% no correction, 37.4% and 33.9% corrected; ^{14}C hydroxyproline 14 and 18% no correction, 13 and 17% corrected). Thus, it would appear that acceptable recovery percentages may be calculated without correction for quenching in these carefully controlled cases.

Approximately 50% (45-61% range; 8 animals) of the injected ^{14}C proline was given off as CO_2 ; approximately 45% (36-55% range; 2 animals) was present in the whole body remains at sacrifice; an insignificant amount, approximately 0.3-0.4%, was estimated as present in the blood (2 animals); 2-5% was estimated to be in the urine (8 animals); 5% was present in the liver of one animal and 0.4-0.6% was in the lung (2 animals). Approximately 1000-1500 dpm of the total lung dpm were estimated to be due to blood remaining in the lung of these two animals (1969-1970 Annual Report, LF-43, pp. 112-115). The assumption was made that 3% of the estimated blood volume was present in the lung, a value intermediate between exsanguinated and non-exsanguinated animals, since complete exsanguination is difficult to achieve in the Syrian hamster. This whole-body retention data was somewhat variable.

More than half of the radioactive uptake into pulmonary tissue appeared to be incorporated into non-collagenous proteins. Small to negligible amounts of proline uptake were noted into the crude acid mucopolysaccharide fraction and the insoluble elastin fraction of the lung connective tissue. However, readily measurable quantities of radioactive uptake were obtained in both the soluble and the insoluble collagen fractions (Table 1). There seemed to be little difference in the quantities

Table 1
Uptake of Radioactive Labeled Proline into Various Pulmonary
Connective Tissue Constituents in an Approximate 50 mg Sample

Days Post Injection	Isotope	Activity Hydrolysate (cpm $\times 10^3$)	Activity Insoluble Collagen (cpm)	Activity Soluble Collagen (cpm)	Activity Insoluble Elastin (cpm)	Activity Crude Acid Muco- polysaccharide (cpm)
1 (2)	^{14}C	13-17	1800-4000	2300-2900	130-240	30-50
2 (1)	^{14}C	15	2100	2500	120	40
3 (1)	^{14}C	11	2300	2200	160	40
7 (2)	^{14}C	8	1800-2600	1000	100-150	20-40
Blank*		N. D.	< 100	< 10	N. D.	< 100
1 (2)	^3H	19-21	3400	3100-4200	100-150	20-30
2 (2)	^3H	14-23	3900-4400	2200-3700	90-120	10-20
3 (2)	^3H	9-16	3600-4200	2000-2700	90-100	20
7 (2)	^3H	9-12	1200-1500	1500-2200	60- 70	20-30
Blank*		N. D.	< 200	< 10	< 150	< 100

N. D. = not determined

< = less than

() = number of animals

* Unlabeled hamster tissue run as a control

incorporated into the two collagen fractions at day 1 and day 2 post-injection. The uptake on day 3 was variable, and by day 7 appreciable reductions in total uptake into soluble collagen and insoluble collagen incorporated had been noted; an exception was insoluble collagen (^{14}C , day 7). Similar trends were noted in proline uptake into pulmonary tissue (Table 1). Such values were uncorrected for quenching effect (which was consistent) but were corrected for recovery percentage. Greater than 66% of the pulmonary ^{14}C radioactivity was present as proline and ^{14}C hydroxyproline, indicating the stability of the label. However, ^3H proline or hydroxyproline apparently composed more than 100% of the ^3H pulmonary radioactivity. This is probably due to the difference in counting efficiency between hydrolyzed pulmonary tissue and extracted amino acid products (Table 2). Tissue ^{14}C hydroxyproline, in most cases, was somewhat variable and rather low (Table 2). The radioactive compounds could not be localized to individual cells such as fibroblasts in any of the tissues examined by autoradiography. A higher diffuse autoradiographic image was present in the liver than in the other tissues.

Table 2
Uptake of Proline and Hydroxyproline Content in Hamster
Pulmonary Tissue as a Function of Time Post-Injection

Days Post Injection	Isotope	Total Lung Uptake (1000 cpm)	Lung Hydrolysate (cpm/5 mg)*	Proline (cpm/5 mg)*	Hydroxyproline (cpm/5 mg)*	Amount Present As Proline Or Hydroxy- proline (%)
1 (2)	^{14}C	49-64	1300-1700	1200	40-90	76- 90
2 (2)	^{14}C	40-43	1300-1500	1200-1300	40-90	85-100
3 (1)	^{14}C	35	1100	800	40	75
7 (2)	^{14}C	24-27	800	500- 700	10-40	67- 91
1 (2)	^3H	66-68	1900-2100	2400-2600	0-50	123-124
2 (2)	^3H	58-67	1400-2300	1800-2700	10-15	117-125
3 (2)	^3H	46-77	900-1500	1100-1400	0-20	89-124
7 (2)	^3H	29-35	900-1200	1000-1300	0	113-114

() = number of animals

* = dry weight

DISCUSSION

More than half of the radioactive uptake into pulmonary tissue appeared to be incorporated into non-collagenous proteins. Moreover, pulmonary radioactivity decreases moderately to day 3 post-injection and more slowly to day 7. These findings suggest that significant proline incorporation has occurred into proteins with relatively short biological half-lives and agrees with the trend in net total pulmonary uptakes indicated by hydrolyzed pulmonary tissue. Minimal incorporation occurred into either the crude acid mucopolysaccharide (CAMPS) or the insoluble elastin fractions of lung tissue. However, proline is not thought to be a useful indicator of CAMPS synthesis. In previously published work⁴ lysine had a low incorporation into insoluble elastin suggesting the possibility of a low biosynthetic rate of this compound. This reason may be considered a possible explanation for low proline incorporation into insoluble elastin.

Radioactive proline is incorporated into both collagen fractions in readily measurable amounts suggesting that collagen is being synthesized and cross-linked in the Syrian hamster lung. The radioactivity of both collagen fractions decreased with time in the case of tritium-labeled compounds. However, only soluble collagen has a similar decrease in ^{14}C -labeled pulmonary tissue. This suggests that soluble collagen may be metabolized. However, such data fail to clearly rule out the possibility that this collagen is merely being cross-linked. Radioactive hydroxyproline in

pulmonary tissue was low and variable suggesting either that little hydroxylation of proline had occurred or that the soluble collagen fraction may have been contaminated, possibly by non-scleroprotein. The variability may be due to large amounts of radioactive proline being present, a limitation of direct extraction from hydrolyzed pulmonary tissue, however, such variability is only of moderate quantity.

Thus, the findings suggest that either one or two days post-injection of radioactive proline are equally acceptable as sacrifice periods for studying pulmonary collagen biosynthesis, and lend confidence to the use of this particular procedure for estimating the extent of collagen biosynthesis at various periods following pulmonary insult. The method, in addition, allows more detailed chemical analyses of the various connective tissue fractions following such insults. The ability to detect such a low content of tissue radioactive hydroxyproline in pulmonary tissue indicates that a response of increased radioactive hydroxyproline content would be relatively simple to detect following pulmonary injury. Just how much insult is required to produce this increase of radioactive hydroxyproline content and at what time this increase of radioactive hydroxyproline content occurs remains undetermined at this time.

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THE RESPONSE OF SYRIAN HAMSTERS TO PARTIAL BODY ^{60}Co
EXTERNAL IRRADIATION

ABSTRACT

To study changes in pulmonary collagen biosynthesis resulting from radiation exposure, groups of Syrian hamsters received weekly or bi-weekly fractionated ^{60}Co radiation (~ 500 R per exposure) to the thoracic region to achieve cumulative total doses of 6000, 4000 or 2000 R. In addition, one group received sham exposure. Animals received an injection of ^{14}C proline 1 day prior to sacrifice at 7-8, 13-14, 18-19 and 21-22 weeks after the initial 500 R fraction. Incorporation of ^{14}C proline into the soluble collagen fraction at the 6000 R and 4000 R dose levels was elevated at 7-8 weeks after the initial fraction of exposure; but, no increase in pulmonary tissue ^{14}C hydroxyproline or in the ^{14}C -proline incorporation into insoluble collagen was observed. Increased uptake of ^{14}C proline into both soluble and insoluble collagen and an increased pulmonary tissue ^{14}C hydroxyproline concentration was observed at 13-14 weeks after the initial fraction of exposure. At 18-19 weeks post-exposure, neither an increased ^{14}C proline uptake nor an increased tissue concentration of ^{14}C hydroxyproline was noted. These findings suggest that ^{60}Co external irradiation may alter collagen biosynthesis at a relatively early time period by increasing the synthesis of collagen but not pulmonary tissue hydroxyproline concentration. At a later time period, collagen is still being synthesized in increased amounts with increased hydroxyproline tissue concentration and is more heavily cross-linked. At a still later time period, there is no longer an increase in collagen synthesis or an increase in the tissue hydroxyproline concentration. At this later time period, macroscopic and histologic pulmonary lesions were observed in the Syrian hamster. Such findings demonstrate the usefulness and sensitivity of monitoring collagen biosynthesis following pulmonary insult.

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INTRODUCTION

The development of pulmonary fibrosis is an insidious late-occurring sequelae following protracted radiation exposure to the lung. Biochemical techniques¹ have revealed a fairly consistent increase of collagen content in canine pulmonary tissue receiving beta radiation exposure from inhaled ^{144}Ce in fused clay. The tissue content of hydroxyproline and hydroxylysine, indicators of collagen, apparently increased with either increasing dose or increasing duration of exposure (1969-1970 Annual Report, LF-43, pp. 212-217; 218-223). However, the measurement of amino acids from tissue hydrolysates to assess the changes in collagen content have certain specific disadvantages, i.e., (1) the specific amino acids are relative indicators of collagen content, but are not actual measurements, (2) content only indicates total quantity of collagen present at a given time period, saying nothing about the biochemical changes producing this content, and (3) acid hydrolysis completely degrades the pulmonary tissue, and further studies cannot be done on the specific connective tissue constituents. Therefore, extraction procedures were developed to provide material of a purity required for determining amino acid content of normal and potentially altered collagen (1970-1971 Annual Report, LF-44, pp. 263-271). Such extraction techniques also permit

one to assay the relative quantity of soluble and insoluble collagen. This assay is useful since salt soluble collagen is believed to be a very newly synthesized collagen.² The ratio of soluble/insoluble collagen allows one to estimate the degree of cross-linking of the collagen present in radiation-damaged lung. However, if one is to better understand the mechanisms responsible for radiation-induced pulmonary fibrosis, techniques must be developed to measure the rate of collagen biosynthesis in normal and damaged lung tissue.

The incorporation of labeled amino acids into the aorta, uterus and tendon connective tissue constituents is an effective way of monitoring collagen biosynthesis.³ Therefore, measurement of ^{14}C -proline incorporation into pulmonary tissue was examined. Most of the uptake of ^{14}C -proline in the pulmonary tissue appears to be in proteins with relatively short half-lives. Negligible incorporation of ^{14}C -proline occurs in the insoluble elastin or crude acid mucopolysaccharide fractions. However, a significant portion is incorporated into both the soluble and insoluble collagen fractions. These results suggest that this technique is useful in monitoring collagen biosynthesis (this report, pp. 250-254).

Having used ^{14}C -proline incorporation to monitor collagen synthesis in normal lung tissue, it was important to determine whether radiation exposure of pulmonary tissue altered the rate of collagen synthesis and thus resulted in progressive pulmonary fibrosis. Prior observations had demonstrated that fractionated external thoracic radiation exposure can produce pulmonary fibrosis in several animal species^{4, 5} as well as man.⁶ This procedure was applied to Syrian hamsters and utilized to study pulmonary collagen synthesis occurring in the presence of radiation pneumonitis.

MATERIALS AND METHODS

The apparatus used for exposing the thorax of Syrian hamsters to ^{60}Co gamma radiation is shown in Figure 1. Lead shielding was used to provide columnated linear slits approximately 4 cm in length and 14 to 18 mm in width. To establish the relative dose to various parts of the body, 16 hamster carcasses ranging in weight from 100 to 150 grams, were implanted with thermoluminescent dosimeters (TLD). The areas of implantation included lung, liver-diaphragm, liver-stomach, neck and abdomen. These carcasses then received a single unilateral exposure of approximately 500 R at a dose rate of ~ 50 or 100 R/minute; after exposure, the dosimeters were recovered and read in a TLD chip reader.

Approximately 180 hamsters, ranging in weight from 80 to 150 grams were either exposed to fractionated thoracic ^{60}Co gamma radiation or received sham exposures. A dose of 500 R (~ 85 to 100 R/min) was delivered unilaterally to either the right or left side and exposures were repeated on a once or twice a week basis until individual groups had received a cumulative dose of 2000, 4000, or 6000 R. In addition, one group received sham exposures. Sixty-two animals were lost to study due to death associated with experimental conditions at time of or subsequent to exposure. The exposure protocol and achieved dosages are shown in Table 1. Thermoluminescent chips were used at each exposure to monitor the dose delivered to each group of hamsters.

At specified times after the initial 500 R fraction (see Table 2), animals were intraperitoneally injected with uniformly labeled ^{14}C -proline (specific activity of > 225 mCi/mmole). Representative animals were monitored for carbon dioxide exhalation as described (this report, pp. 250-254). At one day after injection of the ^{14}C -proline, the animals were anesthetized by an intraperitoneal injection of Nembutal. An aortic bleed-out was effected and the lungs, a skin sample, the pelt remains, and the animal remains were weighed and saved for biochemical processing. At the first sacrifice period, different animals were used for histopathological and biochemical measurements. The lungs of animals assigned to pathology were intratracheally perfused with buffered formalin.

Figure 1. Apparatus for multiple Syrian hamster thorax only exposures to ^{60}Co -external irradiation.

Table 1

Exposure Protocol and Dose Received From
 ^{60}Co External Irradiation to the Thoracic Area
of Syrian Hamsters

Group	Number Of Exposures	Number Of Animals Surviving At End Of Exposures	Calculated Average Dose (R)
I	12	30	5900-6000
II	8	25	3900-4000
	9	5	4500
III	4	30	2000
IV	12	32	0(<28)

Table 2

Sacrifice Protocol for Syrian Hamsters Either Exposed to ^{60}Co
External Thoracic Irradiation or to Sham Exposure

Approximate Dose Level (R)	Number Animals, Sacrificed, Sex and Time			
	7-8 Weeks After Initial Exposure* 3 ♂, 3 ♀	13-14 Weeks After Initial Exposure** ♂	18-19 Weeks After Initial Exposure** ♀	21-22 Weeks After Initial Exposure**
6000	6	3	3	3 ♂
4000	6	3	3	3 ♂
2000	6	3	3	3 ♂
0	6	3	3	2 ♂, 1 ♀

* One-half of the animals used for histopathologic evaluation, one-half for connective tissue analysis.

** Left lung used for histopathologic evaluation, right lung used for connective tissue analysis.

Animals assigned to biochemistry were dissected and saved for processing. The same animal was used for histopathological and biochemical measurements in all subsequent sacrifice periods. A total lung weight was obtained and the left lung was removed and perfused with buffered formalin. The remainder of the lung was saved for biochemical measurements. Tissues saved for chemical evaluation were minced, lyophilized, and the lyophilized tissue analyzed in a manner previously described (this report, pp.250 -254). The amount of pulmonary ^{14}C -radioactivity present as proline and hydroxyproline was > 70% except in one case which was 58%. These data indicate the stability of the label.

RESULTS

The TLD dose measurements from sacrificed animals are shown in Table 3. Although each of these were unilateral right side exposures, no differences were noted in values of the right, left, and midline TLDs implanted in the lung. Means, expressed as a percentage of midline air exposure (MAE), ranged from 93 to 103%; values on individually positioned dosimeters ranged from approximately 80 to 130% of MAE. Both the liver-diaphragm and liver-stomach positioned dosimeters were quite variable; the values were often low (4 to 15% of MAE), but they sometimes exceeded 100% of MAE. Dosimeters implanted in the neck area near the thyroid and in the abdominal area well below the shielding were consistently low, ranging from 2 to 5% of MAE.

Although somewhat variable, $^{14}\text{CO}_2$ production after ^{14}C -proline injection did not seem to be affected by radiation dose, sacrifice time after initial exposure, or appearance of gross pathologic lesions (Table 4).

No consistent macroscopic alterations were noted in animals sacrificed 7-8 or 13-14 weeks after the initial dose fraction was delivered. At 18-19 weeks after initial exposure, greyish lung discoloration and alopecia of the skin over the thorax were noted in some members of the 6000 R group. Numerous small white nodules and greyish-white discoloration in the lungs were found in the 4000 R group, while only a pleuritis was present in the 2000 R group. No lesions were observed in the control animals.

TABLE 3
Quantity of Dose Received by Different Organs After Implantation
of Thermoluminescent Dosimeters into Sacrificed Syrian Hamsters

Trial	I	II	III	IV
Number of Animals	4	4	4	4
Midline Air Exposure (MAE) (R)	499 (473-528)	586 (554-603)	534 (498-585)	530 (483-564)
Lung (% MAE)	98 (80-118)	93 (82-102)	103 (83-129)	100 (84-107)
Liver-diaphragm (% MAE)	53 (19- 94)	71 (9-116)	93 (79-108)	66 (27- 97)
Liver-Stomach (% MAE)	---	---	27 (4- 95)	5 (4- 6)
Neck (% MAE)	3 (2- 5)	3 (2- 3)	3 (3- 3)	4 (3- 4)
Abdomen (% MAE)	3 (2- 4)	2 (2- 3)	3 (2- 3)	3 (2- 3)

--- No observations

() Range of observations

At 21-22 weeks after initial exposure, the 6000 R and 4000 R animals had a greying of hair in the thoracic area. In addition, the lungs revealed numerous greyish-white foci, some of firm consistency and greyish or purplish mottling. In the 2000 R group the same white thoracic hair band was observed but only an occasional pleuritis was visible in the lungs. No visible lesions were present in the controls.

At early sacrifice times (7-8 and 13-14 weeks after the initial exposure), only minimal histologic changes were present consisting of a generalized increase in the number of alveolar macrophages. At 18-19 weeks post-exposure, definite pulmonary lesions were present in some hamsters and consisted of (1) a general increase in number of alveolar macrophages, (2) scattered focal increase in alveolar macrophages usually associated with other lesions, (3) foci of fine alveolar septal fibrosis, usually subpleural, and (4) scattered hemosiderin deposits. However, some hamsters had minimal or no lesions, usually those with lower doses.

Animals sacrificed 21-22 weeks after initial exposure had severe pulmonary lesions which were similar in character to those seen at the 18-19 week sacrifice. In addition, many of the fibrotic alveolar septa were lined by thickened epithelial cells. Again, the lesions were not uniform in all hamsters with the same dose and were generally less severe in the lower dose animals.

The ^{14}C -proline incorporation data relating to radiation dose and sacrifice time after initial exposure are shown in Tables 5 and 6. At 7-8 weeks after initial exposure, there was an increase in the ^{14}C -proline incorporation into soluble collagen of pulmonary tissue in the 6000 R and 4000 R groups. No difference was noted in either the ^{14}C -hydroxyproline concentration or insoluble collagen ^{14}C -proline incorporation. At 13-14 weeks after initial exposure, radioactive incorporation in both the soluble and insoluble collagen of the 6000 R and 4000 R groups was elevated (Table 5). Further, during this sacrifice period, an increase in the ^{14}C -hydroxyproline concentration in pulmonary tissue was also seen in the 6000 R and 4000 R groups (Table 6). By contrast, no notable increase in the pulmonary tissue ^{14}C -hydroxyproline concentration or increase in ^{14}C -proline incorporation was observed in any of the groups at 18-19 weeks after initial exposure and there was also no increase in ^{14}C -hydroxyproline concentration in pulmonary tissue at 21-22 weeks after initial exposure.

DISCUSSION

Ninety-nine percent of the midline air exposure was received in the thermoluminescent dosimeters implanted in Syrian hamster lungs. The amount of exposure received by the liver-diaphragm chip and the liver-stomach chip clearly indicated that, on occasion, both of these areas may have received significant radiation dosage. However, neither the neck (thyroid) area nor the abdominal area received significant radiation dosages.

The increased ^{14}C -proline incorporation into the soluble collagen fraction at 7-8 weeks after initial exposure suggests that increased synthesis of collagen is occurring. However, there does not appear to be a concomitant increase in pulmonary tissue ^{14}C -hydroxyproline concentration, suggesting that either the synthesized collagen was not being adequately hydroxylated or some con-

Table 4
Fraction of $^{14}\text{CO}_2$ Exhaled
From Animals at Various Exposure
Levels and Times After Initial Exposure

Time After Initial Exposure (Weeks)*	Control (%)	2000 R (%)	4000 R (%)	6000 R (%)
7- 8	29-30	29-34	28-32	23-26
13-14	25-31	27-32	25-34	24-25
18-19	25-27	18-26	28-29	21-24
21-22	21-30	27-31	26-30	25-30

* Calculated to nearest week first fraction of animal exposure.

Table 5
Uptake of Radiolabeled Proline Into Insoluble and Soluble
Collagen at Various Times Following Thoracic Irradiation at Different Doses

Time After Initial Exposure (Weeks)*	Radiation Dose							
	Control		~2000 R		~4000R		~6000 R	
	Lung Uptake (cpm)	Specific Activity cpm/mg**	Lung Uptake (cpm)	Specific Activity cpm/mg**	Lung Uptake (cpm)	Specific Activity cpm/mg**	Lung Uptake (cpm)	Specific Activity cpm/mg**
<u>Insoluble Collagen:</u>								
7- 8	1100- 1800†	100	800- 2400	110- 180	1000- 4500	125- 440	900- 1600	90- 200
13-14	2300- 3600	200- 260	2600- 4000	100- 260	4200- 7600	310- 410	6400- 7800	300- 370
18-19	3600- 6300	230- 270	1900- 6300	150- 220	4900- 6100	200- 260	3200- 6600	210- 260
<u>Soluble Collagen:</u>								
7- 8	7800- 10,200	600- 800	7800- 10,500	800- 900	11,200- 13,500	900- 1000	9500- 12,000	700- 1000
13-14	6200- 8000	700- 800	7400- 9300	600- 800	10,900- 14,900	900	12,000- 12,900	800- 1000
18-19	3400- 7100	600- 700	7200- 10,200	700- 800	6700- 9300	600	8700- 12,000	700- 800

* Calculated to nearest week from initial fraction of animal exposure.

** cpm/mg of extracted connective tissue constituent.

† Range of determined values.

Table 6
Incorporation of ^{14}C -Proline and ^{14}C -Hydroxyproline Concentration

Time After Initial Exposure (Weeks)	Radiation Dose					
	Control			~2000 R		
	Lung*	Proline*	Hydroxy- proline*	Lung*	Proline*	Hydroxy- proline*
7- 8	2100- 2400**	1200- 1800	50- 90	2700- 2800	2000- 2500	90- 150
13-14	2000- 2200	1100- 1800	30- 140	1700- 2500	1100- 2100	50- 170
18-19	2000	1700- 1800	140- 170	2100- 2200	1600- 1700	90- 100
21-22	2100- 2200	1500- 1800	70- 80	2400†	1600	70
Time After Initial Exposure (Weeks)	Radiation Dose					
	~4000 R			~6000 R		
	Lung*	Proline*	Hydroxy- proline*	Lung*	Proline*	Hydroxy- proline*
7- 8	2200- 2400	1900	90- 100	2100- 3100	1600- 2600	90- 160
13-14	2500- 3500	2000- 2600	400- 450	2600- 3000	1800- 2300	390- 450
18-19	1700- 1900	1400- 1600	0- 100	1900†	1300	10
21-22	2000- 2500	1100- 2000	60- 90	2100- 3300	1600- 2500	60- 100

* cpm/5 mg dry wt. pulmonary tissue.

** Range of determined values.

† One animal.

tamination of the fraction had occurred during the extraction procedure. Likewise there appeared to be no increase in the rate of synthesis of insoluble collagen. The results from animals sacrificed 13-14 weeks after initial exposure showed an apparent increase in soluble collagen synthesis and an increase in pulmonary tissue ^{14}C -hydroxyproline concentration. This increase existed even if the potential for slight contamination of the hydroxyproline fraction from the relatively large amounts of proline was considered. An increased incorporation of ^{14}C -proline into insoluble collagen was also present. These findings imply that not only was the rate of collagen synthesis increased, but it was also being more rapidly cross-linked into insoluble collagen. Biochemical alterations were present before macroscopic or histopathologic changes could be detected, indicating the sensitivity of this method. Histologic evidence of pulmonary fibrosis was first observed in animals with cumulative radiation doses of 4000 or 6000 R sacrificed 18-19 weeks following initial exposure. These changes consisted of fine alveolar septal fibrosis and were observed somewhat more consistently in animals sacrificed at 21-22 weeks after initial radiation exposure. In spite of these observations, the failure of increased amounts of ^{14}C -proline to be incorporated into the collagen fractions and the lack of an increased concentration of pulmonary ^{14}C -hydroxyproline suggests that, during this time period, collagen synthesis and cross-linking of collagen is no longer occurring at an accelerated rate. These findings indicate that increased collagen biosynthesis and cross-linking precede observable macroscopic and histologic changes in the lung. It remains to be determined if the lack of increased proline incorporation at the time of appearance of lesions indicates a return to normal pulmonary collagen synthetic rates with an adequate number of cells, elevated synthesis in a lesser number of cells, or some other explanation.

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COMPARISON OF LUNG COMPONENTS FROM VARIOUS SPECIES

ABSTRACT

A comparison of the neutral lipid and phospholipid composition of pulmonary tissue, surfactant and exfoliated lining cells from the lungs of four mammalian species (dog, horse, sheep and hamster) was made. The amount, as well as the composition of lipid present in both lung tissue and surfactant from the four species, was very similar. A species difference was observed in the lipid content of the pulmonary lining cells. The neutral lipid composition of pulmonary tissue showed a consistent pattern between species yet individual variability was evident. Large differences between species were observed in the neutral lipid composition of pulmonary surfactant. In pulmonary tissue, phosphatidyl choline (PC), phosphatidyl ethanolamine and sphingomyelin were the major phosphatides whereas in pulmonary surfactant, PC and phosphatidyl glycerol accounted for $\geq 81\%$ of the phospholipid-P from all four species. The surfactant phospholipid composition from all species was similar even though the phospholipid-P profiles from the pulmonary lining cells appeared quite different. The interspecies similarities and differences in the lipid profiles are discussed.

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INTRODUCTION

The relationship between mammalian lung structure and function is of interest relative to the effect of air pollutants on the human lung. This communication addresses specifically the comparison of some lung parameters (neutral lipid and phospholipid composition of pulmonary tissue, surfactant and exfoliated lining cells) from various mammalian species. The inhalation of various toxic and/or pharmacologically active substances can cause different and quite variable responses in mammals. To better understand the human response, the dog, horse, sheep and hamster were studied to provide a base for extrapolation.

EXPERIMENTAL PROCEDURES

Animals: The Beagle dog values are based upon samples taken from 24 animals ranging in age from newborn to 7 years. The Beagle dogs to be kept alive, were pre-anesthetized with Vetame and then anesthetized with a mixture of nitrous oxide and Halothane. The dogs to be sacrificed were pre-anesthetized with Vetame and then anesthetized with sodium pentobarbital and exsanguinated via cardiac puncture. The trachea, lungs and heart were then exposed or removed together in preparation for perfusion and lavage (described below).

Beagle pup No. 626T, delivered by caesarean section, was the runt of the litter (100 grams) and breathed for ~ 2 hours before it died of respiratory distress. It was immediately exsanguinated, the heart and lungs removed together, the lungs intervascularly perfused and then a pulmonary lavage performed (described below). Beagle pups 643A and 643S were normal litter mates and were anesthetized with the nitrous oxide - Halothane mixture described above in preparation for lavage.

The horse was a 6 year-old gelding that weighed ~ 1,000 lbs. at sacrifice. It received propiormazine and surital to a surgical anesthesia level and was exsanguinated just prior to re-

removal of the trachea-heart-lung block in preparation for perfusion and lavage. Two mature Columbian-Ramboullet Cross female sheep were also studied. They were anesthetized with sodium pentobarbital, exsanguinated and the trachea, heart and lungs removed together in preparation for perfusion and lavage.

Nine Syrian hamsters (12 ± 1 mo. of age), divided into two groups of six and three animals, were also studied. The hamsters were anesthetized with sodium pentobarbital, exsanguinated by anaortic cutdown and the trachea-heart-lung block removed. The lungs were intervascularily perfused and then lavaged.

Intervascular Perfusion Procedure: A tube was inserted into the trachea of the dogs, horse and sheep and connected to a Bennett Positive Pressure Ventilator to ventilate the lungs during this and the lavage procedures. The pulmonary artery was dissected for exposure and an appropriately-sized tube inserted and fastened. A peristaltic pump was used at a flow rate of saline of 300 ml/min and a pressure of 50 cm water. At least 20 liters of saline were perfused through the lungs and out the left atrium. Perfusion was continued until the lungs were white (no color in perfusate) and the judgement was made that additional perfusion would have no gain. In the puppy and hamsters, a 20-gauge needle was inserted into the pulmonary artery, the left atrium cut open and a series of 25 ml syringes used to perfuse the lungs with saline until they were white. Only gentle pressures were employed so as not to damage the vasculature.

Pulmonary Lavage Procedures: The basic pulmonary lavage technique has been described.¹ A Carlens catheter was inserted into the Beagle dog's trachea and each lung lavaged independently while the other was ventilated. In the horse, a bronchial lavage was performed on one lung, and a portion of the other lung was taken and frozen. In the sheep, both lungs were lavaged as a unit intratracheally. For the puppies and hamsters, whole lung lavages were employed by inserting a small catheter into the trachea. For the two puppies that were kept alive, whole lung lavages were performed quickly. The lavage technique for the Beagle dog, horse and sheep utilized filling the lung(s) with saline to its functional residual volume and then adding and withdrawing 3 times a volume of saline equal to an inspiratory volume. For the 3 puppies and 9 hamsters, 5 cc of saline was introduced and withdrawn 3 times. The small size of hamsters necessitated combining the lavage fluid from 3 or more animals in order to have sufficient material for lipid analysis.

Preparation of Total Lipid Samples: The lavage solutions from all species were handled similarly. Fresh solutions were refrigerated and centrifuged at $450 \times g$ for 15 min. to separate a fraction containing cellular material. The supernatant fluids were centrifuged at $40,000 \times g$ for 30 min. at 4°C . This high speed centrifugation yielded a clear saline supernatant fluid and a white acellular surfactant fraction. The three fractions were dialyzed against deionized water under refrigeration, lyophilized, weighed, and lipid extracted twice.²

Lung tissue that had not been lavaged from the dogs and horse was frozen at -75°C until lipid extracted.² Since the sheep, hamsters and puppy 626T lungs had been lavaged, the lipid weight for the three lavage fractions was added to that for tissue to obtain total tissue lipid weight.

The non-polar lipids were separated from polar lipids on a column of Bio-Sil HA by eluting exhaustively with chloroform.² The polar lipids were then eluted with methanol from the column.

Analysis of Neutral Lipids: Among the non-polar lipids, only the four major groups (cholesterol, free fatty acids, triglycerides, and sterol esters) were quantitated. Cholesterol was quantitated gravimetrically and the three other values, obtained by photodensitometry, were converted to weight and all values expressed in terms of percent neutral lipid. The procedures for resolution of neutral lipids by thin-layer chromatography and quantitation by photodensitometry have been reported.²

Analysis of Phospholipids: The polar lipid classes were resolved on thin layers of silica gel HR in an acidic solvent system.² Duplicate phosphorus analysis on each of the four fractions for each species were made by the micromethod of Rouser et al.²

RESULTS AND DISCUSSION

The lipid composition of both lung tissue and surfactant from the four species was very similar. The amount of lipid present in lung tissue based on dry or wet weight for Beagle dog, horse, sheep and hamsters was about equal. Even the amount of the different types of lipid (neutral lipid or phospholipid) showed remarkably uniform values. The pulmonary surfactant from the four species also showed a uniform composition (total lipids represent approximately 60% of the surfactant dry weight and neutral lipids and phospholipids represent approximately 15 and 85%, respectively, of total lipids) (Table 1).

The material remaining in the supernatant fluid following centrifugation of lavage fluid has been postulated² to be a portion of surfactant that is in micellular form and containing less lipid than that portion of pulmonary surfactant which can be centrifuged out. This theory appears valid not only for the Beagle dog material (from which the postulate was derived) but also for horse, sheep and hamster material. The lipids from the supernatant fluid as a percent of dry weight were all less than the corresponding surfactant lipid values but the neutral lipid-phospholipid ratios for surfactant and supernatant fluid within species were very nearly the same (Table 1).

The unique differences between species are seen in the lipid content of the pulmonary lining cells that are obtained from a pulmonary lavage. Notice (Table 1) that hamster cells contained 17% lipid whereas Beagle dog, horse and sheep lining cells contained twice that amount. In addition,

Table 1
Total Lipids of Pulmonary Tissue, Surfactant and Exfoliated
Living Cells From Various Mammalian Species

	<u>Beagle Dog</u>	<u>Horse</u>	<u>Sheep</u>	<u>Hamster</u>
<u>Tissue</u>				
Total Lipids, % of Dry Weight	12 $\pm$ 4	14	12	21
Total Lipids, % of Wet Weight	3.4 $\pm$ 0.6	3	2	4
Neutral Lipids, % of Total Lipids	28 $\pm$ 2	22	30	27
Polar Lipids, % of Total Lipids	72 $\pm$ 2	76	61	73
<u>Surfactant</u>				
Total Lipids, % of Dry Weight	53 $\pm$ 21	53	74	62
Neutral Lipids, % of Total Lipids	17 $\pm$ 2	10	11	15
Polar Lipids, % of Total Lipids	83 $\pm$ 2	87	89	85
<u>Supernatant Fluid*</u>				
Total Lipids, % of Dry Weight	14 $\pm$ 6	20	36	8
Neutral Lipids, % of Total Lipids	21 $\pm$ 0.5	12	19	18
Polar Lipids, % of Total Lipids	79 $\pm$ 0.5	88	81	82
<u>Lining Cells</u>				
Total Lipids, % of Dry Weight	33 $\pm$ 17	35	28	17
Neutral Lipids, % of Total Lipids	23 $\pm$ 3	11	45	41
Polar Lipids, % of Total Lipids	77 $\pm$ 3	85	55	59

* Aqueous layer above surfactant after centrifugation.
Values for the Beagle dog are expressed as mean percentages $\pm$ 1 Std. Dev.
All data were obtained by gravimetric analyses.

the neutral lipid phospholipid ratio ranged from 0.13 in the horse to 0.82 in the sheep; and yet the product, of at least a portion of these cells, pulmonary surfactant, showed a remarkably consistent neutral lipid phospholipid ratio between species.

The neutral lipid composition of pulmonary tissue, surfactant and lining cells is shown in Table 2. The total sterol content (cholesterol and sterol esters) of pulmonary tissue from all four species predominated among the neutral lipids with the neutral fat (triglycerides) composition < 30% of the total neutral lipids. The free fatty acids for 3 of the 4 species were $\leq 6\%$ of the total neutral lipids. Again a rather consistent compositional pattern of material was observed between species and yet individual species variability was inherent.

The neutral lipid composition of pulmonary surfactant, like that from tissue, showed that cholesterol was the predominant component. However, large variability between species was evident. The free fatty acid value from sheep stood out as distinctly different from those for the other 3 species as did the amount of triglycerides from the sheep and horse. The neutral lipid composition of the supernatant fluid, although appearing distinctly different between species, was not entirely different from the corresponding surfactant values with possibly a few exceptions. The supernatant fluid was postulated to be surfactant in a micellular form and therefore one would expect to find a larger amount of free fatty acids in micelles than in the material that was centrifuged out. And indeed, from 3 of the 4 species, the free fatty acid values were greater than the FFA values

Table 2
Composition of Neutral Lipids of Pulmonary Tissue, Surfactants
and Exfoliated Lining Cells From Various Mammalian Species

	% Of Total Neutral Lipids			
	<u>Beagle Dog</u>	<u>Horse</u>	<u>Sheep</u>	<u>Hamster</u>
<u>Tissue</u>				
Cholesterol	52 $\pm$ 3	69	56	54
Sterol Esters	8 $\pm$ 2	2	3	5
Free Fatty Acids	11 $\pm$ 1	5	6	4
Triglycerides	29 $\pm$ 3	19	24	34
<u>Surfactant</u>				
Cholesterol	67 $\pm$ 8	82	53	63
Sterol Esters	9 $\pm$ 4	2	2	9
Free Fatty Acids	8 $\pm$ 4	9	37	14
Triglycerides	14 $\pm$ 6	2	4	8
<u>Supernatant Fluid*</u>				
Cholesterol	52 $\pm$ 8	87	28	56
Sterol Esters	8 $\pm$ 3	5	2	19
Free Fatty Acids	28 $\pm$ 6	9	67	19
Triglycerides	12 $\pm$ 4	<1	2	8
<u>Lining Cells</u>				
Cholesterol	50 $\pm$ 11	65	26	30
Sterol Esters	25 $\pm$ 7	6	13	18
Free Fatty Acids	20 $\pm$ 6	18	54	27
Triglycerides	3 $\pm$ 2	7	3	11

* Aqueous layer above surfactant after centrifugation.

Values for the Beagle dog are expressed as mean percentages $\pm$ 1 S.D.

Cholesterol was determined gravimetrically and other neutral lipids photodensitometrically based on a known weight of lipid spotted on the TLC plate.

in the surfactant. The relatively small percentage of cholesterol in the sheep supernatant fluid was probably a reflection of the very large percentage of FFA, again pointing up individual species variability.

The neutral lipid composition of the pulmonary lining cells points up individual species uniqueness. The cholesterol values ranged from 26 to 65% of the neutral lipids and the FFA values fell between 18 and 54%. The spread in the values between species for the other neutral lipids was about as great as those just mentioned. However, the amount of triglycerides from all species was relatively low.

The phospholipid composition from all of the pulmonary constituents (tissue, surfactant and lining cells) from all species was dominated by phosphatidyl choline. This was to be expected since we are dealing with lung tissue and since pulmonary surfactant material is the unique entity in lung and since its surface active property resides in and is dominated by the special dipalmitoyl phosphatidyl choline. In addition to phosphatidyl choline, phosphatidyl glycerol, first shown to occur in pulmonary surfactant in this laboratory,^{2,3} was present in the pulmonary surfactant lining cells and lung tissue from all four species (Table 3).

Table 3
Phospholipid Composition of Pulmonary Tissue, Surfactant and
Exfoliated Living Cells From Various Mammalian Species

	% of Phospholipid Phosphorus						
	<u>LL</u>	<u>SPHI</u>	<u>PC</u>	<u>PI</u>	<u>PS</u>	<u>PG</u>	<u>PE</u>
<u>Tissue</u>							
Beagle Dog		7±5	48±2	4±0.6	8±1	3±0.7	24±0.6
Horse	2	16	45	8	3	5	17
Sheep	2	19	35	4	10	4	21
Hamster	3	11	49	4	7	8	12
<u>Surfactant</u>							
Beagle Dog		5±2	74±2	3±0.6	2±0.9	10±1	4±0.9
Pup # 626T	4	5	70	6	2	3	8
Pup #643A	3	4	69	4	1	7	7
Pup #643S	2	6	63	7	2	8	5
Horse	1	7	70	3	2	11	5
Sheep	6	3	70	0.5	1	11	5
Hamster	4	2	71	2	3	10	3
<u>Supernatant Fluid*</u>							
Beagle Dog		8±3	74±3	3±0.8	2±0.6	8±1	4±1
Horse	1	3	74	2	2	13	3
Sheep	18	4	64	1	2	7	3
Hamster	7	5	69	2	2	8	5
<u>Lining Cells</u>							
Beagle Dog		7±3	70±5	5±1	2±1	7±2	6±2
Horse	2	7	61	5	3	10	9
Sheep	16	18	37	4	3	3	12
Hamster	15	9	50	2	3	6	8

* Aqueous layer above surfactant after centrifugation.
Values for the Beagle dog are expressed as mean percentages ± 1 Std. Dev.
Lysophosphatidyl choline (LL); Sphingomyelin (SPHI); Phosphatidyl Choline (PC); Phosphatidyl Inositol (PI); Phosphatidyl Serine (PS); Phosphatidyl Glycerol (PG); Phosphatidyl Ethanolamine (PE).

The phospholipid composition of pulmonary tissue showed sphingomyelin and phosphatidyl ethanolamine as two other major phosphatides (in addition to phosphatidyl choline) with individual variability between species.

In pulmonary surfactant, phosphatidyl glycerol (PG) and choline, both surface active lipids, accounted for $\geq 81\%$ of the phospholipid-P from all four species (adult animals). The values from pup 626T showed that phosphatidyl glycerol was about one-third the adult value whereas phosphatidyl ethanolamine was two times the adult Beagle value. Since he died of respiratory distress and the only low value was in the PG fraction, the lack of PG may be implicated. Another point that may shed some light on this pup was that 80% of the material removed from his lungs was in the supernatant fraction and only 11% in the surfactant fraction which was very similar to values for adult Beagles (75 and 18%, respectively) but entirely different from the values from the two normal puppy litter mates who showed 38% of the lavageable material in the supernatant fraction and 37% in the surfactant fraction. So only 11% of the lavageable material was true surfactant which may have led to the pup's difficulty; it just did not have enough of the surface-active material to survive even though from other data (unpublished) what was present was surface-active. Also, it has been shown that newborn puppies⁴ in respiratory distress release massive amounts of phospholipids from lung tissue into the alveolar spaces and indeed the phospholipid profile of pup 626T was not so uniquely different from the adult dogs profile but somewhat different from the other pups' profiles who showed a lesser amount of PC than the adult but more phosphatidyl inositol and ethanolamine.

An interspecies comparison of the surfactant phospholipid composition can hardly be made. All four species show a phospholipid profile that was very similar. PC was 70+% and PG 10+% of all phospholipid-P values, attesting to the remarkably uniform composition of mammalian surfactant.

A somewhat greater variability was observed in the interspecies comparison of the phospholipid-phosphorus profiles from the supernatant fluid. The most outstanding difference occurred in the sheep lysophosphatidyl choline value (18% of phospholipid-P). However, the other values in the sheep's profile were not unlike the surfactant values. And again, the lipid composition in the supernatant fluid appeared very similar to the surfactant lipid composition.

The phospholipid-P profiles from the pulmonary lining cells appeared quite different between species. The values for sheep and hamster showed very high LL values and the SPHI from sheep was $\geq$ two times that from hamster, dog and horse. Sheep PC, however, was almost one-half the value from dog lining cells PC.

This study demonstrates the interspecies similarities and differences in the lipid composition observed in pulmonary tissue, surfactant and lining cells. It also illustrates the uniform composition of mammalian pulmonary surfactant.

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MEASUREMENT OF THE QUANTITY OF SURFACTANT REMOVED BY SERIAL LAVAGE IN BEAGLE DOGS AND COMPARISON WITH TOTAL LUNG SURFACTANT

ABSTRACT

The quantity of surface active material removed in serial lavages of Beagle dogs was measured and compared with the amount of surface active material remaining in the lavaged lung. The amounts of surfactant removed by each lavage at weekly and biweekly intervals were approximately equal and equivalent to that calculated to be required to cover the alveolar surface area with a monomolecular layer. The amount of surfactant removed by lavage was a small portion (15-20%) of the total surface active material of the lungs, and consistent with the observed rapid replacement of lavageable surfactant. The data indicate that the amount of surfactant removed by pulmonary lavage is not sufficient to be deleterious to the animal.

PRINCIPAL INVESTIGATORS

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INTRODUCTION

A method for quantitation of the surfactant material obtained by pulmonary lavage from Beagle dogs has been previously reported (1970-1971 Annual Report, LF-44, pp. 256-262). This method, with slight modifications, has been used to assay the surfactant material from four serially lavaged Beagle dogs and from three samples of Beagle dog lung. The study was designed to determine how the quantity of surfactant material obtained by lavage varies throughout a series of lavages and how this quantity relates to the total surfactant material in a lung.

METHODS

The basic methods were the same as those previously described. The surfactant material was concentrated from the lavage fluid by centrifugation. A slow speed centrifugation (3,000 g-min) was used to remove the cellular material and a subsequent high speed centrifugation (1.2×10^6 g-min) was used to spin down the surfactant material. This high speed pellet (HSP) was extracted with hexane:ethanol (4:1, v/v). This extracted material (hereafter referred to as HE soluble) contains all of the lipids in the HSP and may contain some protein. The latter possibility is being investigated. The surface active lipids were purified from the HE soluble fraction by precipitation from cold acetone.¹ In order to approach quantitative recovery, two successive precipitations from acetone were made. The acetone precipitable material (AP) contained sphingomyelin and highly saturated phosphatidyl glycerol and phosphatidyl choline. These are the only three phospholipids in Beagle dog pulmonary lavage fluid found to be highly surface active, i.e., which upon compression have a surface tension less than 20 dynes/cm.

The stages in the purification of the surfactant material may be summarized as follows: Lavage fluid $\longrightarrow$ HSP $\longrightarrow$ HE soluble $\longrightarrow$ AP. Surface tension measurements were made on both the HE soluble and the AP fractions. Aliquots of the material were layered on 0.15 M saline and the surface tension of the film measured during cyclic compression and expansion of the film. The previously reported method of quantitation was based on the area of hysteresis of the surface tension values obtained during the cycle. The method was modified as follows: The surface tension of the phospholipid film during compression and expansion was recorded on an XY recorder. Surface pressure, π , may be calculated by subtracting the surface tension of the phospholipid film, γ_{PL} , from the surface tension of the subphase, γ_S . $\pi = \gamma_S - \gamma_{PL}$. The area on the chart between

the surface tension of the saline subphase (73 dynes/cm) and the dynamic surface tension of the phospholipid film during compression represents a summation of the surface pressure (dynes/cm) of the film acting over the area of the film (cm^2) and is the sum of the surface work (dyne-cm or ergs) of which the film is capable during compression. This "work area" on compression was used instead of the area of hysteresis as a quantitative measure of surfactant because:

1. The work area is meaningful in estimating the functional capacity of the surfactant to do work in the lung during expiration, whereas the area of hysteresis is only a measure of the dissipation of energy during the cyclic compression and expansion of the film.

2. The work area depends only on a compression phase of the cycle. These surface tension values vary directly with the quantity of surfactant in the film, while the expansion values do not.

Another parameter was investigated as a means of quantitating the surface active material. During the compression of the phospholipid film (both the HE soluble and AP fractions), the area occupied by the film when the surface tension dropped below 20 dynes/cm was noted. This was used to calculate the area which the total sample would occupy upon compression to this surface tension value. This value should give an estimate of the maximum alveolar surface area which such a film might occupy and be functionally useful to the lung.

The balance and recorder were calibrated so that each cm^2 of area on the chart of the recorder represented 6.33 ergs and the data are reported in those units. For the serial lavage samples, the cycle time was changed from 4 min to 1-1/3 min. This did not affect the measurements on either the chemically pure phospholipids or the AP fractions. It did, however, decrease the dynamic surface tension upon compression of the HE soluble fraction, which is the least purified fraction measured. The work area or ergs per μg phospholipid was increased by $138 \pm 2\%$. In order to compare these results with our previous data and with the work on lung tissue, the data on HE soluble samples previously run on 4-min cycles must be adjusted upward by 138%. This variation in results due to cycle time is presently under investigation.

The four serially lavaged dogs were healthy young adults (2 males and 2 females) approximately one year old. They were each lavaged five times; the interval between the first two lavages was one week and thereafter approximately 2 weeks.

The lung tissue used for analyses was from three healthy one-year-old dogs. The right lung of two of the dogs was lavaged prior to sacrifice by exsanguination under pentobarbital sodium anesthesia and then removal of the heart-lung block. All three lungs were exhaustively perfused with 0.15 M saline prior to homogenization and lyophilization. Total lipid samples were obtained from each lobe of the lung by extraction using the Folch procedure. The total lipids were then separated into neutral and phospholipid samples on a Bio-Sil HA column and the acetone-precipitable material obtained from the phospholipid fraction. Surface tension studies similar to these done with the lavage fluid were made on the acetone precipitable fraction of the lung lipids.

The methods for lavage,² perfusion (this report, pp. 262-267), and thin layer chromatography³ have been previously described.

RESULTS

The dry weight and work areas of the material in the HE soluble and in the AP fractions of the serial lavages appears in Table 1. The HE soluble was $80 \pm 7\%$ of the HSP fraction. The amount of AP obtained from the HE soluble fraction was $54 \pm 9\%$.

The area occupied by a film of the total phospholipid from each lavage when the film was compressed until it had a surface tension of 20 dynes/cm was somewhat variable. On a body weight basis, the mean area for the 35 HE soluble samples was $3.9 \pm 1.0 \text{ m}^2/\text{kg}$ and for the AP samples

Table 1
Dry Weight and Work Areas of Surface Active Fractions from Serial Lavage Fluids

Dog	556A		556B		560T		559U	
Body Weight	11.25 kg		9.25 kg		6.5 kg		8.0 kg	
Lung	Right	Left	Right	Left	Right	Left	Right	Left
<u>Hexane: Ethanol Soluble Material and Work Areas from Lavage Fluid</u>								
Lavage 1	129 (22.0)*	64 (9.9)	83 (13.8)	68 (10.8)	---	36 (6.4)	73 (13.6)	74 (11.7)
2	157 (25.6)	106 (17.7)	120 (19.6)	51 (7.5)	47 (10.4)	71 (10.7)	83 (14.7)	47 (9.5)
3	151 (32.4)	99 (17.5)	58 (11.7)	58 (9.6)	56 (10.4)	46 (8.0)	98 (15.9)	68 (13.3)
4	97 (21.2)	85 (13.9)	71 (14.9)	46 (8.8)	50 (8.5)	43 (5.8)	65 (12.5)	---
5	100 (16.1)	77 (12.6)	74 (10.2)	---	70 (11.5)	46 (8.8)	---	---
<u>Acetone Precipitable Material and Work Areas from Lavage Fluid**</u>								
Lavage 1	71 (12.4)	34 (5.3)	40 (8.7)	31 (5.6)	---	13 (2.0)	38 (6.2)	41 (5.6)
2	89 (15.6)	59 (9.7)	64 (14.8)	26 (4.8)	21 (2.8)	27 (4.0)	49 (8.9)	23 (4.2)
3	88 (18.6)	53 (8.3)	31 (6.0)	35 (6.9)	31 (6.1)	33 (6.0)	53 (10.4)	50 (10.0)
4	50 (8.9)	42 (7.9)	40 (8.2)	29 (6.0)	37 (6.0)	22 (3.3)	40 (7.9)	---
5	61 (11.3)	40 (9.3)	37 (7.7)	---	36 (6.5)	16 (3.1)	---	---

* Surface active material in mg; figures in parentheses are work areas (10^6 ergs)

** AP = 54 + 9% of HE soluble

was $2.5 \pm 0.8 \text{ m}^2/\text{kg}$. The value for the HE soluble fractions indicates enough phospholipid to cover an alveolar surface area of approximately 40 m^2 for a 10-kg dog, a value close to the 46 m^2 which has been reported as the alveolar surface area for dogs.⁴

If one assumes that each of the lavages is equally effective, then the values for each half lung may be averaged, and reported as the value per kg dog. This provides a means of comparison between dogs. The average value for each half lung was increased to that of the whole lung (assuming the right lung is 58% of the total lung) and divided by the body weight of the dog in kg (Table 2).

The relationship between the dry weight of the HE soluble and the AP fractions and the functional quantity represented by the work areas is shown in Figure 1. The equation for the best fitting straight line was obtained by regression analysis. For the HE soluble data, $y = 5.9x + 1.0$ with a correlation coefficient of 0.97, where $y = \text{mg amount}$ and $x = \text{the area}$. For the AP data, $y = 5.2x + 2.1$ with a correlation coefficient of 0.97.

Table 2
Amount of Surface Active Material per Kg Body Weight

Dog	556A		556B		560T		559U	
Lung	Right	Left	Right	Left	Right	Left	Right	Left
HE soluble (mg/kg)	19.3	18.3	15.1	14.4	14.8	17.7	17.2	18.8
AP (mg/kg)	11.1	9.7	7.9	7.8	8.3	8.1	9.7	11.3
HE soluble work area (ergs $\times 10^6/\text{kg}$)	3.6	2.6	2.6	2.4	2.7	2.9	3.1	3.4
AP work area (ergs $\times 10^6/\text{kg}$)	2.1	1.7	1.7	1.5	1.4	1.4	1.8	2.0
HE soluble film area at 20 dynes/cm (m^2/kg)	4.6	3.6	3.3	3.4	3.7	4.1	3.9	4.7

Figure 1. Relation of work area to dry weight in acetone precipitable and hexane-ethanol soluble fractions.

The results of analyses of lung tissue are shown in Table 3. All dynamic surface tension measurements were made with a 4-min cycle. The values for total lipid in each lobe indicate the size of each lobe. The ratio of the work area to the total lipids illustrates that the work area is dependent on lobe size. The lavage material from the right lung appeared to represent 21% and 14%, respectively, of the total surface active material of dog 433B and 503S. These are initial estimates of the amount of the surface active material present in the lung which is removed upon lavage.

The fraction of lung tissue being used for surface tension measurements (i.e., the acetone precipitable phospholipids) is not equivalent to the AP fraction of lavage fluid. Thin layer chromatography showed that the lung tissue fraction contains all the major phospholipids and not just the three highly surface active ones found in lavage fluid AP. This fact is reflected in the minimum surface tension reached by the acetone precipitable fraction from the lung. The minimums range from 10 to 15 dynes/cm, similar to the values for the HE soluble fraction from lavage fluid. The minimums for the AP fraction of lavage fluid range from 1 to 5 dynes/cm, similar to the chemically pure surface active phospholipids. Thus both the acetone precipitable fraction from lung tissue and the HE soluble fraction from lavage fluid contain non-surface active substances which act to increase the minimum surface tension. Experiments with mixtures of chemically synthesized dipalmitoyl lecithin (minimum surface tension on compression = 0.5 dynes/cm) and dioleoyl lecithin (minimum surface tension on compression = 26 dynes/cm) showed that a film of the mixture could contain up to 30% dioleoyl lecithin and still reach a surface tension below 5 dynes/cm upon compression (Fig. 2). In lavage fluid, the largest amount of non-surface active lipid is cholesterol which is present in a ratio of 1:3 to dipalmitoyl lecithin. Dynamic surface tension studies of mixtures of these substances showed that at just this ratio the minimum surface tension of the compressed film rises above 5 dynes/cm (Fig. 2). The significance of 1:3 molecular ratios in mixed surfactant systems has also been reported by Shah⁵ who proposed that the unusual properties of such mixed films were due to a closer molecular packing and a greater stability of mixed films at interfaces.

DISCUSSION

The amount of material obtained in each of the serial lavages appeared to be approximately equal for each dog. Variations were rather large but showed no definite trend. The data in Table 2 allows a comparison between dogs. The variation was in the range of ± 10 to 15%. In general, then, one can expect to remove about the same amount of surfactant in each of a series of lavages (at the intervals used here) as in single lavages, within the limits of variation demonstrated. This shows that rapid replacement of the surfactant material is possible. The correspondence between

Table 3
Work Areas of Lung Tissue Surface Active Lipids

Dog	462B					433B					503S				
Body Weight	11.5 kg					12.5 kg					7.6 kg				
	Total Lipids (mg)	APPL (mg)	APPL Work Area (10 ⁶ ergs)	Work Area APPL (10 ⁶ ergs/mg)	Work Area Total Lipids (10 ⁶ ergs/mg)	Total Lipids (mg)	APPL (mg)	APPL Work Area (10 ⁶ ergs)	Work Area APPL (10 ⁶ ergs/mg)	Work Area Total Lipids (10 ⁶ ergs/mg)	Total Lipids (mg)	APPL (mg)	APPL Work Area (10 ⁶ ergs)	Work Area APPL (10 ⁶ ergs/mg)	Work Area Total Lipids (10 ⁶ ergs/mg)
Lobe LD	508	287	43.2	.15	.085	529	300	45.7	.15	.086	365	175	35.2	.20	.096
LA	288	184	<u>22.4</u>	.12	.078	254	140	<u>22.9</u>	.16	.090	265	144	<u>26.0</u>	.18	.098
Sum			65.6					68.6					61.2		
% of Whole Lung			42%					41%					42%		
RI	172	94	13.0	.14	.076	139	91	10.6	.12	.076	105	49	9.3	.19	.089
RC	129	79	11.8	.15	.092	176	104	15.7	.15	.089	78	37	6.1	.16	.078
RA	322	198	27.9	.14	.087	268	167	25.0	.15	.093	230	107	18.5	.17	.080
RD	414	242	34.3	.14	.083	520	213	29.0	.14	.056			39.9*		
Lavage (HE soluble)			No Lavage					20.6					12.0		
Sum			87.0					100.9					85.8		
% of Whole Lung			58%					59%					58%		

* Sample lost and work area estimated from the wet weight of the lobe compared to the wet weight of the other right lung lobes

Abbreviations used: LA - left apical lobe; LD - left diaphragmatic lobe; RI - right intermediate; RC - right cardiac lobe; RA - right apical lobe; RD - right diaphragmatic; APPL - acetone precipitable phospholipid.

Figure 2. Effect of surfactant mixtures on minimum surface tension. Abbreviations: DPL - dipalmitoyl lecithin; DOL - dioleoyl lecithin. The arrow on the abscissa indicates the molar ratio of cholesterol to DPL in lavage fluid.

the alveolar surface area of the dog and area covered by a film of the HE soluble fraction when it is compressed to the point that its surface tension is 20 dynes/cm suggests that the lavage procedure is removing no more than a single layer of surfactant from the lungs. Apparently sufficient surface active material remains in the lung either stored in alveolar type II cells and/or as sublayers to the material removed by lavage, to prevent any respiratory problem due to lack of surfactant.

The question arises as to what fraction of the surfactant material from lavage best represents the surfactant *in vivo*. The HE soluble material is the least purified and therefore may be closer to the composition of the surfactant in the lung, but surface tension data for this fraction vary with the cycling time. The AP fraction is purer, and the data do not vary with the cycling time, but the composition may not reflect that of the surfactant lining the alveoli. Also, the further the material is purified, the greater is the possibility of loss of the material due to technical manipulations.

It would appear that cholesterol acts as an "expander" for the highly surface active phospholipids since it is present in just enough quantity to dilute the phospholipids without having much effect on the minimum surface tension. Thus, the lung is able to function efficiently with a smaller amount of specialized phospholipids. The cholesterol might also have some other function in the properties of the film *in vivo*. With this in mind, the area covered by the HE soluble film when it drops to a low surface tension may better represent the alveolar area covered by the surfactant than the area occupied by a similar film of the AP fraction. In considering data from the AP fraction, one must remember that this material can be expanded or diluted by one-third with substances such as cholesterol or unsaturated lecithins and still act efficiently to lower surface tension in the lung. Therefore, it may be helpful to use both fractions for comparison of lavage material from different dogs (and other animals), being certain that comparisons are made between equivalent fractions run under equivalent conditions.

In order to estimate the quantity of surfactant removed from the lung, the amount of highly surface active material present in the whole lung must be determined. Ideally, if the three surface active phospholipids found in the AP fraction of lavage fluid could be separated from lung extracts, the two quantities could very readily be compared. This type of separation is presently being developed in this laboratory but has not yet been achieved. Instead, the acetone precipitable fraction of the lung phospholipids has been used. This fraction showed a minimum surface tension of 10 to 15 dynes/cm, similar to the HE soluble fraction of lavage fluid, but the expander or diluent here is not cholesterol but other phospholipids. Both fractions were diluted with material that is not highly surface active to the extent that minimum surface tensions had been raised to 10 to 15 dynes/cm. Therefore in Table 3 the data for the lung tissue have been compared with the data for the HE soluble fraction of the lavage.

The data presented here demonstrate that pulmonary lavage in no way depletes the lung of its supply of surface active phospholipids and lends further support to the safety of pulmonary lavage for removing inhaled radioactivity.

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EFFECT OF INHALED ^{90}Y IN FUSED CLAY ON PULMONARY CLEARANCE
OF STAPHYLOCOCCUS AUREUS IN MICE: STUDY II

ABSTRACT

Seven hundred and twenty (720) mice were exposed by inhalation to ^{90}Y in fused clay. Two groups with initial lung burdens of 5 to 7 μCi and 8 to 12 μCi ^{90}Y were tested at 1, 2, 3, 4, 7 and 14 weeks post-inhalation exposure to ^{90}Y to determine the pulmonary clearance of inhaled Staphylococcus aureus as compared to sham exposed mice and mice that had inhaled stable yttrium in fused clay. A slight increase in the clearance of inhaled bacteria was evident at 1 week in the mice with ILBs of 8 to 12 μCi followed by a slight suppression at 3 weeks. No other differences in bacterial clearance were observed between the control mice and those that had inhaled ^{90}Y in fused clay. Hematological parameters, histological observations and weight gain were similar among all groups of mice.

PRINCIPAL INVESTIGATORS

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INTRODUCTION

Several factors, including ionizing irradiation have been demonstrated to suppress the pulmonary clearance of inhaled bacteria.¹⁻³ In a previous study, mice with initial lung burdens (ILB) of 7 to 76 μCi of ^{90}Y (physical half-life 64.2 hours) in fused clay exhibited a suppressed ability to clear inhaled Staphylococcus aureus from their lungs.⁴ In the study, however, there were not enough animals with ILBs in the range of 7 to 76 μCi available for study beyond 7 weeks post- ^{90}Y inhalation exposure. Mice with ILB of 31 μCi or greater were all dead by day 70 post- ^{90}Y inhalation exposure, 75 percent of those with ILB of 21 to 30 μCi were dead by 90 days and 5 percent of those with ILB of 20 μCi or less died through day 220.

The present study was undertaken in an effort to: (1) confirm the suppression of the pulmonary clearance of S. aureus in mice with ILBs in the range of 7 to 12 μCi of ^{90}Y in fused clay and (2) to extend these studies beyond 7 weeks to at least one year post- ^{90}Y inhalation exposure.

EXPERIMENTAL PROCEDURES

Seven hundred and twenty conventional male mice of the CFW strain (Carworth Farms) 8 to 10 weeks of age were exposed by inhalation to an aerosol of ^{90}Y in fused clay as previously described (1970-1971 Annual Report, LF-44, pp. 204-210). The ILBs in the exposed mice ranged from 3.1 to 18.7 μCi . The infinite beta radiation dose to the lung was estimated at approximately 400 rads per μCi ILB. After inhalation exposure the mice were housed three per cage. One hundred and sixty control mice sham exposed to filtered ambient air and 160 additional control mice were exposed by inhalation to stable yttrium in fused clay and housed five per cage. All mice were given food (Wayne Lab Blox) and water ad libitum. From the mice exposed to ^{90}Y in fused clay, two groups were selected for studying the effect of the inhaled ^{90}Y on the pulmonary clearance of bacteria. The first group had ILBs ranging from 5 to 7 μCi ^{90}Y and the second group had ILBs ranging from 8 to 12 μCi . The remaining mice are being held for observations of the toxic effects of inhaled ^{90}Y in fused clay.

The procedures used in studying the clearance of inhaled bacteria were essentially similar to those previously reported (1970-1971 Annual Report, LF-44, pp. 272-278). The bacteria used for

this study was a coagulase positive strain of S. aureus (American Type Culture Collection No. 6358). Bacterial cultures were maintained in Trypticase Soy Broth shake cultures, incubated at 37°C for 18 hours and stored at 4°C. A new bacteria culture for use in generating aerosols to which the mice were exposed was cultured for each exposure and washed with 0.1 M phosphate buffer at pH 7.4. The final bacterial suspension contained approximately 1×10^{10} colony forming units (CFU) of S. aureus per ml. Gram stains of all cultures were prepared to determine if pure cultures were being used.

At 1, 2, 3, 4, 7 and 14 weeks post- ^{90}Y inhalation exposure, groups of 10 sham control mice, 10 fused clay control mice, and 20 mice from each of the two groups with different ILBs were exposed to a nebulizer-produced aerosol of S. aureus for 20 minutes. Mice with ILBs of 5 to 7 μCi ^{90}Y were not tested at weeks 1 and 2. Qualitative and quantitative control of each bacterial aerosol was maintained by collecting samples with both all-glass Porton type impingers and an Anderson Air Sampler for viable particles.

The rate of clearance of S. aureus from the lungs of mice was determined by sacrificing half of the mice from each group being tested immediately following the 20-minute exposure to the bacterial aerosols and then sacrificing the remaining mice 4 hours later. Clearance is defined here as the proportion of originally inhaled bacteria which were either rendered non-viable in the lungs, or removed from the lungs by 4 hours after the bacteria were inhaled. All mice were sacrificed by clamping the trachea with a hemostat and severing the cervical vertebrae. Immediately after death, the lungs were aseptically removed, and individually ground in a chilled mortar with a pestle and sterile alumina. The ground lungs were then suspended in 10 ml of cold Trypticase Soy Broth, dispensed into screw capped tubes and placed in crushed ice. The CFU of S. aureus per mouse lung were determined by preparing tenfold serial dilutions of the lung homogenates in 0.1 M phosphate buffer at pH 7.4 and using three Trypticase Soy Agar pour plates per dilution. The plates were incubated at 37°C for 2 to 3 days and the resulting colonies counted. The standard error of the ratio of the bacteria cleared from the lungs of mice between 0 and 4 hours after S. aureus were inhaled, was analyzed by the Theorem of Wilks.⁵ The degree of significance of the percent of bacteria cleared between the mice that inhaled ^{90}Y and the control mice was calculated by the t test and the percentile (p) values obtained from a two-tailed table for t distribution.

All mice were weighed prior to exposure by inhalation to ^{90}Y in fused clay and again on the day they were exposed to bacterial aerosol. In addition, 3 mice from each of the groups being tested for bacterial clearance were bled for hematological study and then sacrificed for pathological study. Baseline information was obtained from normal mice sacrificed on day 0 of ^{90}Y in fused clay exposure. All mice were observed daily for deaths and the cumulative percent survival calculated.⁶

RESULTS AND DISCUSSION

The cumulative survival of the mice with ILBs of 3.1 to 18.7 μCi ^{90}Y through 100 days post-inhalation exposure was 97.8%, which is consistent with our previous findings.⁴ None of the control mice in either the sham or fused clay control groups died during this interval. The body weights of the mice that had inhaled ^{90}Y in fused clay, stable yttrium in fused clay, or that were sham exposed, did not vary significantly throughout this study. This is in contrast with our previous observation that mice with ILBs in the range of 7 to 12 μCi of ^{90}Y in fused clay had decreased weight gains as compared to control mice (1970-1971 Annual Report, LF-44, pp. 204-210). The hematological parameters of the various groups of mice were similar through 14 weeks post-inhalation exposure to ^{90}Y in fused clay.

The histopathological findings from the mice sacrificed in this study revealed minimal alterations attributable to radiation injury from inhaled ^{90}Y in fused clay. Increased numbers of vacuolated alveolar macrophages were noted in some mice at most sacrifice times, but the change was irregular. In addition, small perivascular and peribronchiolar cuffs of lymphocytes were found in many mice, including controls. This change is probably not related to radiation injury. These findings are in contrast to those previously observed in mice with similar ILBs (1970-1971 Annual Report, LF-44, pp. 204-210). It was previously observed that the ILBs in the mice being studied resulted in an increased number of vacuolated alveolar macrophages at 2 weeks; increased numbers of alveolar macrophages and slight perivascular and peribronchiolar inflammation at 3 weeks; increased numbers of foamy alveolar macrophages and mild perivascular and peribronchiolar infiltrates at 4 weeks; and a few small peribronchiolar infiltrates and a slight increase in normal alveolar macrophages at 7 weeks.

No significant differences were observed in the bacterial pulmonary clearance between the sham and stable yttrium in fused clay control mice so the data from these two groups were combined and compared with the bacterial clearance in the mice that had inhaled ^{90}Y in fused clay (Table 1). A slight stimulation in bacterial clearance was evident in mice with ILBs of 8 to 12 μCi when tested 1 week post- ^{90}Y fused clay inhalation exposure. No differences were observed at 2

Table 1
Pulmonary Clearance of Inhaled Staphylococcus aureus in Control Mice
and in Mice that had Inhaled ^{90}Y in Fused Clay

<u>Weeks Post- Exposure</u>	<u>Mouse Group</u>	<u>Bacteria Cleared (%)</u>	<u>p Value**</u>
1	Control	$89.7 \pm 1.6^*$	-
	5 - 7 μCi ILB***	Not evaluated	-
	8 - 12 μCi ILB	91.2 ± 1.0	$> 0.02, < 0.05$
2	Control	89.3 ± 2.0	-
	5 - 7 μCi ILB	Not evaluated	-
	8 - 12 μCi ILB	85.6 ± 3.6	> 0.05
3	Control	93.7 ± 1.0	-
	5 - 7 μCi ILB	90.2 ± 2.0	> 0.05
	8 - 12 μCi ILB	87.2 ± 2.4	$< 0.02, > 0.01$
4	Control	94.0 ± 1.0	-
	5 - 7 μCi ILB	91.7 ± 1.7	> 0.05
	8 - 12 μCi ILB	93.0 ± 1.1	> 0.05
7	Control	90.1 ± 2.0	-
	5 - 7 μCi ILB	93.3 ± 1.4	> 0.05
	8 - 12 μCi ILB	91.3 ± 2.2	> 0.05
14	Control	94.4 ± 0.9	-
	5 - 7 μCi ILB	93.1 ± 1.1	> 0.05
	8 - 12 μCi ILB	92.3 ± 1.9	> 0.05

* Standard error

** Compared with the bacterial clearance in control mice

*** Estimated initial lung burden

weeks, but a slight suppression of bacterial clearance was evident at 3 weeks in the mice with ILBs of 8 to 12 μCi but not in those with 5 to 7 μCi of ^{90}Y . No significant differences in bacterial clearance were observed among the mice tested at 4, 7 and 14 weeks post-inhalation exposure to ^{90}Y . It was noted, however, that among the mice with ILBs of 8 to 12 μCi tested at 14 weeks, a marked suppression of clearance was evident in a few of the individual mice that was not evident when the data were analyzed for the entire group of mice. This was the only instance where marked variations were evident among individual mice within any of the groups tested. The general lack of suppression of bacterial clearance in the mice with ILBs of 8 to 12 μCi ^{90}Y reported here differs from our previous findings⁴ where similar ILBs resulted in suppressed bacterial clearance. The lack of bacterial clearance suppression is consistent with the other negative findings in the present study; i.e., lack of suppression of weight gain, lack of suppression of lymphocyte level and lack of apparent radiation injury, all of which were previously observed in mice with ILBs of 8 to 12 μCi of ^{90}Y in fused clay. At the present time, no explanation for the variance in the observations reported here with the previous findings is readily available.

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PULMONARY FUNCTION MEASUREMENTS IN LABORATORY PONIES

ABSTRACT

Methods currently used for measuring the pulmonary function of Beagle dogs were adopted for use with laboratory ponies. Four series of tests were performed on each of two ponies. Data obtained are compared to results from similar tests performed on Beagle dogs and humans.

PRINCIPAL INVESTIGATOR

J.L. Mauderly

INTRODUCTION

The Beagle dog has been the primary experimental subject in this laboratory because of its moderately long lifespan, its size, and its use in other long-term radiation toxicity programs. More limited studies have been conducted with smaller laboratory animals to make use of unique species characteristics and to aid in the extrapolation to man. Recently, studies using laboratory ponies have been initiated to take advantage of certain similarities between horse and human beings.

The subgross anatomy of the horse lung is more similar to that of the human than is the dog lung.¹ Lungs of horses and humans have a thick vascular pleura and are partially lobulated by thick interlobular septae. Distal airways are composed of numerous true terminal bronchioles with occasional poorly developed respiratory bronchioles. In contrast, dog lungs are characterized by a thin membranous pleura, an absence of secondary lobules, and distal airways composed of well developed respiratory bronchioles leading into large alveolar ducts. The horse is susceptible to several respiratory disorders that appear in similar forms in humans including asthma,² viral influenza,³ chronic pulmonary emphysema,⁴ and newborn distress syndrome (hyaline membrane disease).^{5, 6} The size and lifespan of the pony are also more similar to the human than are those of the smaller laboratory animals.

The reported use of the pony as a laboratory model for studying cardiopulmonary function⁷ and the desirability of using the horse lung as a step toward the extrapolation of inhalation toxicity studies to humans, led us to investigate the adaptation of current pulmonary function testing techniques for use in ponies.

METHODS

One male (gelding) and one female grade pony, weighing 205 and 161 kg, respectively, were purchased locally. Although their ages and backgrounds were unknown, both were estimated to be approximately 8 years old. The ponies were fed a pelleted commercial ration and housed in either sheltered stalls or an outside corral. The ponies were prepared for arterial blood sampling by surgically relocating a 5 cm loop of the left common carotid artery to a subcutaneous position on the left side of the neck.

A valving apparatus for controlling the composition and collection of respired gases were constructed from one-inch diameter pipe fittings and fastened to an adjustable cart. A two-way breathing valve was constructed using two 3.2 cm I. D. one-way valves ("V" valve, Warren E. Collins, Inc., Braintree, Mass.). The breathing valve was connected to the valving apparatus with flexible tubing to allow for limited movement of the subject's muzzle. The cart carrying the breathing apparatus was positioned in front of the apparatus used for testing dogs and connections were made which allowed the same gas sampling, analyzing and recording equipment to be used for both species.

In preparation for testing, the ponies were led into a mobile restraining cart outside the building, the halter rope was tied to the cart, and the cart was then rolled into the laboratory. The hair around the muzzle and over the left carotid artery was clipped and surgical lubricant was applied to the area to be contacted by the mask. A fiberglass mask with a latex rubber diaphragm seal was placed over the muzzle and secured by a leather headstall. Chains connecting the sides of the headstall to the cart restricted lateral movement of the muzzle. Figure 1 shows a pony restrained in the cart and wearing the mask. Figure 2 shows the pony connected to the gas collecting and analyzing apparatus.

Four series of measurements were performed on each pony by procedures identical to those used for dogs (1969-1970 Annual Report, LF-43, pp. 205-211 and 1970-1971 Annual Report, LF-44, pp. 227-236) except that the arterial blood samples were obtained with the subjects in a standing position. Parameters measured included respiratory frequency, tidal

Figure 1. Pony restrained in the cart, rolled into the laboratory and fitted with the breathing mask.

Figure 2. Pony connected to the testing apparatus. All measurements are performed in the position shown.

Table 1

Pulmonary Function Measurements* Obtained on Healthy Ponies, Beagle Dogs and Human Subjects

Parameter	Units	Male Pony		Female Pony		Combined Ponies	Beagle Dogs		Humans	
		Mean	Range	Mean	Range	Mean	Mean	Range	Mean	Range
Body weight	kg	205		161		183	9.1	6.6-13.6	74.3	56.5-84.5
Respiratory frequency	breaths/min	16	12-20	30	26-34	23	20	10-31		
Tidal volume	liters BTPS**	1.30	1.17-1.51	1.52	1.26-1.72	1.41	0.186	0.099-0.350		
Minute volume	liters BTPS/min	19.87	17.9-23.4	45.24	39.1-49.7	32.56	3.72	2.29-6.02	9.37	7.70-12.9
Functional residual capacity	ml/kg body wt.	35	32-38	43	41-44	39	48	27-58	54	44-71
Functional residual capacity	liters BTPS	7.22	6.63-7.73	6.88	6.54-7.14	7.05	0.43	0.28-0.67	4.08	3.41-4.56
Lung clearance index	liters expired/liter FRC	14	12-15	15	14-15	15	23	15-36	10	9-11
Steady state CO diffusing capacity	ml/min/mmHg	48.4	30.3-55.3	49.5	39.2-58.1	49.0	9.0	2.9-16.6	36.9	23.1-63.1
Fractional uptake of CO	decimal fraction	.354	.308-.428	.284	.230-.344	.319	.480	.194-.749		
Alveolar O ₂ tension	mmHg	83	82-86	89	88-91	86	83	71-97	85	77-100
Arterial O ₂ tension	mmHg	72	71-73	79	77-80	76	75	64-90	73	65-83
Alveolar-arterial O ₂ gradient	mmHg	11	9-15	10	8-13	11	8	1-15	12	3-24
Arterial CO ₂ tension	mmHg	42	41-42	39	37-40	41	41	31-50	36	32-42
Alveolar CO ₂ tension	mmHg	37	36-39	34	30-37	36	37	27-45	33	28-39
Arterial-alveolar CO ₂ gradient	mmHg	5	3-6	5	3-7	5	4	0-7	3	-2-6
O ₂ saturation of hemoglobin	%	93	92-93	94	93-94	94	91	86-94	94	93-96
Arterial pH	pH units	7.42	7.40-7.44	7.42	7.39-7.45	7.42	7.38	7.31-7.45	7.45	7.41-7.50
O ₂ uptake	ml STPD*** /min.	420	378-511	551	513-603	486	90	54-169	280	240-314
CO ₂ output	ml STPD/min.	444	434-451	779	733-839	592	85	50-155	242	203-304
Respiratory exchange ratio	O ₂ uptake/CO ₂ output	1.06	0.87-1.19	1.42	1.39-1.43	1.24	.95	.78-1.16	.86	.68-1.04
Specific ventilation	ml expired/ml O ₂ uptake	47	46-49	83	69-96	65	40	28-57	34	24-60
Alveolar ventilation	% of minute volume	53	45-56	45	38-55	49	57	45-71	71	62-78
Effective ventilation	% of minute volume	47	39-51	39	32-44	43	51	40-66	65	54-78
Alveolar dead space ventilation	% of minute volume	6	5-7	6	4-11	6	6	2-9	7	0-10

* Four measurements were made on two 8-year old ponies; single measurements on 60-100 dogs; and single measurements on 8 humans (human data provided by Dr. U. C. Luft, Department of Physiology, Lovelace Foundation). All measurements were made at an altitude of approximately 1620 meters.

** BTPS = Body temperature and pressure, saturated

*** STPD = Standard temperature and pressure, dry.

volume and minute volume, functional residual capacity, lung clearance index, steady-state carbon monoxide diffusing capacity, fractional uptake of carbon monoxide, alveolar P_{O_2} and P_{CO_2} , arterial P_{O_2} and P_{CO_2} , alveolar-arterial gradients of O_2 and CO_2 , O_2 saturation of hemoglobin, arterial pH and base excess, O_2 uptake, CO_2 output, respiratory exchange ratio, specific ventilation, alveolar ventilation, effective ventilation, and alveolar deadspace ventilation.

RESULTS AND DISCUSSION

The means and ranges for each parameter measured are shown in Table 1. For comparison, results of similar tests performed on healthy adult Beagle dogs in the same laboratory⁸ and on healthy adult human subjects in the Lovelace Foundation Department of Physiology laboratory are also listed. The values for alveolar and arterial O_2 and CO_2 tensions, arterial pH and O_2 saturation of hemoglobin are very similar for the three species. The other parameters are related to body size, as their differences would indicate.

The female pony had consistently higher values for minute volume and O_2 uptake than the male even though she weighed less. She also had a lower percent alveolar ventilation and a much higher specific ventilation. These findings may reflect a difference in attitude between the ponies. The female tolerated the procedures but was very excitable and never appeared to relax while the male appeared quite relaxed throughout the measurements. A slight, nonspecific functional impairment in the female could have contributed to the differences in ventilation and gas exchange. The collection of data from a larger number of ponies of known age and background is necessary for establishing values to which an individual pony can be compared.

The development of measurement techniques and the collection of these preliminary data indicate the practicality of handling ponies as experimental subjects in the laboratory, and point the way for their use in studies to assess the toxicity of inhaled radionuclides.

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PERIOD ANALYSIS OF THE DEVELOPMENTAL ELECTROENCEPHALOGRAM OF THE BEAGLE DOG

ABSTRACT

Electronic analysis of electroencephalographic (EEG) data can be useful in the detection of abnormalities that are not readily detectable on visual analysis. Period analysis of the EEG of the normal Beagle dog from a 3-month puppy to a young adult at 12 months of age were made in order to better interpret the change with age in the frequency of the EEG of the developing Beagle dog. Electroencephalograms of 50 dogs were recorded at 3, 6 and 12 months of age on magnetic tape and analyzed with a period coder. The mean and standard deviation of the percent possible baseline crosses was determined for the above age groups for each EEG band and bipolar lead. The percent of possible baseline crosses for the slowest EEG band showed a decrease with age. There was an increase in activity in the two highest EEG bands through 1 year of age. Other Bands showed no consistent change with age.

PRINCIPAL INVESTIGATORS

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INTRODUCTION

The visual analysis of the electroencephalogram (EEG) of the Beagle dog is a tedious, time-consuming task. Although rewarding from the standpoint of detection of gross EEG abnormalities, the subtle changes in frequency that are not readily detectable by the visual analysis may be important from the standpoint of representing a significant abnormality. The use of electronic signal decomposition and data reduction such as period analysis for abstracting useful information from EEG signs provides another tool to assist in the detection of EEG abnormalities.¹ The period analysis of the EEG is a time-domain analysis which demonstrates the amount of EEG activity present in each of the EEG band widths, i.e., delta (1.0-3.5 Hz), theta (3.5-7.5 Hz), alpha (7.5-10.5 Hz), beta 1 (10.5-20.5 Hz), and beta 2 (20.5-30.0 Hz).

In order to be able to better interpret the developing EEG of the normal Beagle dog in which it is known that changes in the basic EEG frequencies do occur with age,^{2,3} the following study was conducted.

MATERIALS

Dogs:

The 148 Beagles used in the study were raised in the AEC-Lovelace Foundation Fission Product Inhalation Laboratory in accordance with previously reported procedures.⁴ These dogs were produced from a breeding program designed to provide for the production of dogs from a stable gene pool over an extended period for entry into a series of sequentially and interrelated experiments,⁵ and were produced from sires and dams that had not been observed to have any spontaneous convulsive seizures. The animals used in this study have not been observed to have any spontaneous convulsive seizures or abnormality in the electroencephalogram. The dogs used in this study were born between January 25, 1971 and June 28, 1972. Electroencephalograms were obtained on animals at 3, 6 and 12 months of age. Electroencephalograms were recorded on two dogs at two ages. All other dogs were used at only one age.

Data Collection Equipment:

An 8-channel electroencephalogram was used for EEG recordings, the paper speed was 30 cm per second and the calibration was maintained at a 5 mm or 1 cm deflection equal to 50 μ v. Electrode placements were designed to obtain an overall survey of the brain. The montage used in this study is illustrated in Figure 1. Electrodes used were 27-gauge tungsten platinum alloy subdermal needle electrodes. The electroencephalogram was recorded visually on paper. The signal was also recorded on analog magnetic tape.

Figure 1. The EEG montage and relationship of the surface electrodes to the brain are shown. The lead designations are as follows: right frontal (2), right parietal (10), right temporal (6), right occipital (14), left frontal (1), left parietal (9), left temporal (5), left occipital (13) and vertex (20). The bipolar run C₂ was used in this study.

Period Analysis Equipment:

The EEG data on magnetic tape was analyzed by the use of an EEG period coder. This period coder with its logic systems divides the major periods into the EEG bands containing 1.0 to 3.5 Hz, 3.5 to 7.5 Hz, 7.5 to 13.5 Hz, 13.5 to 19.5 Hz and 19.5 to 30.0 Hz.

METHODS

A canvas sling was used to hold the dog during the EEG recording session. The dog was placed in the sling with its legs extending through holes and its head resting on a foam rubber pad. The dog was blindfolded using a light gauze and cotton bandage. Each dog was preconditioned for periods of 15 minutes to an hour, prior to the recording session. The amount of time necessary to precondition the dog to the sling depended upon the temperament of the dog. No medication was given to any of the animals in this study. The recordings were obtained while the dog was awake. In the case of the young 3-month-old puppies, it was necessary to apply external stimuli by periodically clapping the hands to assure a constant wakeful condition. The electroencephalogram was recorded by a direct ink writer on paper and was simultaneously recorded on analog magnetic tape. The EEG montage used in this study is illustrated in Figure 1 (Run C₂). This montage is a bipolar lead system which measures the leads in the following sequence: right frontal-right parietal, left frontal-left parietal, right parietal-right occipital, left parietal-left occipital, right parietal-right temporal, and left parietal-left temporal.

Methods Data Analysis:

The number of baseline crosses obtained in each band for each bipolar lead was recorded from the period coder by age group. The percent of possible baseline crosses was calculated for

Table

Percent of Possible Baseline Crosses per EEG Band of Normal Beagle Dogs at 3, 6 and 12 Months of Age

EEG Frequency Band (Hz)	Age (Mo.)	Sample Size	EEG Leads					
			RF-RP	LF-LP	RP-RO	LP-LO	RP-RT	LP-LT
			Mean (± 1 S.D.)	Mean (± 1 S.D.)	Mean (± 1 S.D.)	Mean (± 1 S.D.)	Mean (± 1 S.D.)	Mean (± 1 S.D.)
1.0 - 3.5	3	50	17.16 $\pm$ 13.04	15.33 $\pm$ 9.55	15.91 $\pm$ 10.55	15.91 $\pm$ 10.40	18.17 $\pm$ 10.51	19.65 $\pm$ 13.01
	6	50	10.77 $\pm$ 7.86	10.24 $\pm$ 7.35	7.64 $\pm$ 7.41	6.62 $\pm$ 5.72	10.70 $\pm$ 10.01	10.04 $\pm$ 8.02
	12	50	8.89 $\pm$ 6.48	10.58 $\pm$ 6.77	8.35 $\pm$ 7.11	6.49 $\pm$ 5.76	7.73 $\pm$ 6.82	8.24 $\pm$ 5.88
3.5 - 7.5	3	50	37.75 $\pm$ 12.09	40.25 $\pm$ 8.92	35.02 $\pm$ 6.31	33.31 $\pm$ 8.61	36.87 $\pm$ 9.96	35.29 $\pm$ 9.71
	6	50	43.65 $\pm$ 12.87	42.15 $\pm$ 14.03	37.36 $\pm$ 11.68	37.93 $\pm$ 11.37	35.03 $\pm$ 11.58	38.58 $\pm$ 12.49
	12	50	34.96 $\pm$ 11.94	36.98 $\pm$ 13.86	33.02 $\pm$ 10.11	33.31 $\pm$ 12.84	33.16 $\pm$ 10.88	34.47 $\pm$ 10.45
7.5 - 13.5	3	50	25.07 $\pm$ 6.76	23.95 $\pm$ 8.53	28.95 $\pm$ 8.20	28.99 $\pm$ 9.16	23.75 $\pm$ 5.88	24.00 $\pm$ 8.05
	6	50	24.10 $\pm$ 8.21	25.05 $\pm$ 10.47	29.77 $\pm$ 9.69	29.14 $\pm$ 9.97	23.65 $\pm$ 7.87	24.34 $\pm$ 8.74
	12	50	26.30 $\pm$ 8.97	24.30 $\pm$ 10.05	30.10 $\pm$ 9.61	31.20 $\pm$ 10.45	24.82 $\pm$ 8.24	26.93 $\pm$ 8.31
13.5 - 19.5	3	50	8.14 $\pm$ 3.48	9.26 $\pm$ 4.26	9.89 $\pm$ 3.80	9.94 $\pm$ 4.33	9.73 $\pm$ 6.09	8.61 $\pm$ 3.62
	6	50	10.01 $\pm$ 5.38	8.47 $\pm$ 3.97	12.52 $\pm$ 5.68	12.93 $\pm$ 5.31	12.44 $\pm$ 6.46	12.37 $\pm$ 5.71
	12	50	13.35 $\pm$ 5.56	12.18 $\pm$ 6.33	15.76 $\pm$ 6.52	14.85 $\pm$ 6.99	16.99 $\pm$ 6.56	15.95 $\pm$ 6.86
19.5 - 30.0	3	50	3.82 $\pm$ 1.95	3.36 $\pm$ 1.61	3.66 $\pm$ 2.01	3.98 $\pm$ 2.23	3.92 $\pm$ 1.68	4.23 $\pm$ 2.21
	6	50	4.90 $\pm$ 3.19	4.44 $\pm$ 2.17	5.70 $\pm$ 2.69	5.61 $\pm$ 3.44	6.05 $\pm$ 3.39	6.79 $\pm$ 3.55
	12	50	6.28 $\pm$ 3.11	5.53 $\pm$ 2.37	6.91 $\pm$ 3.34	6.44 $\pm$ 3.39	8.41 $\pm$ 3.70	7.85 $\pm$ 4.32

each point. The number of possible baseline crosses for each frequency band was assumed to be the mean of the frequency range present within the band. The mean and standard deviation of the percent of possible baseline crosses was determined for each point.

RESULTS AND DISCUSSION

Percent of possible baseline crosses and standard deviation per EEG band for each bipolar lead of the normal Beagle dog at 3, 6 and 12 months of age is shown in Table 1. The mean for the 1.0 to 3.5 Hz frequency band decreased for all leads from 3 months to 12 months of age, with the exception of the left frontal-left parietal and right parietal-right occipital leads in which there was a slight increase from 6 months to 12 months of age. There was a slight increase in activity in all leads in the 3.5 to 7.5 Hz band from 3 months to 6 months of age; however, from 6 to 12 months of age there was a decrease in this frequency band with the exception of the right parietal-right temporal lead. There was, however, a decrease in the percent of possible baseline crosses for this band in the 12-month dogs in comparison with the 3-month dogs. There was an increase in the percent of possible baseline crosses in the 7.5 to 13.5 Hz band in the 12-month dogs as compared to the 3-month dogs. There was, however, little change between the 3- and 6-month dogs for this frequency band. There was an increase in the percent of possible baseline crosses in the 13.5 to 19.5 Hz band in all leads with the exception of the left frontal-left parietal lead in the 6-month dogs as compared to the 3-month dog. The percent of possible baseline crosses in the 13.5 to 19.5 Hz band for the 12-month-old animals was higher in all leads compared to either the 3-month or 6-month-old dogs. The percent of possible baseline crosses present in the 19.5 to 30.0 Hz showed an increase in the 6-month over the 3-month dogs and an increase in the 12-month over the 6-month animals.

In summary, the percent of possible baseline crosses for the slowest (1.0 to 3.5 Hz EEG frequency band) showed a decrease in activity as the dog increased from 3 months to 1 year of age. There is a corresponding increase in activity in the two higher frequency bands (13.5 to 19.5 Hz and 19.5 to 30.0 Hz EEG bands) as the dog increased from 3 months to 6 months of age. The other EEG frequency bands (3.5 to 7.5 Hz and 7.5 to 13.5 Hz) show no consistent change with increasing age from 3 to 12 months in any of the bipolar leads used in this study. The basic frequency changes of the EEG with age in the dog have been visually observed, however, until now they have not been quantitated. Cognizance of the decrease in percent of possible baseline crosses of the lowest EEG frequency band as a dog increases in age from 3 months through one year is important in determining if there are brain abnormalities present which are characterized by slow wave activity. The knowledge gained from this study will assist in obtaining a better interpretation of the EEG of the developing Beagle dog.

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SECTION V

THERAPY FOR INHALED RADIONUCLIDES

BRONCHOPULMONARY LAVAGE AND DTPA TREATMENT OF AN ACCIDENTAL INHALATION ^{239}Pu EXPOSURE CASE

ABSTRACT

Bronchopulmonary lavage was performed on three occasions on an individual who had accidentally inhaled and deposited in his lung an estimated 450 nCi of ^{239}Pu . The right lung was lavaged on the 8th and 17th day post-exposure and the left lung on the 12th day using large volumes of isotonic saline. Daily treatment with diethylenetriaminepentaacetic acid (DTPA) intravenously was initiated on day 8 post-exposure. Fifty-nine nCi of ^{239}Pu recovered in the lavage fluid was associated predominantly with lung macrophages and relatively little with recovered surfactant. Eighty-seven nCi of ^{239}Pu were excreted in the 11 days following the first lavage; 16 nCi in feces and 71 nCi in urine. In total, 146 nCi of ^{239}Pu and 3.55 nCi of ^{241}Am were recovered during the first 11 days after treatment was initiated. The $^{239}\text{Pu}/^{241}\text{Am}$ ratio of material removed via lavage was slightly higher, urine was slightly lower, and feces about the same as the $^{239}\text{Pu}/^{241}\text{Am}$ ratio of air filter samples obtained at the time of the accident, thus indicating the feasibility of using ^{241}Am lung counting to estimate the ^{239}Pu lung burden at early times following the accident. These data indicate the effectiveness of both bronchopulmonary lavage and DTPA in reducing the body burden of ^{239}Pu following accidental inhalation exposure.

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INTRODUCTION

The treatment of accident cases involving inhalation and pulmonary deposition of ^{239}Pu in man is a complex problem. One approach to treatment is to remove radioactivity from the lung and other tissues, thereby reducing the radiation exposure and minimizing the potential for radiation induced disease. Chelating agents, such as diethylenetriaminepentaacetic acid (DTPA), have been evaluated extensively in experimental animals and man as a means for removing internally deposited plutonium.¹ In situations where the material enters the body in a relatively soluble form and DTPA treatment is initiated promptly, the body burden of ^{239}Pu can be substantially reduced. In other situations where the material is relatively insoluble and/or DTPA treatment is delayed only very small amounts of ^{239}Pu are removed. This poor success with DTPA is frequently the case when relatively insoluble forms of ^{239}Pu are inhaled and deposited in lung.

Recognizing these limitations in treatment of accidental inhalation exposure cases, experimental animal studies were conducted which demonstrated the usefulness of bronchopulmonary lavage for removing inhaled radioactivity from the lung.^{2,3} Results of other research⁴ demonstrated the relative safety of the procedure when used on healthy dogs, and combined with significant experience with pulmonary lavage in treating respiratory disease in man,⁵ lent confidence to the potential usefulness of the procedure for treating human accident cases. This view was further supported by

a report⁶ from a French laboratory where the basic procedure developed in this laboratory was used to remove significant amounts of inhaled ^{239}Pu from the lungs of baboons that had inhaled $^{239}\text{PuO}_2$.

In August 1972, an individual who had been accidentally exposed to a ^{239}Pu aerosol and had an estimated lung burden of ~ 450 nCi was referred to this laboratory for discussion concerning the possible use of bronchopulmonary lavage to reduce his ^{239}Pu lung burden. This report will briefly describe the treatment and recovery of ^{239}Pu from the individual.

PRE-TREATMENT

The exposed individual with his wife, accompanied by the industrial physician from the installation where he was employed, visited this laboratory on the fifth day after the accidental exposure. A majority of the day was spent advising the subject on the bronchopulmonary lavage procedure and its use in man and experimental animals including both potential risks and benefits associated with its use. On the sixth day, the subject indicated he would like to have the lavage procedure performed. Detailed arrangements were immediately developed for the hospitalization and treatment of the subject including arrangements for Dr. J. A. Kylstra and Dr. K. D. Hall of Duke University Medical Center to perform the first lavage in view of their extensive experience with the procedure.⁵ The two subsequent lavages were to be performed by individuals from the Lovelace-Bataan Medical Center. On the seventh day the subject was admitted to the hospital and an extensive pre-lavage medical work-up was performed. Special attention was directed to evaluating the subject's cardiopulmonary function.

TREATMENT

On the eighth day bronchopulmonary lavage was performed on the subject's right lung using the basic procedure of Kylstra.⁵ The right lung was lavaged first because *in vivo* lung counts indicated it contained the largest quantity of ^{239}Pu . Subsequently on the 12th day the left lung was lavaged and on the 17th day the right lung was lavaged a second time. On all three occasions the medical course of the subject was free of complications and similar to that previously reported by Kylstra.⁵ Isotonic saline warmed to body temperature was used as the lavage media. Approximately 95% of the fluid was recovered; a finding in agreement with previous results in experimental dogs.³ The fluid contained appreciable numbers of cells (Table 1), primarily pulmonary macrophages. The cell count decreased with each successive sample obtained at a particular lavage session indicating that the more available cells were removed early. The cell count increased with each lavage treatment suggesting a cellular response to the lavage procedure. Such a response would be anticipated based on histopathological study of lavaged dog lungs.⁴

RECOVERY OF ^{239}Pu and ^{241}Am

Samples of lavage fluid and excreta were analyzed for ^{239}Pu and ^{241}Am using ^{236}Pu and ^{243}Am as tracers with dissolution, chemical separation, electrodeposition and alpha pulse height analysis. The quantity of ^{239}Pu and ^{241}Am recovered in the lavage fluid is shown in Table 1. The quantity of radioactivity recovered decreased in successive fractions for a given treatment paralleling the cell recovery results. That this would be the case is readily apparent from autoradiographs of the recovered cells (Fig. 1) which revealed that the majority of ^{239}Pu was associated with cells. Similar radiochemical results were obtained when supernatant, surfactant and cells fractions were radioanalyzed (Table 2). The material was first centrifuged at 1500 RPM for 10 minutes (4000 g-min) to centrifuge out the cells. The supernatant was then decanted off the cell button and centrifuged at 15,000 RPM for 60 min (1.2×10^6 g-min) or 20,000 RPM for 30 min (1.05×10^6 g-min) to yield a surfactant fraction in the bottom of the tube.

Table 1
Recovery of ^{239}Pu , ^{241}Am , and Cells in Lung Lavage Fluid
in Accidental Inhalation Exposure Case

Lavage Date	Days Post- Exposure	Sample No.	Volume (liters)	Total Cell Count (10^6)	Total Radioactivity		$^{239}\text{Pu}/^{241}\text{Am}$
					^{239}Pu (nCi)	^{241}Am (nCi)	
8/30	8 (right lung)	1	4	48	12.3	0.235	52
		2	4	32	5.4	0.069	78
		3	4	23	4.5	0.066	68
		4	4	16	4.0	0.058	68
		5	4	14	2.3	0.046	51
		6	4	14	1.0	0.035	28
		7	4	<u>6.7</u>	<u>1.8</u>	<u>0.031</u>	<u>58</u>
Sub-Total				150	31.3	0.54	58
9/3	12 (left lung)	1	4	109	6.5	0.219	30
		2	4	32	1.9	0.034	56
		3	4	46	1.5	0.027	54
		4	4	81	1.3	0.021	60
		5	4	56	0.9	0.016	58
		6	4	35	0.9	0.017	54
		7	4	<u>18</u>	<u>0.8</u>	<u>0.017</u>	<u>47</u>
Sub-Total				375	13.8	0.35	40
9/8	17 (right lung)	1	4	1200	11.7	0.218	54
		2	4	170	1.4	0.029	47
		3	4	<u>95</u>	<u>0.8</u>	<u>0.020</u>	<u>52</u>
Sub-Total				1480	<u>13.9</u>	<u>0.27</u>	<u>51</u>
TOTAL - All Lavages					59.0	1.16	51

Figure 1. Microautoradiograph of cells recovered with lavage fluid. Note alpha "stars" associated with pulmonary macrophages.

Table 2
²³⁹Pu Analysis of Subfractions of Lavage Material
 (% Distribution/Lavage Sample)

Sample	Rt. Lung (8/30)	Lt. Lung (9/3)	Rt. Lung (9/8)	Average
# 1 Supernatant	4	10	8	7
Surfactant	2	3	4	3
Cells	94	87	88	90
# 2 Supernatant	3	9	36	16
Surfactant	2	12	11	8
Cells	95	79	53	76
# 3 Supernatant	4	10	20	11
Surfactant	8	32	14	18
Cells	88	58	66	71
# 4 Supernatant	5	14		
Surfactant	4	15		
Cells	91	71		
# 5 Supernatant	4	12		
Surfactant	9	14		
Cells	87	74		
# 6 Supernatant	10	7		
Surfactant	14	10		
Cells	76	83		
# 7 Supernatant	1	13		
Surfactant	1	12		
Cells	98	75		
Average				
Supernatant	3	10	14	9
Surfactant	3	11	6	7
Cells	94	79	80	84

Less radioactivity was recovered in subsequent lavages performed on the 12th and 17th day (Table 1). The removal of substantially less radioactivity in the lavage performed on the 12th day (the first lavage of the left lung) is probably related both to the smaller amount of radioactivity initially present in the left lung as well as the greater elapsed time from exposure. The lower recovery in the lavage performed on the 17th day (the second lavage of the right lung) is no doubt related to the elapse of additional time as well as the fact that a portion of the radioactivity had been removed in the first lavage. These results are similar to observations obtained in dog experiments (see this report, pp. 295 - 304).

Substantial radioactivity was recovered in both urine and feces (Tables 3 and 4). The marked increase in urinary excretion of ²³⁹Pu and ²⁴¹Am following the first lavage is undoubtedly related to the simultaneous initiation of daily intravenous administration of DTPA. DTPA therapy was considered immediately after the accident, however, at that time the accident aerosol was assumed to have been quite insoluble and thus it was presumed that DTPA would be relatively ineffective. On the 7th day, information was obtained to suggest that the accident aerosol may have been more soluble than was originally thought, so the use of DTPA was reconsidered. DTPA was given intravenously; it was not added to the lavage fluid since previous research with inhaled ¹⁴⁴CeCl₃ had suggested that the effectiveness of DTPA in lavage fluid was primarily mediated through absorption of DTPA from the lung into the blood and thus, the DTPA could be as effective if given intravenously with no added benefit from addition of DTPA to the lavage fluid.^{7, 8} The magnitude of the increase in urinary excretion suggests that the ²³⁹Pu and ²⁴¹Am were available in a relatively soluble form. This assumption was subsequently supported by *in vitro* solubility data (see this report, pp. 29 - 36) which indicated a solubility rate (fraction/day) of 10⁻³ to 10⁻¹ for a sample of aerosol

Table 3
Recovery of ^{239}Pu and ^{241}Am in Urine in Accidental Inhalation
Exposure Case - Lung Lavage and DTPA Therapy
(Exposure on 8/22/71)

<u>Date</u>	<u>Pre- or Post-Treatment*</u>	<u>Volume (cc)</u>	<u>^{239}Pu (nCi)</u>	<u>^{241}Am (nCi)</u>	<u>$^{239}\text{Pu}/^{241}\text{Am}$</u>
8-29	Pre	1150	<u>0.112</u>	<u>0.004</u>	<u>28</u>
8-30 & 8-31	Post	3490	10.21	0.36	28
9-1	"	2370	6.61	0.17	39
9-2	"	2590	12.00	0.32	38
9-3	"	1640	5.25	0.14	38
9-4	"	3690	9.48	0.28	34
9-5	"	2200	5.48	0.14	39
9-6	"	1500	3.45	0.14	25
9-7	"	1580	4.95	0.11	45
9-8	"	2840	6.09	0.18	34
9-9	"	2860	5.51	0.12	46
9-10	"	2080	<u>2.37</u>	<u>0.06</u>	<u>40</u>
Post-Treatment Total			71.4	2.02	35

* Lung lavage on 8/30, 9/3 and 9/8
1 gm Dicalcium salt of DTPA daily starting on 8/30

Table 4
Recovery of ^{239}Pu and ^{241}Am in Feces in Accidental
Inhalation Exposure Case - Lung Lavage and DTPA Therapy
(Exposure on 8/22/71)

<u>Date</u>	<u>Pre- or Post-Treatment*</u>	<u>Weight (gm)</u>	<u>^{239}Pu (nCi)</u>	<u>^{241}Am (nCi)</u>	<u>$^{239}\text{Pu}/^{241}\text{Am}$</u>
8-29	Pre	40.4	<u>0.45</u>	<u>0.0046</u>	<u>98</u>
8-31	Post	162.3	1.55	0.031	50
9-1	"	150.3	2.66	0.064	42
9-2	"	64.8	1.01	0.027	37
9-2	"	65.8	2.07	0.049	42
9-3	"	47.0	1.92	0.039	49
9-5	"	32.5	0.55	0.0097	57
9-6	"	71.6	1.80	0.040	45
9-7	"	222.2	1.57	0.038	41
9-8	"	301.9	1.25	0.034	37
9-9	"	140.0	0.82	0.020	41
9-10	"	93.4	<u>0.57</u>	<u>0.0087</u>	<u>66</u>
Post-Treatment Total			15.8	0.36	44

* Lung lavage on 8/30, 9/3 and 9/8
1 gm, Dicalcium salt of DTPA daily starting on 8/30

collected from the accident compared to 10^{-7} to 10^{-6} for high temperature treated $^{239}\text{PuO}_2$ aerosols generated in the laboratory. This finding emphasizes the potential usefulness of in vitro solubility test results as guides in the treatment of accidentally exposed individuals. If the results for day 1 shown in Figure 5 (see this report, pp. 29 - 36) had been available the day after the accident, DTPA treatment could have been immediately indicated.

The rate of fecal excretion of radioactivity appears to be increased post-lavage treatment although recognizing variations in fecal excretion, it is uncertain if the single pre-treatment value is representative. An increased rate might be related to increased numbers of pulmonary macrophage mobilized by the lavage procedure and an associated increase in mucociliary clearance.

Sometime after the accident a portion of an air filter sample collected at the time of the accident became available. Three aliquots were radioanalyzed and another aliquot used in an in vitro solubility study. The radioanalytical results are shown in Table 5. Of special interest are the $^{239}\text{Pu}/^{241}\text{Am}$ activity ratios. The average ratio (42) was similar to that estimated for the material known to be the source of the radioactivity released. The variation (34-46) in the three aliquots may be real and related to variations in different areas of the filter recognizing that the filter represents a summation of all activity collected with possible differences in the $^{239}\text{Pu}/^{241}\text{Am}$ ratio of material released at various times during the accident and in different particle size fractions.

Table 5
Recovery of ^{239}Pu and ^{241}Am on Air Filter Collected Near
Location of Individual Accidentally Exposed via Inhalation

Sample Aliquot	^{239}Pu (μCi)	^{241}Am (μCi)	$^{239}\text{Pu}/^{241}\text{Am}$
#1	1.20	0.0262	46
#2	0.506	0.0148	34
#3	0.984	0.0232	42
Weighted Average			42

A summary of the radioactivity recovered in lavage fluid and excreta is shown in Table 6. A total of 146.2 nCi of ^{239}Pu or about one-third of the subject's lung burden was removed. The $^{239}\text{Pu}/^{241}\text{Am}$ ratios of activity removed from the lung are higher, that of urine lower, and that of feces about the same as that of the air filter sample. The differences are slight and of unknown significance.

SUMMARY

This case representing the first use of bronchopulmonary lavage for removing inhaled radioactivity from man has a number of interesting aspects. First, both the lavage and DTPA treatment contributed to a substantial reduction in the individual's lung burden with no apparent untoward health effects attributable to either treatment. The relative effect of the combined lavage and DTPA treatment may be assessed by comparing whole-body retention of this subject with that of a second individual exposed in the same accident.⁹ The second individual's lung burden was a factor of three lower and he received only DTPA treatment. Using whole-body counter data from the installation where the individuals were employed and normalizing the individuals' body burdens, it is possible to estimate their potential cumulative radiation doses to the lung. On a relative basis, using normalized lung burden, the individual who received both lavage and DTPA treatment, is estimated to receive about one-third the dose of the individual who received only DTPA. This

Table 6
Recovery of ^{239}Pu and ^{241}Am Following Lung Lavage and
DTPA Therapy in an Accidental Inhalation Exposure Case
(Exposure on 8/22/71, Recovery for 11 Days Following First Lavage on 8/30/71)

	^{239}Pu (nCi)	^{241}Am (nCi)	$^{239}\text{Pu}/^{241}\text{Am}$
Lung Lavage			
Right Lung (8/30)	31.3	0.54	58
Left Lung (9/3)	13.8	0.35	39
Right Lung (9/8)	<u>13.9</u>	<u>0.27</u>	<u>52</u>
Total	59.0	1.16	51
Feces	15.8	0.36	44
Urine	<u>71.4</u>	<u>2.02</u>	<u>35</u>
Total	146.2	3.55	41
Air Filter			42

reduction in dose may be attributed both to removal of radioactivity by lavage and DTPA therapy and to a more rapid rate of loss of radioactivity in the lavage and DTPA treated subject than in the individual who received only DTPA. The extent to which this difference in retention is related to the lavage treatment or individual variability is unknown.

The excellent agreement observed between the results of *in vitro* solubility determinations and the enhanced urinary excretion related to DTPA treatment offer strong encouragement to the continued development of *in vitro* solubility test procedures to aid in the selection of therapy regimens for accidental exposure cases.

The relative uniformity of the $^{239}\text{Pu}/^{241}\text{Am}$ ratios for the exposure aerosol, the material removed by lavage, and excreta lends confidence to the assessment of the lung burden of ^{239}Pu by accuracy of such assessments depends upon knowing the $^{239}\text{Pu}/^{241}\text{Am}$ ratio of the exposure aerosol and having this ratio maintained in the lung in the absence of differential behavior of the two radionuclides.

The favorable results obtained in this case with lavage treatment should lead to earlier consideration of lavage in future cases where substantial quantities of relatively insoluble forms of radioactive material are accidentally deposited in the lung. At this time, it is our impression that lavage treatment should be delayed until the second day following an accidental exposure. This allows sufficient time for early mucociliary clearance of material deposited in the trachea and bronchii, thus minimizing the risk of inadvertently washing material from these regions into the deep lung during the lavage procedure. Further, this allows additional time for rigorous external decontamination of the individual if this is necessary as well as careful measurement of the individual's radioactivity burden and critical medical and health physics assessment of the case. Based on animal experience, it is our impression that lavage could be performed at approximately four-day intervals as long as significant radioactivity was being removed to warrant the medical risks associated with use of the procedure. The major medical risk is that associated with anesthetizing the subject for each lavage.

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REMOVAL OF INHALED ^{144}Ce FUSED CLAY FROM THE BEAGLE DOG

LUNG BY BRONCHOPULMONARY LAVAGE

ABSTRACT

The effectiveness of bronchopulmonary lavage for removing ^{144}Ce in fused clay from the lungs was investigated in Beagle dogs. Twelve Beagle dogs were exposed to a ^{144}Ce in fused clay aerosol and 8 of these dogs received 10 saline lavage treatments, 5 on each lung. Four dogs received no lavage treatment. The initial lung burden was reduced by 54% in 6 of the treated dogs and 22 and 26% in 2 dogs. This reduction in initial lung burden over a 56-day treatment period resulted in a reduction in the radiation dose to lung to 90 days by 44% and the potential cumulative dose by 48%. An additional group of 4 dogs was exposed to an aerosol of fused clay only and were given 10 lung lavages over a 56-day period. No significant clinical changes due to treatment were observed in these animals. All of the dogs in this study survived 90 days and are now under observation in the colony.

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INTRODUCTION

Although the safety record of the nuclear industry has been excellent, the increasing use of nuclear energy increases the potential for an accidental release of an aerosol of radioactive particles. Inhalation of these radioactive particles poses a serious potential hazard for individuals who might be exposed in this manner. Once these particles are deposited within the body, the tissues surrounding these particles are subjected to a chronic irradiation which persists until the source of radioactivity is removed by physical, chemical or biological means.

Previous reports^{1,2} from this laboratory have indicated the efficacy and safety of bronchopulmonary lavage for removing inhaled radionuclides from lung. The purpose of this study was to evaluate the therapeutic value and efficacy of bronchopulmonary lavage in the removal of significant amounts of radioactive material from the lung following the inhalation of a potentially fatal dose. The study also evaluates the potential hazard of the treatment when performed on a weekly schedule. The aerosol used in this study, ^{144}Ce in fused clay, was selected because ^{144}Ce in this form is relatively insoluble in body fluids with an effective half-time of retention in the lung of approximately 170 days. The toxicity of ^{144}Ce inhaled in this form by the Beagle dog has been characterized in other experiments in this laboratory (this report, pp. 157 to 166).

MATERIALS AND METHODS

Sixteen Beagle dogs (8 males, 8 females) ranging from 13 to 15 months in age at the time of inhalation exposure were used in this experiment. These dogs were judged to be free from apparent abnormalities following a complete physical examination as well as thoracic radiographs, hematology and serum chemistry studies of the venous blood. The dogs were randomly divided into three groups: Group I (8 dogs) was exposed by inhalation to a ^{144}Ce -containing fused clay aerosol and subjected to repeated lavage; Group II (4 dogs) was exposed by inhalation to a ^{144}Ce -containing fused clay aerosol and not subjected to lavage; and Group III (4 dogs) was exposed by inhalation to a fused clay aerosol containing only stable cerium and subjected to repeated lavage.

Food was withheld on the day of exposure until completion of the exposure. Pre-exposure sedation consisted of 60 mg phenobarbital given orally at 2.5 hours and 0.25 mg triflupromazine/

lb. body weight given 0.75 hours before exposure. The exposure took place in an apparatus designed to permit "nose-only" inhalation exposures and a measure, by whole-body plethysmography, of the dog's respiratory pattern during the exposure period.³ Procedures for the preparation, generation and characterization of the exposure aerosol were the same as those reported previously.⁴ The particle sizes in the exposure aerosol were distributed log-normally with activity median aerodynamic diameters (AMAD) that ranged from 1.6 to 2.1 μm for individual exposures with a σ_g from 1.5 to 1.9. Duration of the exposure was determined individually through the use of a NaI scintillation detector which monitored the buildup of radioactivity in the thorax during the inhalation exposure.

The dogs were housed individually in metabolism cages for two weeks prior to exposure for cage acclimation and pre-exposure sampling. They were returned to these cages following the inhalation exposure and remained there during the first 70 days post-exposure during which time daily collections of urine and feces were made for analysis. At 70 days post-exposure, the dogs were transferred from the metabolism facility to the kennel facility where they will be maintained for the duration of the experiment.

General clinical observations were made daily throughout the 70-day period. Pulmonary function tests were performed on each dog using a routine set of procedures described previously (1969-1970 Annual Report, LF-43, pp. 205-211). Two sets of baseline measurements were obtained before exposure and these were repeated at 30, 60 and 90 days post-exposure. Whole-body counting and scanning procedures were used to assess the initial body burden, the initial lung burden and changes in retained burden of ^{144}Ce due to removal by the lavage procedure as well as by physical and biological reduction. The ^{144}Ce activity removed in the lavage fluid was divided into one-liter quantities and assayed for ^{144}Ce content by gamma counting in a simulated annular detector. This detector as well as a more sensitive 5 in. x 8 in. well detector using a liquid scintillator were used to assay the urine and feces samples by gamma counting. Whole-body counting was performed on several systems; a vertical system in which the dog is supported above two shielded 4" x 4" NaI crystals, a $\sim 4\pi$ whole-body counter using a liquid scintillator, and a longitudinal whole-body scanner.

The bronchopulmonary lavage procedure used was described by Kylstra *et al.*⁵ and modified for use in the Beagle dog. A lavage was performed on the right lung of each treated dog on days 2, 9, or 10, 21, 35 and 49 post-exposure and on the left lung on days post-exposure 7, 14, 28, 42 and 56. At the end of each lavage procedure the dogs that had been exposed to ^{144}Ce in fused clay aerosol (Group I) were given 50 mg of Ca DTPA diluted in 1.5 ml of normal saline by slow intravenous injection.

A 60 ml aliquot (10 ml/liter) of the recovered lavage fluid from each lavage was removed and cell counts performed. A cytocentrifuge specimen was prepared and the slides were stained with May-Grunwald Giemsa and differential cell counts performed.

RESULTS

The whole-body retention of ^{144}Ce inhaled infused clay particles is typified by the data illustrated in Fig. 1(a) obtained from one of the four dogs in this experiment that were exposed by inhalation to ^{144}Ce -labeled fused clay particles but not subjected to lavage. The sharp reduction in body burden during the first two days accompanied by excretion primarily via the feces, represented clearance by mucociliary activity of ^{144}Ce deposited initially in the upper respiratory tract, followed by swallowing and transit through the gastrointestinal tract. Beyond that time, the remaining ^{144}Ce was retained more tenaciously. The curve shown in Fig. 1(a) was fitted to these data by a computerized, non-linear least square method and has an effective half-life of retention

Figure 1. Whole-body retention of ^{144}Ce in dogs exposed by inhalation to ^{144}Ce in fused clay. Dog 11 received no post-exposure treatment while Dog 5 was lavaged 10 times during the first 56 days after exposure. DTPA was given intravenously after each lavage. The dashed vertical lines represent the decrease in body burden with each treatment.

for this longer-term component of 170 days. Previous studies in this laboratory have shown that the initial lung burden of ^{144}Ce in fused clay is closely approximated by the zero-time intercept of this long-term retention component (1968-1969 Annual Report, LF-41, pp. 19-35).

Estimates of initial lung burden for both lavaged and non-lavaged dogs were computed on the basis of the longitudinal scanning data obtained prior to lavage on day 2. A relationship between the integral of the counts under the lung peak in the early post-exposure period and the initial lung burden determined from the whole-body counting data extrapolated to zero time had been derived in earlier experiments in this laboratory involving dogs exposed by inhalation to similar activity levels of ^{144}Ce in fused clay. This relationship was then used with the integrals of the lung peaks on day 2 to determine the values for initial lung burden.

Table 1 lists the dogs in this experiment by their group designation and also their initial lung burdens of ^{144}Ce determined by the above method. The mean $\mu\text{Ci/kg}$ values for Groups I and II are 54 and 52, respectively, with similar ranges in both groups. During the inhalation exposure, the individuals dogs inhaled from 25 to 66 L of ^{144}Ce -contaminated air containing 32 to 54 $\mu\text{Ci/L}$. From the values for individual dogs along with their initial body and lung burdens of ^{144}Ce , it is possible to calculate the percentages of total and lung deposition. A mean total deposition of 62% of the activity inhaled with a range from 40 to 73% was observed. Corresponding values for deposition in the lung are a mean of 23% with a range of 17 to 31%.

From Fig. 1(b), it is apparent that some ^{144}Ce was removed in each of the 10 lavages even though they spanned a period from 2 to 56 days after the inhalation exposure. Table 2 lists the percentage of initial lung burden removed with each lavage for each dog. Since these values have been corrected for the physical decay of the ^{144}Ce , the general decrease reflects a decreased availability with increasing time post-exposure. The recovery of ^{144}Ce from 6 of the 8 dogs (excluding dogs 6 and 7) was quite similar with about 30% of the initial lung burden being removed in 5 lavages of the right lung and about 20% removed from the left lung in 5 lavages. The recoveries from dogs 6 and 7 were approximately one-half of these values. Regardless of these poorer recoveries, the right and left lung recoveries maintained their same relative importance for all 8 dogs as may be seen from the ratios of right and left lung activity to total lung activity.

Another method of analyzing these data is to express the quantity of recovered ^{144}Ce for a given lavage as a percentage of the existing lung burden at the time of lavage. In Figure 2, the presentation is based on lavage recoveries as percentages of the total body burden of ^{144}Ce . Since other experiments with ^{144}Ce in fused clay in this laboratory have shown that the ^{144}Ce in lung represents approximately 95% of body burden during this time period post-exposure, these values are within about 5% of what they would be when expressed as percentage of existing lung burden. The upper curve represents the mean and range for the 6 dogs having the best recovery while the lower 2 curves represent the individual values for dogs 6 and 7. Their data points are on the low side of the range for the first 3 lavages and fall outside the range of the 6 dogs for all of the final 7 lavages. After a decreasing trend for the first 4 lavages, the percent of existing body burden appears to level off at a rather steady value during the period of 21 to 56 days post-exposure.

Urine and feces collections were made daily for all 12 dogs exposed to the fused clay containing ^{144}Ce . Typical sets of data for one dog each from Groups I and II expressed as percentages of the initial lung burden are shown in Fig. 3(a) and (b). The excreta data for dog 9, who was not lavaged, are shown in Fig. 3(a). Both the urine and feces show a slight decrease during the 70-day period due in part to the physical decay of ^{144}Ce (16% in 70 days). The amount of ^{144}Ce excreted in the urine was approximately 2 orders of magnitude less than that in the feces.

Table 1
Experimental Design and Initial Lung Burdens of ^{144}Ce

Group Number	Dog Number	Tattoo Number	Weight Kg	Sex	Lavage	DTPA I. V.	Initial Lung Burden	
							μCi	$\mu\text{Ci/kg}^*$
I	1	555A	10.5	M	x	x	680	64
	2	558A	10.9	M	x	x	680	62
	3	560U	7.3	F	x	x	410	55
	4	558S	6.9	F	x	x	370	54
	5	560S	7.5	F	x	x	400	53
	6	561S	7.9	F	x	x	410	51
	7	556C	9.1	M	x	x	440	48
	8	558C	8.4	M	x	x	410	48
II	9	559B	10.2	M			520	47
	10	562S	7.7	F			610	60
	11	561T	6.4	F			320	50
	12	559A	11.0	M			520	47
III	13	556A	11.1	M	x		-	-
	14	560T	6.2	F	x		-	-
	15	556B	9.1	M	x		-	-
	16	559U	7.9	F	x		-	-

*Kg = Total body weight

Table 2
Removal of ^{144}Ce in Fused Clay Particles by Bronchopulmonary Lavage

	Days PE	% Initial Lung Burdens*								
		Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6	Dog 7	Dog 8	$\bar{X}$
Right Lung	2	15	11	21	23	22	9.4	12	15	16
	9	6.2	8.3	6.2	3.0	4.9	1.5	4.5	9.4	5.5
	21	3.0	1.9	2.9	2.1	6.0	1.5	.53	1.8	2.5
	35	3.2	1.7	1.5	1.8	2.0	.47	.65	3.5	1.9
	49	2.9	3.6	.72	1.4	.40	.23	1.2	2.2	1.6
	Total	30.3	26.5	32.3	31.3	35.3	13.1	18.9	31.9	27.5
Left Lung	7	9.0	4.4	12	7.6	1.8	3.0	4.9	8.1	6.4
	14	6.9	5.6	4.0	5.6	9.8	3.2	1.8	6.4	5.4
	28	2.0	4.1	2.5	2.3	2.5	1.3	.23	4.1	2.4
	42	1.7	1.3	1.9	1.5	2.0	.83	.37	2.0	1.5
	56	2.8	2.1	.84	1.3	1.2	1.4	.29	1.7	1.5
	Total	22.4	17.5	21.2	18.3	17.3	9.7	7.6	22.3	17.0
	Total Lung	52.7	44.0	21.2	18.3	17.3	9.7	7.6	22.3	17.0
Right Lung										
Total		.58	.60	.60	.63	.67	.58	.71	.59	.62
Left Lung										
Total		.42	.40	.40	.37	.33	.42	.29	.41	.38

* Corrected for $T_p = 285$ days

Figure 2. ^{144}Ce recovered in each lavage as a percentage of the body burden immediately prior to that lavage. The upper curve represents the mean of 6 dogs. Two other dogs with lower recoveries are plotted separately.

Figure 3. Excretion of ^{144}Ce in urine and feces expressed as percent initial lung burden. Dog 9 received no post-exposure treatment while Dog 4 received 10 lavage treatments followed by intravenous injections of DTPA.

The dog whose urine and feces data are plotted in Fig. 3(b) received 10 lavages and an intravenous injection of DTPA following each lavage. Excretion of ^{144}Ce in the feces was initially similar to that seen in the unlavaged dog but decreased more rapidly with time post-exposure. Urinary excretion was increased by about one order of magnitude over that in the dog without lavage or DTPA administration. The shape of the urinary excretion curve closely parallels the fecal excretion curve.

Mean cell counts in the recovered lavage fluid decreased from lavages performed on days 2, 7 and 10 by more than 50% by day 14 post-exposure (Table 3). The dogs exposed to ^{144}Ce in clay had higher initial cell count than did the unexposed dogs. Neutrophil percentages in the differential cell counts of the recovered cells on days 7, 10 and 14 post-exposure were greatly elevated in the exposed vs unexposed dogs. The percent neutrophils decreased by day 21 but remained higher in the ^{144}Ce in clay exposed dogs out to day 56 post-exposure, whereas the percent lymphocytes, epithelial cells and other cells did not change significantly at any lavage time.

Slight changes were found in ventilation and gas exchange during pulmonary function tests performed up to 90 days post-exposure. These changes from baseline values of each individual dog were not large enough to fall outside of normal ranges established for this colony.

DISCUSSION

A volume of 6 liters was used for each of the lavages in the present work to insure that adequate washing would occur. Although the largest recovery was associated with the first 2 liters, it is important to note that significant quantities of ^{144}Ce were recovered with each of the 6 liters.

Based on previous studies in this laboratory on dogs exposed by inhalation to ^{144}Ce in fused clay, it is anticipated that the 4 untreated dogs in this experiment with initial lung burdens at 47 to 60 $\mu\text{Ci } ^{144}\text{Ce/kg}$ will die within a period from approximately 6 to 14 months post-exposure. (1968-1969 Annual Report, LF-41, pp. 19-35). Previous deaths in this time period have been due to the progressive loss of normal pulmonary function and eventual death due to pulmonary fibrosis.

Table 3

Cell Counts and Differential Cell Count Values of Lung Cells in Recovered Fluids from Bronchopulmonary Lavages Performed at Various Times Post-Exposure

Parameter*	Days Post-Exposure									
	2	7	10	14	21	28	35	42	49	56
	Dogs 1-8, Group I, ^{144}Ce in Clay									
Cell Count X $10^3/\text{cc}$	199	204	137	98.5	150.5	67	65	49	51	65
% Macrophages	91.1	80.0	52.6	78.1	84.7	89.4	91.2	89.0	90.2	87.3
% Lymphocytes	4.5	3.4	3.0	3.0	2.8	1.2	2.0	2.1	2.2	2.1
% Neutrophils	3.0	15.9	43.8	19.1	10.0	7.1	6.6	7.9	6.5	9.8
% Epithelial cells	1.6	0.5	0.7	0.4	2.3	2.2	2.3	1.1	1.0	0.8
	Dogs 13-16, Group III, Controls									
Cell Counts X $10^3/\text{cc}$	133	123	113	80	281	85	65	57	64	77
% Macrophages	88.3	88.1	92.0	98.3	96.2	96.5	94.8	96.1	91.5	94.1
% Lymphocytes	4.9	2.4	2.3	1.2	1.9	2.3	2.9	1.5	4.6	3.4
% Neutrophils	4.2	7.8	3.5	0.1	1.1	0.5	1.4	1.3	1.7	1.0
% Epithelial cells	2.4	0.9	0.5	0.4	0.5	0.7	0.5	1.2	1.8	1.0

* All values are arithmetic means

The decreasing amount of radioactivity removed by lung washing with increasing time post-exposure has been previously established (1967-1968 Annual Report, LF-34, pp. 207-216). In spite of this decrease, some activity was removed with each successive lavage. After reviewing the ^{144}Ce recovery data for the individual lavages, it was decided that treatment would be terminated after 10 lavages since ^{144}Ce activity recovered for the tenth lavage represented less than 5% of the existing body burden in over one-half of the dogs at that time.

The removal of 44 to 54% (6 dogs) and 23 and 26% (2 dogs) of the initial lung burden by 10 bronchopulmonary lavage procedures significantly reduced the potential for early, serious biological effects from irradiation of the lung. Of the total removed, 62% came from the right lung and 38% from the left. These percentages are similar to the physiological fractions of the total lung even though the left lung was always a few days behind the right lung in the lavage schedule.

The absorbed radiation dose to the lungs of the 4 exposed, untreated dogs was calculated in the following manner. The whole-body retention data for each dog were combined with a function expressing lung burden as a fraction of total body burden to establish an expression for lung burden as a function of time post-exposure, $L(t)$. The cumulative absorbed beta dose to lung was

$$\text{cum rads} = \frac{51.2 \bar{E} A_0}{.014W} \int_0^t L(t) dt$$

Where $\bar{E}$ = average beta energy of ^{144}Ce - ^{144}Pr , 1.25 Mev.

A_0 = initial μCi in lung

W = body weight in grams

$L(t)$ = lung burdens at time t as a fraction of the initial lung burden

Similar calculations were performed for the 8 dogs that were lavaged except that the integral had to be solved graphically due to its stepwise reductions. Cumulative absorbed beta doses to lung to 90 days and their potential infinite values are given in Table 4. The 10 lavages performed during the first 56 days produced an average reduction of 44% in the dose accumulated to 90 days and a 48% reduction in the potential cumulative dose.

The level of activity of ^{144}Ce in the urine was increased in those dogs that received intravenous DTPA but the total removal of ^{144}Ce was less than 2% at 70 days post-exposure. The use of DTPA to remove relatively insoluble particles of ^{144}Ce from the lung could not be justified from the data obtained in this study. The decrease in fecal excretion of ^{144}Ce in the lavaged Group I dogs as compared to the unlavaged Group II dogs appears related to the decreasing body burden in the Group I dogs. The continuing removal of ^{144}Ce from the lung decreased the available material for clearance from the respiratory tract resulting in a decreased excretion rate in the feces.

The higher total cell count removed in the lavage fluid in the first two procedures (day 2 - right lung, day 7 - left lung) of the ^{144}Ce exposed dogs as compared to the unexposed dogs cannot be explained from the existing data. A relative neutrophilic response was found from day 7 lavage to the day 21 post-exposure lavage which appeared to be a result of radiation. Such a response was not observed in any of the lavaged unexposed dogs in Group III. The relative number of neutrophils in the recovered lung cells remained higher in the exposed dogs out to the 56-day lavage which appeared to be a radiation injury response and not related to the lavage procedure itself. The total cell count fell in all dogs lavaged beginning with either the 2nd or 3rd procedure and leveled out at about 50% of the original counts by day 28 post-exposure. It appears as if the cell population was depleted to a specific replacement rate related to one lavage (one lung) per week. In addition to a sparing effect related to reduction in the radiation exposures it is possible that removal of radiation damaged macrophages from the lung may minimize development of pulmonary disease recognizing that cell exudates have been incriminated in the development of chronic lung disease.⁶

Table 4
Cumulative Absorbed Beta Doses to Lung

Dog No.	90 Days		Potential Dose	
	Cumulative Rads	Cumulative Rads ($\mu\text{Ci/kg}$)	Cumulative Rads	Cumulative Rads ($\mu\text{Ci/kg}$)
1	11,000	170	30,000	460
2	13,000	210	40,000	650
3	7,800	140	22,000	390
4	8,800	160	25,000	470
5	8,000	150	23,000	430
6	14,000	280	48,000	940
7	11,000	240	36,000	750
8	8,700	180	24,000	500
	$\bar{X} = 190$		$\bar{X} = 570$	
9	19,000	320	60,000	1000
10	17,000	330	53,000	1000
11	16,000	330	52,000	1000
12	18,000	390	56,000	1200
	$\bar{X} = 340$		$\bar{X} = 1100$	

The small changes in pulmonary function tests were observed in all lavaged dogs but the alterations were non-specific and no significance was ascribed to the changes. The treatment regime used in the present study of 10 lavages in a 56-day period did not appear to pose any serious health hazard to the dogs.

The removal of a significant percent of the ^{144}Ce activity and the resulting decrease in the 90-day and potential cumulative radiation dose support the therapeutic efficacy of bronchopulmonary lavage for the prevention or reduction in severity of radiation-induced diseases of the lung. The reduction in radiation dose to the lungs is probably sufficient, with the possible exception of dogs 2, 6 and 7, to result in the dogs being spared from death due to radiation pneumonitis and pulmonary fibrosis during the first year post-inhalation of ^{144}Ce . It is quite likely, however, despite the treatment that their radiation exposure will be high enough that their lifespan will be shortened by radiation-induced disease such as pulmonary neoplasia.

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SECTION VI

RESPIRATORY TRACT DEPOSITION MODELS

CASTING THE LUNGS *IN SITU*

ABSTRACT

A method for producing flexible silicone rubber casts of the airways of the lungs in situ is described. Casts are made to correspond to lung volumes occurring during normal breathing. The lung is prepared for casting by replacing the air within with CO₂ followed by filling with degassed physiological saline. The saline dissolves the CO₂ gas within the airways allowing for a bubble-free finished cast. Casting compound is then slowly injected through the trachea. The saline diffuses out of the lung and passes out of the thorax through several small slits in the thoracic wall. After the injection is completed the cast lung is allowed to cure in situ before it is removed and the tissue digested away. Finished casts have an overall shape corresponding closely to the shape of the thorax. Casts produced by this in situ method appear to have more realistic geometrical relationships than those produced from excised lungs.

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INTRODUCTION

The accurate description of the anatomical details of respiratory airways is essential for producing satisfactory lung models for predicting particle deposition and localized dose patterns for inhaled materials within the respiratory tract in man and other animals. Morphometric data currently available are incomplete and therefore inadequate for these purposes. Equations for predicting regional particle deposition within the lungs are commonly based on the inertial, gravitational and diffusional forces acting on inhaled particles. The anatomical features considered to be of importance in particle deposition are airway segment shapes and sizes (usually diameters and lengths), the inclination of airways to the force of gravity, and the branching angles. Radii of curvature of airway segments and branch regions, airflow patterns, air mixing within the lungs and cyclic changes in structural relationships during breathing must also be considered in meaningful deposition calculations.

Flexible casts of the airways are useful for replicating anatomical detail. Two major criteria for evaluating such casts are fidelity to original airway anatomy and suitability of the cast for precise morphometry. This report presents a method for producing acceptable flexible casts of the respiratory system by the injection of a casting material in situ. The described method appears to overcome some short-comings inherent in making casts from excised lungs.

METHODS

After an examination of several casting materials, including paraffin, latex, low melting point metals, and several varieties of silicone molding compounds, two silicone rubber industrial molding compounds (Table 1) were chosen. These two materials produce strong, flexible casts with good representation of anatomical detail of structures as small as 100 microns. They also cure at room temperature and have very small shrinkages during curing.

Table 1

Physical Properties of Silicone Rubber Industrial Molding Compounds

	<u>Silastic E*</u>	<u>RTV-700**</u>
<u>Uncured Properties</u>		
color	white	white/green/red
density	1.12 gm/cm ³	1.07 gm/cm ³
viscosity	1,200 poises	600 poises
<u>Cured Properties</u>		
tensile strength	700 psi	600 psi
elongation at rupture	400 percent	400 percent
temperature stability (upper limit)	500° F	---
shrinkage (after 7 days)	0.1 percent	about 1.2 percent

* From Dow Corning Bulletin 61-008a, 1970, Midland, Michigan

** General Electric Product data sheet, Silicone Products Department, Waterford, New York

The procedure for making lung casts in the thorax is outlined in Figure 1. Before the cast is made, several measurements of the functional residual capacity and the tidal volume are taken if possible. The resulting values are used to determine the injection volume and to evaluate the final cast fidelity.

The experimental animal is anesthetized with sodium pentobarbital and the trachea is intubated. The animal is then placed on its back and the tracheal tube connected to a CO₂ reservoir. Breathing this gas kills the anesthetized animal and replaces O₂ and N₂ in the lungs with CO₂. The CO₂ is considerably more water soluble than O₂ and N₂ and helps to produce a bubble-free cast. When casting the lungs of cadavers, as in the case of the human, the lungs are flushed with CO₂ using a respiratory pump.

The inside of the thorax is then vented to the atmosphere by making six or more small ventral-lateral incisions between the ribs and through the thoracic wall allowing the lungs to collapse. The space between the lungs and parietal pleura is filled with physiological saline that has been previously degassed under vacuum. This provides support for the lungs during the remainder of the casting procedure. In the case of a rodent, the entire body may be suspended in degassed saline.

PRE-CASTING
PROCEDURE

MAKE LUNG VOLUME
MEASUREMENTS ON
ANIMAL

CASTING
PROCEDURE

ANESTHETIZE AND
INTUBATE ANIMAL

FLUSH LUNGS*
WITH CO₂ GAS

INFUSE SALINE INTO
THORAX AND LUNGS

INJECT CASTING MATERIAL
INTO LUNGS

POST-CASTING
PROCEDURE

EXCISE LUNGS

DIGEST TISSUE

MEASURE CAST
VOLUME
AND EVALUATE

* - At this time, the animal dies.

Figure 1. Flow chart for preparing a lung cast in situ.

Degassed saline is next slowly introduced into the lungs through the tracheal catheter using the pressure of a 5- to 10-cm column of saline. This pressure inflates the lungs and enhances the diffusion of saline out of the lungs which are permeable to saline. Several (3 to 5) lung volumes of saline are infused, dissolving the CO_2 gas within. This saline infusion requires about 15 to 30 minutes. Fluid, diffusing from the lungs, flows out of the thoracic incisions during the infusion. These incisions, on each side of the thorax, at the apex, mid-region and near the diaphragm, are situated so that fluid will not be trapped within the thorax and interfere with full inflation of the lungs. The animal has now been prepared to receive the casting material.

The casting material is mixed with its catalyst using an electric drill and is then degassed under vacuum. A syringe-type injection system (Fig. 2) is used to slowly inject catalyzed silicone rubber into the lungs through the tracheal catheter. Syringes with volumes of 50 cc, 1 liter and 4 liters are used for injecting rodents, dogs and humans, respectively. A filling time for the lung of about one hour is considered optimal. This avoids excessive pressures within the lungs and is within the working time of the casting material. Saline in the lung diffuses freely out of the lungs and through the pleural membrane as it is displaced by the casting material. The injected casting material is metered and the injection stopped when filling corresponds to a predetermined lung volume.

Casts are made to volumes that correspond to lung volumes occurring during normal breathing. Three methods are used for determining the filling volume of the lungs. Whenever possible we use the results of measurements of functional residual capacity and tidal volume made on unanesthetized dogs in our pulmonary physiology laboratory. When such data are not available, as in the cases of the rodent or human, published values of lung volumes for the appropriate species, size, sex and age are used.^{1,2} In those cases where the lung volume values from the literature are inappropriate, molding compound is injected until the lungs appear to be well-filled and just fill the available space in the thorax without distention of the chest wall. Ideally, 12 hours at room temperature are allowed for curing of the cast before it is removed from the thorax. The cast lungs are then carefully excised from the thorax and placed in a fresh 1 M NaOH solution for about 24 hours at room temperature for tissue digestion. A final washing with a weak acid solution may be used to provide a more durable cast.

Figure 2. Air-driven syringe for the metered dispensing of silicone rubber mold-making compounds.

DISCUSSION

Although airway casts in excised lungs have been prepared by many investigators,³⁻⁸ the in situ method was developed to provide casts of the lungs in their normal anatomical configuration. Gross lung shape is determined by the interplay of the lungs' intrinsic structural properties and constraints imposed by the thoracic walls, diaphragm, heart, major vessels, and other tissues within the thoracic cavity. When detailed airway geometry, including branch angles, must be replicated, these shape constraints become significant. Casts made by three techniques, (1) excision - air drying - vacuum filling;⁷ (2) excision - saline replacement;⁸ and (3) in situ - saline replacement, as herein described, were compared to the casts of their respective thoraces (Fig. 3). The cast made in situ had an overall shape that better approximated that of its thorax. The lobes of excised, inflated lungs have a tendency to overinflate and repel each other, thus changing the branch angles and other anatomical features. Also, the trachea enters the lungs at an abnormal angle in excised lung casts. The in situ method minimizes these and other distortions caused by the lack of normal anatomical support.

The in situ technique has three basic drawbacks: the technique requires a relatively complete animal or human body which, in many instances, may not be available; it requires more time and more careful attention than excised lung techniques; and artifacts may be produced by filling the lungs with 100% CO₂ or saline. For example, flushing the lungs with CO₂ gas causes a condition of lowered pH that may lead to constriction of the smooth muscle of the airways.

Upon microscopic examination of the casts, alveoli with slightly angular shapes are clearly seen and the transition of conducting airways to respiratory surfaces is evident (Fig. 4). The in situ technique, which has been applied successfully to hamsters, rats, Beagle dogs and humans, has provided our laboratory with lung casts suitable for detailed airway morphometry.

Figure 3. A comparison of rat lung casts made by using excised lungs (1 and 2) with a cast made using the in situ technique (3). A cast of the thoracic cavity of a rat (4) is shown for comparison.

Figure 4. Distal airway regions from an in-situ case of a dog lung. The cast has been trimmed so that distal airway detail is visible. Due to the low filling volume the alveoli are not completely cast.

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USE OF A HEAT TRANSFER ANALOGY FOR THE MATHEMATICAL MODEL OF RESPIRATORY TRACT DEPOSITION

ABSTRACT

Mathematical models, predicting the aerosol deposition in the respiratory tract, are reviewed. Data in the literature indicated not only that the air flow in the trachea and major bronchi may not be laminar, but also that the entrance effect of the tube or airway has not been considered. A new approach to a mathematical model of respiratory tract deposition, based on the analogy of the heat and mass transfer, is discussed.

PRINCIPAL INVESTIGATOR

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INTRODUCTION

The deposition of inhaled aerosol particles in the human and experimental animal respiratory tract has been the subject of a number of experimental and theoretical studies. Because of the complexity of the lung anatomy and the calculations involved, most of the mathematical models have been made with fairly simple lung models. Data obtained from these models indicate that the effects of entrance and entrance configuration in each segment, the effect of curvatures of the airways and the effect of turbulent flow at larger airways have not been considered. This report presents a new approach to a mathematical model of the lung.

USE OF HEAT TRANSFER ANALOGY FOR THE CALCULATION OF PARTICLE DEPOSITION PROBABILITIES

There are two ways to improve the mathematical model of respiratory tract deposition. The first is the development of better lung model input data. Data that more closely describe the living lung are currently being collected in our laboratory in morphometric studies (this report, pp. to). The second is the improvement in equations which describe the deposition probabilities due to inertial impaction, sedimentation and Brownian diffusion in each region of airways.

For the small airways where the flow is laminar, most of the deposition probability equations given by Landahl^{1,2} and Beeckmans³ are quite adequate. Based on the equation of motion of a particle, rather than the stopping distance concept, the following probability equation for inertial impaction deposition can be derived

$$P_I = 1 - \frac{2}{\pi} \cos^{-1} (\theta \cdot St) + \frac{1}{\pi} \sin [2 \cos^{-1} (\theta \cdot St)] \quad (1)$$

where θ is the bending angle or change of direction of the airway, $St = \frac{2 \rho_p r_p^2 v}{9 \mu d}$ is the Stokes number, d is the diameter of the tube, ρ_p is the density of the particle, r_p is the radius of the particle, v is the flow velocity in the region and μ is the flow viscosity.

For the larger airways where the flow is turbulent, other equations must be chosen. The influence of turbulent flow on aerosol deposition due to inertial impaction and sedimentation is rather weak compared to diffusion mechanism. The effect of turbulence is to create more uniform

particle concentration across the tube cross-section which is the basis upon which most investigators derive the deposition probabilities due to inertial impaction and sedimentation in the respiratory tract. Therefore, the following will concentrate on the turbulent diffusion deposition. Fuchs⁴ and Davies⁵ reviewed several equations for turbulent diffusion deposition in a circular tube. If, however, the following equations of convective heat transfer with constant properties, and of convective mass transfer are considered, we find that

$$\frac{dT}{dt} = \alpha \nabla^2 T \quad (2)$$

$$\frac{dn}{dt} = D \nabla^2 n \quad (3)$$

where

$$\frac{d}{dt} = \frac{\partial}{\partial t} + u \frac{\partial}{\partial x} + v \frac{\partial}{\partial y} + w \frac{\partial}{\partial z}$$

$$\nabla^2 = \text{Laplace operator} = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}$$

α = thermal diffusivity

D = diffusion coefficient of the particle

T = temperature

n = particle concentration

Accordingly, if the boundary conditions in both cases are similar, such as constant wall temperature, T_w , in heat transfer versus zero concentration at the wall in mass transfer, then the two cases are similar. Because of this similarity between the heat transfer and the diffusion equations, the solutions for both equations must also be similar. Therefore, from the solution of the heat transfer equation, the solution of convective diffusion equation is obtained when the concentration is substituted for the temperature T and the diffusion coefficient D for the thermal diffusivity α . For example, if we replace:

Heat Transfer		Mass Transfer
$Nu = \text{Nusselt number} = \frac{h \cdot 2R}{k}$	by	$Sh = \text{Sherewood number} = \frac{k_m \cdot 2R}{D}$
$Pr = \text{Pradtl number} = \frac{\nu}{\alpha}$	by	$Sc = \text{Schmidt number} = \frac{\nu}{D}$
$Pe = \text{Peclet number} = Pr Re$	by	$Pe = \text{Peclet number} = Sc Re$

where

Re = Reynolds number

k_m = mass transfer coefficient

R = radius of the tube

D = diffusion coefficient

ν = kinetic viscosity of median

k = heat conductivity

h = heat transfer coefficient

α = thermal diffusivity

then, the solution of heat transfer can be applied directly to mass transfer if they have similar boundary conditions. From the particle flux to the tube wall, one can derive the deposition probability due to diffusion as

$$P_D = 1 - \exp \left(-Sh \frac{Dx}{R^2 \nu} \right) \quad (4)$$

where x is the tube length. The Sherewood number in the above equation is not a local Sherewood number, but rather the average Sherewood number over the length x . Since the technology of heat

transfer is well-developed, there exists a number of solutions to the tube flow. In the respiratory tract, however, each segment is rather short, so that the entrance effect plays an important role. In the following, some steady state heat transfer solutions which are suitable for applying to the respiratory tract model are summarized.

Laminar Flow Heat and Mass Transfer, $T_w = \text{Constant}$ or $n = 0$ at Tube Wall

Leveque⁶ gives a solution for the Nusselt number for laminar flow in the entrance region of a circular tube as

$$Nu = 1.077 (Pe)^{1/3} \left(\frac{2R}{x}\right)^{1/3} \text{ for } 100 < Pe \frac{2R}{x} < 5,000$$

Here Nu is the local Nusselt number. The mean Nusselt number can be calculated as

$$\overline{Nu} = \frac{1}{x} \int_0^x Nu \, dx' = 1.616 (Pe)^{1/3} \left(\frac{2R}{x}\right)^{1/3} \text{ for } 100 < Pe \frac{2R}{x} < 5,000$$

Therefore, by similarity, we have the following equation for mass transfer

$$\overline{Sh} = 1.616 (Sc)^{1/3} (Re)^{1/3} \left(\frac{2R}{x}\right)^{1/3} \text{ for } 100 < Sc \, Re \frac{2R}{x} < 5,000 \quad (5)$$

In small airways, where the flow is laminar, the entrance effect can be taken into account by using equation (5), with equation (4), if the ratio of the radius to the length of the airways is satisfied in equation (5).

Turbulent Flow Heat and Mass Transfer, $T_w = \text{Constant}$ or $n = 0$ at Tube Wall

Although there has been a considerable amount of investigation of mean and local turbulent heat-transfer coefficients in entrance regions of tubes, only a few general correlations show any effect of entrance conditions on such coefficients. Nusselt⁶ studied the effect of entrance length and recommended the following equation

$$\overline{Nu} = 0.036 (Re)^{0.8} Pr^{1/3} \left(\frac{x}{2R}\right)^{-0.054} \text{ for } 10 < \frac{x}{2R} < 400$$

This equation has been used extensively for predicting heat transfer coefficients in entrance sections. In terms of mass transfer, it becomes

$$\overline{Sh} = 0.036 (Re)^{0.8} Sc^{1/3} \left(\frac{x}{2R}\right)^{-0.054} \text{ for } 10 < \frac{x}{2R} < 400 \quad (6)$$

Hausen⁷ gave the expression for the average Nusselt number for developing region, turbulent flow as

$$\overline{Nu} = 0.116 [(Re)^{2/3} - 125] (Pr)^{1/3} \left[1 + \left(\frac{2R}{x}\right)^{2/3}\right] \left(\frac{\mu_B}{\mu_W}\right)^{0.14} \text{ for } Re > 2,300$$

where μ_B is the viscosity at bulk liquid temperature and μ_W the viscosity at tube-wall temperature. In the diffusion case, $\mu_B = \mu_W$ because there is no temperature gradient. Therefore

$$\overline{Sh} = 0.116 [(Re)^{2/3} - 125] (Sc)^{1/3} \left[1 + \left(\frac{2R}{x}\right)^{2/3}\right] \text{ for } Re > 2,300 \quad (7)$$

For the fully developed turbulent flow, Prandtl⁷ and Taylor⁷ derived an equation for heat transfer

$$Nu = \frac{0.0384 (Re)^{3/4} Pr}{1 + A (Re)^{-1/8} (Pr - 1)}$$

where

$$A = 1.5 Pr^{-1/6}$$

and

$$A \approx 0.7 \text{ for } Pr \geq 150$$

In the case of mass transfer, this becomes

$$Sh = \frac{0.0384 (Re)^{3/4} Sc}{1 + A (Re)^{-1/8} (Sc - 1)} \quad (8)$$

where

$$A = 1.5 Sc^{-1/6}$$

and

$$A \simeq 0.7 \text{ for } Sc \geq 150$$

In the larger airways where the flow is turbulent, either equation (6) or (7) can be used with equation (4) to calculate the diffusion deposition probability with entrance effect taken into account. If fully developed turbulent flow can be assumed, then equation (8) can be used

Effect of Entrance Configurations

The effect of entrance configurations on the heat-transfer coefficient in a circular tube has been studied by Boelta et al.⁶ They recommended that the heat-transfer coefficient be increased by a factor, f_e

$$f_e = 1 + C_1 \frac{2R}{x} \text{ for } \frac{x}{2R} > 10 \quad (9)$$

where the constant C_1 has different values depending on the entrance configuration. For example, $C_1 \simeq 5$ for a 45°-angle-bend entrance whereas for a 90°-angle-bend entrance, $C_1 \simeq 7$.

In lung deposition calculation, the angle-bend entrance configuration was used in calculating inertial impaction deposition, but not taken into account in diffusion mechanism. The use of equation (9) may improve this situation.

Laminar Flow in Curved Pipes

The influence of curvature is stronger in laminar flow than in turbulent flow. This influence has been studied by several investigators and has been summarized by Schlichting.⁸ Akiyama⁹ used the boundary vorticity method to study the laminar flow heat transfer in curved pipes and gave the formula

$$\frac{Nu}{(Nu)_0} = 0.181 Q (1 - 0.839 Q^{-1} + 35.4 Q^{-2} - 207 Q^{-3} + 419 Q^{-4})$$

where

Nu is for curved pipe

$(Nu)_0$ is for straight pipe

$$Q = (Re^2 \frac{R}{r_c} Pr)^{1/4}$$

r_c = radius of curvature

This means by introducing a factor $f_c = Nu/(Nu)_0$, to the straight pipe, we can take into account the effect of curvature. In the case of mass transfer, it becomes

$$f_c = 0.181 Q (1 - 0.839 Q^{-1} + 35.4 Q^{-2} - 207 Q^{-3} + 419 Q^{-4}) \quad (10)$$

where

$$Q = (Re^2 \frac{R}{r_c} Sc)^{1/4}$$

and

$$Sh = f_c (Sh)_0 \quad (11)$$

In lung deposition calculation, the airways segments were assumed to be straight. In reality, they are curved. The use of equation (11) with equation (4) may overcome this situation.

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APPENDIX A

On the following pages detailed data are presented on selected parameters relative to (a) total body and lung content of radionuclides, and (b) the resultant radiation dose received by individual dogs that have been assigned to longevity or sacrifice studies. In addition, information is provided on the current interpretation of the most prominent clinico-pathological features associated with death of animals. The data are presented recognizing that they are not only valuable reference for scientists in this laboratory but also for scientists in other laboratories who desire to follow in detail the progress of these important studies. The data represent the best information presently available and it must be noted that, with time, certain values and diagnoses will be modified and updated as new and better information becomes available. The methods used in establishing the radiation dose parameters presented have been described in the text of the report.

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1. ⁹⁰SrCl₂ Longevity Dogs

⁹⁰Sr LONGEVITY DOGS
(front and back)

Number			Inhalation Exposure			Initial Body Burden			Long-Term Retained Burden		Radiation Dose to Skeleton								Date of Death	90 Days Since SrCl Inhalation		Comment***
			Date	Age Days	Wt. (kg)						Dose Rate (rads/day)				Cuml. Dose (rads)					9-30-72	Death	
Tattoo	Radiobiol.	Sex				Rank	µCi/kg*	µCi	Rank	µCi/kg*	Initial	Days	9-30-72	Potential at 5000 days	At Death	730** Days	To 9-30-72	Potential to 5000 days	To Death			
157E	01-416	F	67115	431	9.7	1	280	2700	1	120	55	21		7.0	21	18000		73000+	19000	69143	759	E-Fibrosarcoma
164A	02-419	M	67124	387	9.0	9	210	1900	2	120	54			1.3	24			52000+	17000	68344	585	D-Epileptic Seizures
158E	02-416	F	67115	429	10.2	6	240	2400	3	120	54	16		5.3	15	14000		55000+	17000	69311	927	E-Hemangiosarcoma
195C	03-456	F	67275	397	9.3	3	270	2500	4	110	48				30				810	67296	21	D-Hemat. Dyscrasia
195B	02-456	M	67275	389	10.1	4	260	2600	5	100	48				34				1100	67303	28	D-Hemat. Dyscrasia
162F	01-419	F	67124	436	11.2	2	270	3000	6	100	47	21		3.7	20	18000		61000+	22000	69279	886	E-Osteochondrofibrosarcoma
158B	03-416	M	67115	429	9.3	5	240	2200	7	100	47				36				1300	67146	31	E-Hemat. Dyscrasia
159B	02-417	F	67117	430	9.8	8	220	2200	8	98	45				29				660	67135	18	E-Hemat. Dyscrasia
160B	02-418	M	67122	435	9.5	7	230	2200	9	97	44	18		6.2	17	15000		62000+	17000	69255	864	E-Osteosarcoma & Hemangiosarc.
23C	01-261	M	65229	408	9.1	11	160	1500	10	83	37	15		5.7	14	13000		54000+	18000	68233	1099	E-Osteochondrosarcoma
159A	01-417	M	67117	430	11.3	10	180	2000	11	74	34				25				850	67146	29	D-Hemat. Dyscrasia
160C	03-417	F	67117	430	10.4	12	160	1700	12	69	31	7.5		3.2	6.8	7000		28000+	9900	70163	1142	E-Hemangiosarcoma
23B	02-256	M	65208	387	8.0	17	110	880	13	59	27	8.1		2.3	5.9	7600		27000+	15000	70169	1787	E-Osteosarcoma
26F	03-263	F	65231	384	7.8	15	120	940	14	52	24	9.0		3.7	6.4	8900		33000+	18000	70343	1938	E-Osteosarc. & Hemangiosarc.
13A	02-228	M	65123	381	8.3	19	99	820	15	51	23	6.6		1.9	5.4	6400		22000+	10000	69023	1361	D-Cerebellar Hemorrhage
12F	01-228	F	65123	401	8.1	18	110	890	16	50	26	8.6		4.5	8.0	7900		35000+	10000	68074	1046	E-Hemangiosarcoma
162A	01-418	M	67122	434	11.9	13	130	1500	17	50	23	9.5		2.3		8800		32000+	17000	71363	1702	E-Osteochondrosarcoma
22E	02-257	F	65209	396	6.7	21	93	620	18	44	20	4.7	2.8	1.7		4700	11000	17000		2621		
26A	01-262	M	65230	383	7.8	14	120	940	19	41	19	6.4		2.4	5.3	6400		23000+	10000	69173	1404	D-Fibrosarcoma
19B	01-252	M	65201	404	6.4	23	84	540	20	40	18	3.8	2.4	1.4		3900	9700	14000		2629		
22F	01-256	F	65208	395	8.8	16	120	1100	21	34	16	6.2		2.6	5.1	5900		23000+	10000	69287	1540	E-Osteosarcoma
19C	02-252	F	65201	404	7.8	22	87	680	22	28	13	3.3	2.4	1.8		3300	8400	13000		2629		
22A	02-253	M	65202	389	10.5	20	98	1000	23	28	12	6.1		1.5	3.5	6100		20000+	13000	71258	2247	E-Hemangiosarcoma
19D	01-253	F	65202	405	8.7	24	71	620	24	27	12	3.4	2.4	2.0		3500	8700	14000		2628		
40E	03-283	F	65301	383	6.3	28	27	170	25	9.6	4.4	1.5	1.0	.64		1500	3700	5700		2529		
28C	02-271	M	65256	406	7.6	26	30	230	26	9.3	4.3	1.4		.56	.84	1500		5100+	3300	72136	2436	D-Leukemia
39C	02-283	F	65301	385	8.7	29	27	230	27	9.1	4.2	1.0	.62	.33		1100	2500	3700		2529		
38E	01-283	F	65301	391	6.5	27	29	190	28	8.9	4.0	.82	.48	.24		840	2000	2800		2529		
30C	02-272	M	65257	395	8.5	32	19	160	29	8.3	3.8	1.1	.64	.31		1200	2800	3900		2573		
30B	01-272	M	65257	395	8.2	35	17	140	30	7.9	3.7	.91	.53	.26		950	2200	3100		2573		
42D	01-284	F	65302	377	7.8	30	25	200	31	7.7	3.6	1.1	.63	.30		1100	2600	3700		2528		
28B	01-271	M	65256	406	7.2	25	32	230	32	7.1	3.2	1.0	.64	.44		1100	2600	3800		2574		
22D	01-257	M	65209	396	9.1	36	16	150	33	6.8	3.1	.88	.53	.32		930	2200	3200		2621		
30D	03-272	M	65257	395	8.9	31	23	200	34	6.6	3.1	.92	.53	.26		930	2200	3200		2573		
42E	02-284	F	65302	377	8.7	33	19	170	35	6.1	2.8	.83	.52	.29		830	2000	3000		2528		
42F	03-284	F	65302	377	7.3	34	17	120	36	5.7	2.6	.59	.34	.19		670	1500	2100		2528		
26B	01-266	M	65238	391	9.0	37	6.6	59	37	3.2	1.5	.41	.20	.086		410	960	1300		2592		

⁹⁰SrCl₂ Longevity Dogs (Cont. d)

Number		Sex	Inhalation Exposure			Initial Body Burden			Long Term Retained Burden		Radiation Dose to Skeleton				Cuml. Dose (rads)				Days Since ⁹⁰ SrCl Inhalation			Comment***
Tattoo	Radiobiol.		Date	Age Days	Wt. (kg)	Rank	μCi/kg*	μCi	Rank	μCi/kg*	Dose Rate (rads/day)		Potential at 5000 days	At Death	730**	To 9-30-72	Potential to 5000 days	To Death	Death	9-30-72	Death	
35E	02-277	F	65271	380	7.5	38	5.5	41	38	2.3	1.0	.32	.23	.23		350	810	1400		2559		
30G	01-277	F	65271	409	7.0	39	4.6	32	39	2.2	1.1	.37	.23	.12		380	910	1300		2599		
27D	02-267	F	65239	390	10.6	41	4.1	43	40	2.2	.98	.19	.087	.034		190	430	570		2591		
27A	03-266	M	65238	389	9.1	43	4.0	36	41	1.9	.88	.18	.099	.044		180	430	590		2592		
26G	02-266	F	65238	391	7.0	46	3.3	23	42	1.9	.87	.19	.089	.032		180	440	570		2592		
23E	01-265	M	65237	416	7.8	45	3.3	26	43	1.7	.79	.29		.057	.16	270		900	610	71293	2247	D-Bronchial Pneumonia
24B	03-265	M	65237	398	8.2	42	4.0	33	44	1.6	.73	.24	.15	.077		230	580	840		2593		
37F	01-282	F	65300	400	8.1	44	3.3	30	45	1.1	.48	.19	.12	.063		190	460	670		2530		
24A	02-265	M	65237	398	8.0	40	4.2	34	46	1.0	.47	.17	.086	.038		170	400	540		2593		
30E	01-276	M	65270	408	8.1	47	2.8	23	47	1.0	.46	.17	.083	.033		180	400	530		2560		
30F	02-276	F	65270	408	10.4	48	2.6	27	48	1.0	.44	.13	.067	.029		140	320	430		2560		
19A	01-254	M	65203	406	8.7	C	0	0	C	0										2627		
21C	02-254	F	65203	398	8.5	C	0	0	C	0										2627		
24E	01-264	F	65232	393	8.6	C	0	0	C	0										2601		
26E	02-264	F	65232	385	6.9	C	0	0	C	0										2598		
28A	01-273	M	65258	408	9.1	C	0	0	C	0										1572		
30A	03-273	M	65258	396	9.5	C	0	0	C	0										2572		
31A	01-278	M	65272	400	9.1	C	0	0	C	0										2558		
32A	02-278	M	65272	394	8.9	C	0	0	C	0										2558		
33B	03-278	M	65272	388	8.9	C	0	0	C	0										2558		
35F	01-285	F	65305	414	8.1	C	0	0	C	0										2525		
40D	02-285	F	65305	387	9.4	C	0	0	C	0										2525		
42C	03-285	F	65305	380	10.3	C	0	0	C	0										2525		
158A	01-420	M	67115	438	10.2	C	0	0	C	0										1985		
160A	02-420	M	67117	437	9.9	C	0	0	C	0										1983		
162E	03-420	F	67122	436	10.2	C	0	0	C	0										1978		

* kg refers to total body weight.

** Days post-⁹⁰SrCl₂ Inhalation.

***D, E or S --Died, Euthanized or Sacrificed with the most prominent features associated with death.

+ Died before potential infinite dose was received.

2. ⁹⁰SrCl₂ Sacrifice Dogs

⁹⁰Sr SACRIFICE DOGS (front)
¹⁴⁴CeCl₃ LONGEVITY DOGS (back)

				Inhalation Exposure		Initial Body Burden			Long-Term Retained Burden		Radiation Dose to Skeleton					Cuml. Dose (rads)		Date of Death	90 Days Since ⁹⁰ SrCl ₂ Inhalation		Comment**		
Tattoo	Radiobiol.	Sex	Date	Age Days	Wt. (kg)	Rank	μCi/kg*	μCi	Rank	μCi/kg*	Initial	730 Days	On 9-30-72	Potential at 5000 Days	At Death	730 Days	To 9-30-72		Potential to 5000 days	To Death		9-30-72	Death
7B	01-212	M	65081	407	7.6	3	130	990	1	67	29	14		3.0	13	12000		43000+	15000	67279	928	E-Hemangiosarcoma	
4C	02-183	M	64325	405	7.4	2	130	960	2	65	30				23				730	64353	28	S-	
10A	02-215	M	65084	394	10.0	8	100	1000	3	55	22	10		2.2	8.6	9000		31000+	13000	68157	1168	E-Hemangiosarcoma	
8A	02-212	M	65081	402	7.9	1	150	1200	4	51	23	10		2.9	8.4	9000		34000+	15000	68348	1362	E-Osteosarcoma	
9D	01-215	F	65084	398	8.9	4	130	1200	5	47	20	9.5		2.6	8.0	8400		31000+	13000	68305	1316	E-Hemangiosarcoma	
11B	02-216	F	65085	389	9.7	9	91	880	6	47	25				17				600	65116	31	E-Hemat. Dyscrasia	
2B	01-183	M	64325	411	7.7	6	120	920	7	46	21			.001	6.3			7200+	3900	65340	381	S-	
10B	01-216	F	65085	395	7.9	10	87	690	8	44	20	7.1		1.7	4.6	6300		22000+	14000	70293	2034	D-Osteosarc. & Hemangiosarc.	
9B	01-214	M	65083	397	9.6	17	71	680	9	39	18	5.0		.87	3.8	4800		15000+	7600	68355	1367	E-Fibrosarcoma	
9C	02-214	F	65083	397	10.1	15	81	820	10	37	17	7.1		1.0	5.4	6700		20000+	10000	68306	1318	E-Osteosarcoma	
12E	02-230	F	65125	403	8.4	18	69	580	11	36	16	6.0		2.0	3.9	5600		21000+	14000	71314	2380	E-Osteosarc. & Hemangiosarc.	
6B	01-207	M	65054	414	7.6	20	62	470	12	36	16	4.4	2.3	1.1		4400	11000	14000		2776			
5A	02-184	M	64328	391	9.2	7	110	1000	13	35	16			.029	7.2			9600+	3600	65341	379	S-	
8B	01-213	M	65082	403	8.5	16	78	660	14	34	16	5.1		1.7	3.4	4600		18000+	11000	71155	2264	E-Osteochondrosarcoma	
4D	01-184	M	64328	408	9.2	11	83	760	15	31	14				11				350	64356	28	S-	
12B	01-229	F	65124	402	11.0	21	59	650	16	30	13	4.1	2.3	1.2		3900	9900	14000		2706			
6D	03-207	F	65054	414	7.4	13	82	610	17	29	14	4.3	2.5	1.5		4300	11000	15000		2776			
12D	01-230	F	65125	403	7.6	14	82	620	18	28	13			.34	5.4			12000+	4000	66345	585	D-Leukemia	
6C	02-207	F	65054	414	8.2	19	64	520	19	24	11	4.0	2.1	1.1		3800	9700	13000		2776			
9A	02-213	M	65082	396	10.7	22	48	510	20	20	9.3	3.7	2.2	1.5		3500	9000	13000		2748			
4B	01-185	M	64329	409	8.8	23	45	400	21	16	7.1	2.9		1.5	1.9	2800		11000+	7100	72035	2628	D-Baso Squamous Carcinoma	
12C	02-229	F	65124	402	9.6	24	43	410	22	15	6.8	2.3	1.4	.95		2200	5600	8200		2706			
2A	02-182	M	64324	410	6.8	5	120	820	23		33				27				150	64329	5	S-	
4A	01-182	M	64324	404	9.6	12	93	890	24						13				77	64329	5	S-	
5C	03-182	F	64324	387	5.7	C	O	O												64330	6	S-	
4E	03-183	F	64328	405	7.8	C	O	O												64352	24	S-	
2D	03-184	F	64328	414	9.9	C	O	O												65342	380	S-	
6A	04-207	M	65054	414	10.0	C	O	O													2776		
9E	01-217	F	65083	399	8.2	C	O	O												72165	2638	E-Fibrosarcoma	
10C	02-217	F	65085	393	8.9	C	O	O													2745		
12A	01-231	M	65124	403	10.3	C	O	O													2706		
13B	02-231	M	65124	383	9.6	C	O	O												72183	2615	D-Autoimmune Hemolytic Anemia	
13C	03-231	M	65124	383	8.7	C	O	O													2706		
13D	04-231	F	65124	383	6.5	C	O	O													2706		

* kg refers to total body weight.

** D, E, or S -- Died, Euthanized, or Sacrificed and the most prominent features associated with death.

+ Died before potential infinite dose was received.

3. ¹⁴⁴CeCl₃ Longevity Dogs

Number Tattoo Radiobiol.			Inhalation Exposure			Initial Lung Burden Initial Rank μ Ci/kg* μ Ci μ Ci/kg*			Lung				Liver				Skeleton				Date of Death	144 Days Since		Comment***	
			Date	Age (Days)	Wt (kg)				Cumulative (rads)			Cumulative (rads)			Cumulative (rads)			9-30-72	Death						
									730**	To	Potential	To	730**	To	Potential	To	730**			To		Potential	To		
		Sex								Days	9-30-72	Infinite	Death	Days	9-30-72	Infinite	Death	Days	9-30-72	Infinite	Death		9-30-72	Death	
152C	02-407	F	67094	428	8.3	1	360	2900	740			24000+	16000			170000+	41000			25000+	6800	67238	144		D-Pul. Injury
156B	03-408	M	67096	418	8.6	2	320	2800	520			22000+	5800			150000+	5400			22000+	930	67117	21		E-Hemat. Dyscrasia
151B	01-407	M	67094	431	9.8	3	270	2700	460			18000+	5900			130000+	7000			19000+	1200	67125	31		E-Hemat. Dyscrasia
156D	01-408	F	67096	418	10.0	4	210	2100	420			14000+	4000			98000+	3800			14000+	630	67118	22		E-Hemat. Dyscrasia
198E	01-457	F	67278	392	8.6	5	210	1800	400			14000+	13000			98000+	53000			14000+	8400	68288	375		D-Pul. Fibros.
151C	03-407	F	67094	431	10.0	6	190	1900	380			13000+	4200			88000+	5100			13000+	860	67125	31		E-Hemat. Dyscrasia
197D	02-458	F	67279	393	9.0	7	190	1700	360			13000+	4200			88000+	5300			13000+	870	67311	32		D-Hemat. Dyscrasia
199A	02-462	M	67285	397	8.5	8	190	1600	340			13000+	5100			88000+	7600			13000+	1300	67329	44		D-Hemat. Dyscrasia
201G	02-463	F	67290	385	8.3	9	170	1400	310			12000+	3600			79000+	3900			12000+	660	67317	27		D-Hemat. Dyscrasia
153A	02-408	M	67096	430	10.3	10	150	1500	280			10000+	6600			70000+	17000			10000+	2900	67234	138		D-Pul. Injury
195A	01-458	M	67279	401	10.0	11	150	1500	240			10000+	8700			70000+	35000			10000+	5600	68250	336		D-Hepatic Injury
198A	01-462	M	67285	399	8.5	12	140	1200	310	9500		9500	9500	50000		65000+	52000	7800		9700+	8100	69353	799		E-Osteosarcoma
203F	03-463	F	67290	377	6.3	13	140	870	360			9500+	3200			65000+	4300			9700+	730	67326	36		D-Hemat. Dyscrasia
197C	03-462	M	67285	399	8.3	14	130	1100	260			8800+	7500			60000+	29000			9000+	4600	68229	309		D-Hepatic Injury
200A	02-460	M	67283	390	10.2	15	120	1300	190			8200+	7700			56000+	36000			8300+	5800	69062	510		D-Marrow Aplasia
199E	01-460	F	67283	395	7.5	16	110	810	330	7500		7500	7500	40000		51000+	50000	6200		7600	7500	72265	1808		D-Hemangiosarcoma
62F	02-322	F	66082	388	9.6	17	100	960	200	6800		6800	6800	36000		47000+	39000	5600		6900+	6000	68226	874		D-Hepatic Injury
201C	03-460	M	67283	378	8.8	18	94	830	280	6400		6400	6400	34000		44000+	41000	5300		6500	6400	72216	1759		E-Hemangiosarcoma
64A	01-326	M	66096	391	9.0	19	74	660	220	5000		5000	5000	27000		34000	33000	4100		5100	5000	72069	2164		E-Squamous Carcinoma
60B	01-320	F	66075	402	8.6	20	69	590	130	4700		4700	4700	25000		32000	30000	3900		4800	4700	70246	1632		E-Squamous Carcinoma
200E	01-463	F	67290	397	8.3	21	68	560	250	4600		4600	4600	24000		32000	30000	3800		4700	4600	72247	1783		D-Hemangiosarcoma
62E	01-322	M	66082	388	8.1	22	67	540	130	4600		4600	4600	24000		31000	29000	3800		4600	4600	70356	1735		D-Hemangiosarcoma
64C	03-323	F	66084	379	9.3	23	55	520	140	3700		3700	3700	20000		26000	24000	3100		3800	3700	71064	1806		E-Myelogenous Leukemia
62B	02-321	M	66080	386	9.9	24	51	500	110	3500	3500	3500		18000	24000	24000		2900	3500	3500		2385			
63C	02-323	F	66084	383	6.8	25	44	300	170	3000	3000	3000		16000	20000	20000		2500	3000	3000		2381			
66B	03-326	M	66096	385	8.8	26	43	380	120	2900	3000	2900		15000	20000	20000		2400	3000	3000		2369			
65B	02-326	M	66096	386	10.9	27	39	430	110	2700	2700	2700		14000	18000	18000		2200	2700	2700		2369			
61B	01-321	M	66080	397	9.5	28	31	300	68	2100	2100	2100		11000	14000	14000		1700	2100	2100		2385			
60C	02-320	F	66075	402	10.2	29	28	280	130	1900	1900	1900		10000	13000	13000		1600	1900	1900		2390			
63B	01-323	M	66084	383	8.1	30	26	210	130	1800	1800	1800		9400	12000	12000		1500	1800	1800		2381			
54A	01-305	M	66027	407	10.2	31	25	250	43	1700	1700	1700		9000	12000	12000		1400	1700	1700		2438			
54B	02-305	F	66027	407	11.6	32	24	280	43	1600	1600	1600		8600	11000	11000		1300	1700	1700		2438			
52D	01-302	F	66025	410	7.7	33	21	170	43	1400	1400	1400		7600	9800	9800		1200	1400	1400		2440			
55D	02-306	F	66028	407	8.7	34	17	150	45	1200	1200	1200		6100	7900	7900		950	1200	1200		2437			
60D	03-320	F	66075	402	8.8	35	16	140	150	1100	1100	1100		5800	7400	7400		900	1100	1100		2390			
57A	02-308	M	66034	392	8.2	36	15	130	34	1000		1000	1000	5400		7000	6700	840		1000	1000	71034	1826		E-Myeloprolif. Disorder
53B	02-301	F	66024	404	11.0	37	14	150	34	950	950	950		5000	6500	6500		780	970	970		2441			

¹⁴⁴CeCl₃ Longevity Dogs cont'd.

¹⁴⁴CeCl₃ LONGEVITY DOGS, cont'd. (front)

⁹¹YCl₃ LONGEVITY DOGS (back)

Number		Sex	Inhalation Exposure		Wt (kg)	Initial Lung Burden			Initial Body Burden μCi/kg*	Radiation Dose to Tissue												Date of Death	Days Since ¹⁴⁴ Ce Inhalation		Comment***	
			Date	Age (Days)		Rank	μCi/kg*	μCi		Lung				Liver				Skeleton					9-30-72	Death		
										Cumulative (rads)				Cumulative (rads)				Cumulative (rads)								
										730**	To	Potential	To	730**	To	Potential	To	730**	To	Potential	To					
Tattoo	Radiobiol.									Days	9-30-72	Infinite	Death	Days	9-30-72	Infinite	Death	Days	9-30-72	Infinite	Death					
53C	02-302	F	66025	405	9.0	38	14	130	42	950	950	950		5000	6500	6500		780	970	970					2440	
56B	01-308	M	66034	402	10.9	39	14	150	34	950		950	950	5000		6500	6200	780		970	950	71019		1811	D-Myelogenous Leukemia	
52C	01-301	F	66024	409	9.0	40	13	120	44	880	880	880		4700	6000	6000		730	900	900					2441	
55A	01-306	M	66028	407	10.8	41	12	130	33	810	810	810		4300	5600	5600		670	830	830					2437	
57C	02-309	M	66035	393	8.2	42	12	95	24	810	810	810		4300	5600	5600		670	830	830					2430	
51B	01-299	M	66021	408	8.4	43	8.1	68	14	550	550	550		2900	3800	3800		450	560	560					2444	
57B	01-309	M	66035	393	9.3	44	6.9	64	30	470	470	470		2500	3200	3200		390	480	480					2430	
50E	03-297	F	66018	411	8.1	45	6.3	51	12	430	430	430		2300	2900	2900		350	430	430					2447	
50A	01-297	M	66018	411	8.0	46	6.2	50	13	420	420	420		2200	2900	2900		350	430	430					2447	
49A	01-294	M	66013	407	9.9	47	5.5	55	11	370	370	370		2000	2600	2600		310	380	380					2452	
52B	02-299	M	66021	406	10.9	48	5.2	56	10	350	350	350		1900	2400	2400		290	360	360					2444	
49B	02-294	M	66013	407	8.8	49	4.9	43	13	330	330	330		1800	2300	2300		270	340	340					2452	
49D	01-295	F	66014	408	10.9	50	4.7	52	11	320	320	320		1700	2200	2200		260	320	320					2451	
50F	01-298	F	66020	413	8.3	51	4.2	35	13	290	290	290		1500	2000	2000		240	290	290					2445	
49E	02-295	F	66014	408	9.1	52	3.9	36	10	270	270	270		1400	1800	1800		220	270	270					2451	
51A	02-298	M	66020	407	11.1	53	3.6	40	8.6	240	240	240		1300	1700	1700		200	250	250					2445	
50D	02-297	F	66018	411	6.9	54	2.9	20	13	200	200	200		1000	1300	1300		160	200	200					2447	
49G	01-296	F	66017	411	8.4	55	2.6	22	7.5	180	180	180		940	1200	1200		150	180	180					2448	
49C	01-300	M	66013	415	8.7	C	O	O	O																2452	
50C	02-300	F	66017	414	9.1	C	O	O	O																2448	
51C	03-300	M	66021	408	10.4	C	O	O	O																2444	
51E	04-300	F	66021	408	8.4	C	O	O	O																2444	
52A	05-300	M	66021	406	8.5	C	O	O	O																2444	
53A	01-310	F	66024	415	9.3	C	O	O	O																2441	
53D	02-310	F	66024	415	8.1	C	O	O	O																2441	
54C	03-310	F	66027	415	9.2	C	O	O	O																2438	
56A	04-310	M	66034	403	11.8	C	O	O	O																2431	
60A	01-327	M	66075	423	10.1	C	O	O	O																2390	
61C	02-327	F	66080	413	10.0	C	O	O	O																2385	
62A	03-327	M	66080	402	13.2	C	O	O	O																2385	
153D	01-412	F	67094	437	9.3	C	O	O	O									67243				149	S-			
156E	02-412	F	67094	425	6.7	C	O	O	O									67243				149	S-			
197B	01-465	M	67289	410	9.0	C	O	O	O																1811	
198C	02-465	F	67289	410	9.0	C	O	O	O																1811	
201A	03-465	M	67289	391	12.6	C	O	O	O																1811	

*kg refers to total body weight.
 **Days post-inhalation exposure.
 ***D, E, or S -- Died, Euthanized, or Sacrificed and the most prominent features associated with death.
 + Died before potential infinite dose was received.

4. ⁹¹YCl₃ Longevity Dogs

Number Tattoo Radiobiol.			Inhalation Exposure Age (Days) Wt (kg)			Initial Lung Burden Rank μCi/kg* μCi			Initial Body Burden μCi/kg*			Radiation Dose to Tissue												Date of Death	91 Days Y Inhalation 9-30-72 Death			Comment***
												Lung				Liver				Skeleton								
												Cumulative rads				Cumulative rads				Cumulative rads								
												30**	120**	To	Death	30**	120**	To	Death	30**	120**	To	Death					
Sex	Date	(Days)	(kg)	Rank	μCi/kg*	μCi	μCi/kg*	Days	Days	Total	Death	Days	Days	Total	Death	Days	Days	Total	Death	Death	9-30-72	Death						
118E	02-380	F	66320	413	9.3	1	540	5100	1300			+	3400			+	630			+	910	66332		12	D-Hemat. Dyscrasia			
122C	01-383	M	66333	410	9.8	2	300	3000	750			+	2200			+	560			+	800	66353		20	D-Hemat. Dyscrasia			
118F	01-382	F	66326	419	8.0	3	290	2300	780			+	2000			+	460			+	670	66343		17	E-Hemat. Dyscrasia			
119C	01-384	F	66335	423	8.2	4	250	2100	550			+	1900			+	500			+	730	66357		22	D-Hemat. Dyscrasia			
164B	01-423	M	67146	409	9.5	5	250	2300	430			+	1900			+	500			+	730	67168		22	D-Hemat. Dyscrasia			
119D	02-382	F	66326	414	6.5	6	240	1600	720			+	2000			+	600			+	860	66354		28	D-Hemat. Dyscrasia			
123A	02-383	M	66333	409	11	7	240	2600	890			+	1800			+	460			+	670	66354		21	D-Hemat. Dyscrasia			
118A	01-381	M	66322	415	8.1	8	220	1800	550	1900	2400	2640	2640	570	1500	1900	1900	840	2100	2900	2900	72143		2012	E-Squamous Cell Carcinoma			
119A	03-381	M	66322	409	8.3	9	220	1800	550	1900	2400	2640		570	1500	1900		840	2100	2900			2143					
118D	02-381	F	66322	415	8.6	10	200	1800	510	1700	2200	2400		520	1300	1800		760	1900	2600			2143					
120C	03-384	F	66335	420	9.3	11	200	1900	630			+	1600			+	420			+	600	66358		23	E-Hemat. Dyscrasia			
164F	02-423	F	67146	409	9.0	12	200	1800	450			+	1600			+	430			+	620	67170		24	D-Hemat. Dyscrasia			
165A	01-426	M	67153	392	11	13	160	1700	540	1400	1800	1900		420	1100	1400		610	1500	2100			1947					
171F	02-434	F	67163	391	6.3	14	160	1000	710	1400	1800	1900		420	1100	1400		610	1500	2100			1937					
169C	01-434	M	67163	397	8.7	15	150	1300	460	1300	1700	1800		390	1000	1300		570	1400	2000			1937					
118B	01-380	M	66320	413	7.9	16	140	1100	340	1200	1500	1700		360	940	1200		530	1300	1800			2145					
120A	02-384	M	66335	420	11	17	130	1400	550	1100	1400	1600		340	870	1100		490	1200	1700			2130					
164C	03-422	M	67144	407	9.3	18	110	1100	280	950	1200	1300	1300	290	740	970	970	420	1100	1400		68252		473	D-Epileptic Seizure			
169D	01-432	F	67159	393	5.9	19	100	610	240	860	1100	1200		260	670	880		380	960	1300			1941					
164G	01-425	F	67151	414	7.7	20	94	730	170	810	1000	1100		240	630	830		360	900	1200			1949					
174A	01-438	M	67172	385	9.6	21	92	880	190	790	1000	1100		240	620	810		350	880	1200			1928					
171B	02-435	M	67166	394	9.0	22	90	820	230	770	990	1100		230	600	790		340	860	1200			1934					
165F	03-426	F	67153	392	9.2	23	82	750	220	710	900	980		210	550	720		310	790	1100			1947					
166E	02-426	F	67153	390	11	24	73	820	410	630	800	880		190	490	640		280	700	950			1947					
172A	03-435	M	67166	385	8.8	25	68	600	180	580	750	820		180	460	600		260	650	880			1934					
134C	02-385	F	66354	408	9.9	26	66	650	230	570	730	790		170	440	580		250	630	860			2111					
134A	01-385	M	66354	408	9.7	27	62	600	230	530	680	740		160	420	550		240	600	810			2111					
176D	03-438	F	67172	384	9.2	28	60	550	250	520	660	720		160	400	530		230	580	780			1928					
169A	01-435	M	67166	400	10	29	58	600	240	500	640	700	700	150	390	510	510	220	560	750	750	68165		364	D-Epileptic Seizure			
172C	01-433	F	67160	379	7.1	30	53	380	120	460	580	640		140	360	470		200	510	690			1940					
173G	02-433	F	67160	376	7.2	31	52	370	130	450	570	620		140	350	460		200	500	680			1940					
174E	02-438	F	67172	385	8.7	32	51	450	200	440	560	610		130	340	450		190	490	660			1928					
167B	01-431	M	67158	394	11	33	51	540	130	440	560	610		130	340	450		190	490	660			1942					
171E	03-429	F	67156	384	6.4	34	48	310	140	410	530	580		120	320	420		180	460	620			1944					
165G	02-422	F	67144	383	8.2	35	46	380	92	400	510	550		120	310	400		170	440	600			1956					
169B	01-429	M	67156	390	9.9	36	44	440	80	380	480	530		110	290	390		170	420	570			1944					

⁹¹YCl₃ Longevity Dogs cont'd

⁹¹YCl₃ LONGEVITY DOGS, cont'd. (front)
⁹¹YCl₃ SACRIFICE DOGS (front)

¹³⁷CsCl LONGEVITY DOGS (back)

137CsCI LONGEVITY DOGS (back)																														
Number Tattoo Radiobiol.			Sex			Inhalation Exposure Age Wt (Days) (kg)			Initial Lung Burden Rank μCi/kg* μCi			Initial Body Burden μCi/kg*			Radiation Dose to Tissue												Date of Death	91 Y Inhalation 9-30-72	Days Since Inhalation Death	Comment***
															Lung				Liver				Skeleton							
															Cumulative rads			To Death	Cumulative rads			To Death	Cumulative rads			To Death				
Days	Days	Total	30**	120**	Total	30**	120**	Total	30**	120**	Total																			
164D	01-422	M	67144	407	9.3	37	43	400	130	370	470	520	110	290	380	160	410	560		1956										
176E	01-437	F	67170	382	6.8	38	41	280	150	350	450	490	110	270	360	160	390	530		1930										
171A	02-429	M	67156	384	8.2	39	40	320	94	340	440	480	100	270	350	150	380	520		1944										
166C	02-425	M	67151	388	11	40	31	350	64	270	340	370	81	210	270	120	300	400		1949										
174F	02-437	F	67170	381	6.2	41	16	97	85	140	180	190	42	110	140	61	150	210		1930										
167C	04-426	M	67153	389	9.9	42	14	140	64	120	150	170	36	94	120	53	130	180		1947										
118C	01-386	F	66320	447	10.2	C	O	O	O											2145										
119B	02-386	M	66322	442	9.4	C	O	O	O											2143										
121A	04-386	M	66335	435	9.4	C	O	O	O											2140										
164E	01-430	F	67151	420	8.8	C	O	O	O											1949										
165D	02-430	D	67151	396	11.4	C	O	O	O											1949										
165E	03-430	F	67151	396	9.0	C	O	O	O											1949										
166B	04-430	M	67153	394	10.3	C	O	O	O											1947										
167A	01-441	M	67156	413	10.3	C	O	O	O											1944										
167E	02-441	F	67156	413	10.3	C	O	O	O											1944										
171D	03-441	F	67163	405	7.8	C	O	O	O											1937										
174D	04-441	F	67166	390	13.1	C	O	O	O											1934										
176B	05-441	M	67195	389	10.4	C	O	O	O											1905										

*kg refers to total body weight.

**Days post-inhalation exposure.

***D, E, or S -- Died, Euthanized, or Sacrificed and the most prominent features associated with death.

+ Died before total was received.

5. ⁹¹YCl₃ Sacrifice Dogs

Number			Inhalation Exposure			Initial Body Burden			Lung				Liver				Skeleton				Date of Death	91 Y	Days Since Inhalation	Comment***	
									Cumulative rads				Cumulative rads				Cumulative rads								
									30**	120**	To		30**	120**	Total	To	30**	120**	Total	To					
Tattoo	Radiobiol.	Sex	Date	Age (Days)	Wt (kg)	Rank	μCi/kg*	μCi	μCi/kg*	Days	Days	Total	Death	Days	Days	Total	Death	Days	Days	Total	Death	9-30-72	Death		
173F	02-442	F	67179	395	9.1	1	220	2000	260	1900	2400	2600		570	1500	1900		840	2100	2900		1921			
172B	01-442	M	67179	398	7.2	2	220	1600	500			+	1800			+	530			+	750	67206		27	D-Hemat. Dyscrasia
176C	01-443	F	67180	392	8.3	3	170	1500	380	1500		+	1500	440		+	480	650		+	680	67213		33	D-Hemat. Dyscrasia
174C	02-443	M	67180	393	7.9	4	120	970	310	1000	1300	1400		310	800	1100		460	1200	1600		1920			

*kg refers to total body weight.

**Days post-inhalation exposure.

***D, E, or S -- Died, Euthanized, or Sacrificed and the most prominent features associated with death.

+ Died before total was received.

6. ¹³⁷CsCl Longevity Dogs

Number		Injection Exposure		Age		Wt.		Initial Body Burden		Radiation Dose to Whole Body					Cuml. (rads)				Date of Death	137 Days Since Cs Injection		Comment***	
										Rate (rads/day)				At Death	30**	180**	365**	To Death		30**	180**		365**
Tattoo	Radiobiol.	Sex	Block	Date	Days	(kg)	Rank	µCi/kg*	µCi	Initial	30**	180**	365**						At Death			30**	
271D	12-558	F	F	68330	421	7.2	1	4000	29000	72				31				1100	68356		26	D-Hemat. Dyscrasia	
244B	06-522	M	A	68164	402	8.8	2	3900	34000	72	31			30	1300			1400	68197		33	D-Hemat. Dyscrasia	
241F	06-523	F	B	68165	419	8.2	3	3900	32000	71				38				1000	68187		22	D-Hemat. Dyscrasia	
273A	11-558	M	E	68330	405	9.4	4	3800	36000	69				43				950	68349		19	D-Hemat. Dyscrasia	
249D	06-540	M	D	68215	422	10.1	5	3600	36000	68				35				1300	68242		27	D-Hemat. Dyscrasia	
253C	06-539	F	C	68214	393	9.5	6	3500	33000	65				38				970	68236		22	D-Hemat. Dyscrasia	
277F	09-560	F	H	68354	392	7.1	7	3000	21000	54	19	.20	.002		920	1500	1500				1381		
284B	09-562	M	I	69028	394	8.5	8	2900	25000	52	18	.20	.001		900	1500	1500				1341		
282C	10-562	F	J	69028	402	7.6	9	2900	22000	53	25	.59	.020		1000	2000	2000				1341		
280C	09-567	F	L	69052	429	7.9	10	2900	23000	53				32				910	69076		24	E-Hemat. Dyscrasia	
292A	10-567	M	K	69052	377	8.5	11	2900	25000	52	17	.30	.003		790	1500	1500				1317		
241G	05-523	F	B	68165	419	8.6	12	2800	24000	51				27				860	68190		25	D-Hemat. Dyscrasia	
247E	05-539	F	C	68214	428	7.9	13	2800	22000	51				27				910	68241		27	D-Hemat. Dyscrasia	
266C	09-558	M	E	68330	435	7.4	14	2800	21000	50	23	.25	.005		950	1700	1700				1405		
273E	10-558	F	F	68330	405	8.3	15	2800	23000	51	26	.75	.030		1000	2200	2200				1405		
245B	05-522	M	A	68164	392	9.1	16	2700	25000	51	18			5.1	860			1400	68245		81	D-Hemat. Dyscrasia	
279D	10-560	M	G	68354	383	8.1	17	2700	22000	48	22	.50	.010		950	1800	1800				1381		
248A	05-540	M	D	68215	428	9.6	18	2600	25000	48	23	.30	.003		1000	1900	1900				1520		
244E	04-523	F	B	68165	403	7.5	19	2100	16000	37	16	.35	.004		700	1500	1500				1570		
266D	08-558	F	F	68330	435	7.8	20	2100	16000	37	15	.20	.005		670	1200	1200				1405		
279B	07-560	M	G	68354	383	9.9	21	2000	20000	36	19	.60	.020		780	1500	1500				1381		
275E	08-560	F	H	68354	410	7.8	22	2000	16000	37	15	.20	.003		700	1200	1200				1381		
283D	08-562	F	J	69028	423	8.8	23	2000	18000	37	13	.25	.004		640	1100	1100				1341		
292C	08-567	F	L	69052	377	9.0	24	1900	17000	36	14	.40	.008		670	1200	1200				1317		
241A	04-522	M	A	68164	418	10.0	25	1900	19000	36	18			9.0	710			1300	68241		77	D-Hemat. Dyscrasia	
271A	07-558	M	E	68330	421	9.8	26	1900	19000	35	19	.40	.010	.0003	730	1500	1500	1500	70292		693	D-Shock	
283A	07-562	M	I	69028	423	11.2	27	1900	21000	35	14	.30	.005		670	1200	1200				1341		
291A	07-567	M	K	69052	382	10.8	28	1900	21000	35	12	.28	.003		630	1000	1000				1317		
253B	04-539	F	C	68214	393	9.7	29	1800	17000	34	15	.40	.006		650	1300	1300				1521		
247A	04-540	M	D	68215	429	9.8	30	1800	18000	34	17	.60	.010		720	1500	1500				1520		
244C	03-522	M	A	68164	402	6.7	31	1600	11000	28	9.0	.13	.001		430	770	770				1571		
280D	06-562	F	J	69028	405	6.8	32	1600	11000	28	9.8	.25	.003		500	840	850				1341		
279A	05-560	M	G	68354	383	9.5	33	1500	14000	27	11	.11	.002		470	830	840				1381		
278F	06-560	F	H	68354	391	8.4	34	1500	13000	26	13	.30	.010		560	1000	1000				1381		
286D	05-567	F	L	69052	417	8.8	35	1500	13000	27	10	.22	.004		480	820	830				1317		
241E	03-523	F	B	68165	419	9.4	36	1400	13000	26	14	.50	.015		550	1200	1200				1570		
267A	05-558	M	E	68330	435	11.2	37	1400	16000	26	15	.60	.020		580	1300	1300				1405		
268C	06-558	F	F	68330	433	11.0	38	1400	15000	26	17	1.4	.060		630	1500	1500				1405		

137CsCl Longevity Dogs (Cont'd.)

Number				Injection Exposure		Initial Body Burden		Radiation Dose to Whole Body						Cuml. (rads)			Date of Death	137 Days Since Cs Injection		Comment***	
								Rate (rads/day)													
								Tattoo	Radiobiol.	Sex	Block	Date	Age Days	Wt. (kg)	Rank	µCi/kg*		µCi	Initial		30** Days
289D	05-562	M	I	69028	376	9.7	39	1400	14000	26	9.9	.30	.005		480	870	880			1341	
247C	03-540	M	D	68215	429	8.8	40	1300	11000	25	12	.18	.003		470	980	990			1520	
291C	06-567	M	K	69052	382	7.7	41	1200	9200	22	11	.41	.005		520	970	990			1317	
252C	03-539	F	C	68214	407	9.3	42	1200	11000	21	12	.25	.005		480	950	970			1521	
244F	02-523	F	B	68165	403	5.8	43	1100	6400	20	7.2	.083	.001		340	590	590			1570	
278B	04-560	M	G	68354	391	9.5	44	1100	10000	21	10	.20	.003		440	810	820			1381	
241B	02-522	M	A	68164	418	9.8	45	1000	9800	19	7.9	.13	.001		330	640	650			1571	
273F	04-558	F	F	68330	405	8.4	46	1000	8400	19	10	.45	.040		420	880	910			1405	
278D	03-560	F	H	68354	391	10.3	47	1000	10000	19	8.4	.13	.004		350	640	650			1381	
281C	04-562	F	J	69028	404	8.4	48	1000	8400	19	6.5	.15	.006		320	560	570			1341	
281B	03-567	F	L	69052	428	9.8	49	940	9200	17	8.1	.30	.008		350	690	710			1317	
285A	03-562	M	I	69028	393	10.5	50	920	9700	17	6.3	.20	.002		310	540	550			1341	
287A	04-567	M	K	69052	410	10.2	51	900	9200	17	8.0	.28	.007		360	670	690			1317	
249C	02-540	M	D	68215	422	8.8	52	900	7900	17	8.6	.22	.002		340	700	710			1520	
266A	03-558	M	E	68330	435	9.1	53	890	8100	16	7.9	.15	.004		330	630	640			1405	
248C	02-539	F	C	68214	427	8.3	54	880	7300	16	7.6	.11	.002		330	610	610			1521	
241C	01-522	M	A	68164	418	9.7	C	0	0											1571	
244D	01-523	F	B	68165	403	7.2	C	0	0						70081					647	D-Auto Immune Hemolytic Anemis Bacterial Endocarditis
251D	01-539	F	C	68214	408	6.8	C	0	0											1521	
247B	01-540	M	D	68215	429	9.4	C	0	0											1520	
270B	01-558	M	E	68330	423	8.4	C	0	0											1405	
267D	02-558	F	F	68330	435	7.4	C	0	0											1405	
277A	02-560	M	G	68354	392	9.4	C	0	0											1381	
274E	01-560	F	H	68354	419	7.1	C	0	0											1381	
282A	01-562	M	I	69028	402	8.6	C	0	0											1341	
283C	02-562	F	J	69028	395	8.8	C	0	0											1341	
286C	01-567	M	K	69052	417	8.4	C	0	0											1317	
282D	02-567	F	L	69052	426	6.9	C	0	0											1317	

* kg refers to total body weight.
**Days post-137Cs injection.
***D, E or S -- Died, Euthanized or Sacrificed with the most prominent features associated with death.

7. ⁹⁰Y Fused Clay Longevity Dogs

Number		Inhalation Exposure		Age		Wt.		Initial Lung Burden		Initial Body Burden		Radiation Dose to Lung		Cuml. (rads)		Date of Death	Days Since ⁹⁰ Y Clay Inhalation		Comment**
Tattoo	Radiobiol.	Sex	Block	Date	Days	(kg)	Rank	μCi/kg*	μCi	μCi/kg*	μCi	Rate (rads/min)	At Death	∞	To Death		9-30-72	Death	
333A	02-661	M	A	69266	415	10.3	1	5200	53000	6100	62000	12	2.0	64000	55000	69273	7.5		D-Pulmonary Injury
333T	01-661	F	B	69266	415	8.6	2	3600	31000	4800	42000	8.2	0.4	45000	43000	69278	12		D-Pulmonary Injury
347S	02-684	F	D	69322	379	9.8	3	2800	27000	3500	34000	6.3	0	35000	35000	70004	47		D-Pulmonary Injury
340C	03-684	M	C	69322	419	10.6	4	2600	28000	3500	37000	6.0	0	32000	32000	69353	31		D-Pulmonary Injury
332V	01-662	F	B	69267	418	5.5	5	2400	13000	4100	23000	5.4	0	29000	29000	69342	75		D-Pulmonary Injury
339A	04-684	M	C	69322	422	9.8	6	1900	19000	2400	23000	4.4	0	24000	24000	70021	64		D-Pulmonary Injury
335S	03-661	F	B	69266	399	9.6	7	1900	18000	2600	25000	4.3	0	23000	23000	69336	70		D-Pulmonary Injury
334A	04-661	M	A	69266	406	11.4	8	1700	19000	2300	26000	3.8	0	21000	21000	69304	38		E-Pulmonary Injury
341T	03-685	F	D	69323	417	9.0	9	1700	15000	2500	22000	3.8	0	21000	21000	70033	75		D-Pulmonary Injury
340U	01-684	F	D	69322	419	9.8	10	1600	16000	4600	45000	3.8	0	20000	20000	70045	88		D-Pulmonary Injury
341C	02-685	M	C	69323	417	10.1	11	1500	15000	1800	18000	3.5	0	19000	19000	70043	85		D-Pulmonary Injury
340B	05-685	M	C	69323	421	10.6	12	1400	15000	1700	18000	3.3	0	18000	18000	70048	90		D-Pulmonary Injury
334B	02-662	M	A	69267	407	10.6	13	1400	15000	1900	20000	3.2	0	18000	18000	69290	23		D-Pulmonary Injury
332T	04-662	F	B	69267	418	8.0	14	1400	11000	1900	15000	3.2	0	17000	17000	69356	89		E-Pulmonary Injury
347B	04-685	M	C	69323	380	8.5	15	1300	11000	1500	13000	3.0	0	16000	16000	70033	75		D-Pulmonary Injury
335A	03-662	M	A	69267	400	9.6	16	1100	11000	1600	15000	2.5	0	14000	14000	69358	91		D-Pulmonary Injury
343V	01-685	F	D	69323	395	7.1	17	1100	7500	2600	18000	2.4	0	13000	13000	70050	92		D-Pulmonary Injury
406U	04-820	F	H	70258	409	8.4	18	1100	8800	1400	12000	2.4	0	13000	13000	70349	91		D-Pulmonary Injury
339S	05-662	F	B	69267	367	7.7	19	1000	7800	1800	14000	2.3	0	13000	13000	69349	82		D-Pulmonary Injury
406A	03-820	M	G	70258	409	12.0	20	980	12000	1500	18000	2.2	0	12000	12000	71001	108		D-Pulmonary Injury
448U	02-874	F	L	71089	411	8.4	21	900	7600	1800	15000	2.1	0	11000	11000	71230	141		D-Pulmonary Injury
439A	03-863	M	I	71053	402	13.1	22	850	11000	1300	17000	1.9	0	11000	11000	71158	105		D-Pulmonary Injury
343C	03-686	M	C	69325	397	9.3	23	760	7100	860	8000	1.7	0	9400	9400	70077	117		D-Pulmonary Injury
437T	01-863	F	J	71053	406	7.6	24	740	5600	820	6200	1.7	0	9200	9200	71175	122		D-Pulmonary Injury
380B	01-746	M	E	70124	394	9.0	25	730	6600	970	8700	1.7	0	9100	9100	70323	199		D-Pulmonary Injury
451B	04-874	M	K	71089	401	9.5	26	730	6900	1100	10000	1.7	0	9100	9100	71232	143		D-Pulmonary Injury
403T	02-820	F	H	70258	416	6.9	27	710	4900	910	6300	1.6	0	8800	8800	71023	135		D-Pulmonary Injury
449U	01-874	F	L	71089	408	5.9	28	710	4200	1200	7100	1.6		8800			550		
452B	03-874	M	K	71089	401	9.8	29	700	6900	1100	11000	1.6	0	8700	8700	71210	121		D-Pulmonary Injury
341S	02-686	F	D	69325	419	9.8	30	690	6800	940	9200	1.6	0	8600	8600	70123	163		D-Pulmonary Injury
413A	01-821	M	G	70259	383	11.2	31	680	7600	780	8700	1.6	0	8400	8400	71108	214		D-Pulmonary Injury
333B	06-662	M	A	69267	416	11.9	32	680	8000	980	12000	1.5	0	8400	8400	70028	126		D-Pulmonary Injury
448B	04-863	M	I	71053	375	9.8	33	670	6600	850	8300	1.5		8300			586		
402C	01-820	M	G	70258	417	7.0	34	660	4700	790	5500	1.5		8200	8200	71356	463		D-Pulmonary Injury
404U	03-821	F	H	70259	416	5.8	35	640	3700	860	5100	1.5	0	7900	7900	71114	220		D-Pulmonary Injury
434T	02-863	F	J	71053	415	7.3	36	640	4700	810	5900	1.5	0	8000	8000	71176	123		D-Pulmonary Injury
446C	03-864	M	I	71054	380	11.2	37	600	6700	670	7600	1.4		7500	7500	71291	237		D-Pulmonary Injury
436U	01-864	F	J	71054	412	9.1	38	590	5300	930	8500	1.3	0	7300	7300	71259	205		D-Pulmonary Injury

90Y Fused Clay Longevity Dogs (Cont'd.)

Number		Inhalation Exposure					Initial Lung Burden			Initial Body Burden		Radiation Dose to Lung				Date of Death	Days Since Y Clay Inhalation		Comment**
Tattoo	Radiobiol.	Sex	Block	Date	Age Days	Wt. (kg)	Rank	µCi/kg*	µCi	µCi/kg*	µCi	Rate (rads/min) At		Cuml. (rads) To			9-30-72	Death	
												Initial	Death	∞	Death				
371S	03-746	F	F	70124	423	7.8	39	590	4600	670	5200	1.4	0	7300	7300	70306		D-Pulmonary Injury	
400T	04-821	F	H	70259	426	6.5	40	500	3300	560	3600	1.2		6200			745		
378B	04-746	M	E	70124	410	10.3	41	490	5100	700	7000	1.1		6100			880		
333S	02-663	F	B	69268	417	7.6	42	460	3500	590	4400	1.1		5700			1101		
450B	03-875	M	K	71090	406	9.4	43	450	4200	560	5300	1.0		5600			549		
446S	04-864	F	J	71054	380	8.1	44	420	3400	560	4500	.95		5200			585		
332C	01-663	M	A	69268	419	8.5	45	410	3500	500	4300	.95		5100			1101		
449S	04-875	F	L	71090	409	7.9	46	400	3200	560	4400	.92		5000			549		
400U	01-817	F	H	70251	418	7.6	47	400	3000	530	4000	.91		4900			753		
411C	02-821	M	G	70259	394	9.2	48	380	3500	570	5200	.87		4700			745		
439C	02-864	M	I	71054	403	9.7	49	380	3700	700	6800	.87		4700			585		
411D	04-817	M	G	70251	386	9.8	50	380	3700	420	4100	.87		4700			753		
452A	01-875	M	K	71090	402	9.6	51	380	3600	530	5100	.87		4700			549		
449T	02-875	F	L	71090	409	8.2	52	380	3100	540	4400	.87		4700			549		
374T	02-746	F	F	70124	414	8.0	53	370	3000	460	3700	.84		4600			880		
348C	04-686	M	C	69325	376	8.7	54	360	3200	670	5800	.83		4500			1044		
343T	01-686	F	D	69325	397	8.5	55	360	3100	440	3700	.83		4500			1044		
434S	01-867	F	J	71055	417	9.4	56	340	3200	440	4100	.76		4200			584		
407S	02-817	F	H	70251	402	7.2	57	320	2300	440	3200	.73		4000			753		
380D	01-747	M	E	70125	395	9.4	58	300	2900	400	3800	.71		3800			879		
406B	03-817	M	G	70251	402	12.0	59	300	3600	480	5700	.69		3800			753		
446D	04-867	M	I	71055	381	11.4	60	300	3400	460	5200	.69		3700			584		
375U	02-747	F	F	70125	415	7.6	61	290	2200	390	3000	.69		3600			879		
437S	03-867	F	J	71055	408	8.4	62	280	2300	430	3600	.63		3500			584		
441A	02-867	M	I	71055	399	9.0	63	270	2400	340	3100	.62		3400			584		
399A	02-818	M	G	70252	422	9.0	64	260	2300	280	2500	.59		3200			752		
377B	03-747	M	E	70125	412	9.0	65	250	2300	340	3100	.57		3100			879		
450C	01-876	M	K	71091	407	10.4	66	250	2600	270	2800	.57		3100			548		
339U	04-687	F	D	69328	428	7.2	67	240	1700	320	2300	.54		3000			1041		
372S	04-747	F	F	70125	423	9.6	68	230	2200	320	3100	.54		2800			879		
339B	01-687	M	C	69328	428	9.1	69	230	2100	230	2100	.51		2800			1041		
332S	03-663	F	B	69268	419	8.6	70	220	1900	280	2400	.51		2800			1101		
447U	04-876	F	L	71091	414	6.6	71	220	1500	270	1800	.51		2700			548		
335B	04-663	M	A	69268	401	9.8	72	190	1900	280	2700	.44		2400			1101		
408U	01-818	F	H	70252	395	9.0	73	190	1700	260	2400	.44		2400			752		
438S	01-868	F	J	71056	405	9.7	74	190	1800	420	4100	.43		2400			583		
447B	03-868	M	I	71056	379	7.3	75	180	1300	290	2100	.41		2200			583		
377S	01-748	F	F	70126	413	9.9	76	150	1500	190	1900	.34		1900			878		

⁹⁰Y Fused Clay Longevity Dogs (Cont'd.)

Number		Inhalation Exposure					Initial		Initial		Radiation Dose to Lung				Date of Death	Days Since ⁹⁰ Y Clay Inhalation		Comment**
Tattoo	Radiobiol.	Sex	Block	Date	Age Days	Wt. (kg)	Rank	µCi/kg*	µCi	µCi/kg	µCi	Rate (rads/min)	At Death	Cumulative (rads) To Death		9-30-72	Death	
380C	03-748	M	E	70126	396	10.2	77	140	1500	180	1900	.34		1800		878		
339T	02-665	F	B	69269	369	6.4	78	130	830	190	1200	.30		1600		1100		
407B	03-818	M	G	70252	403	10.6	79	130	1300	190	2000	.29		1600		752		
450E	03-876	M	K	71091	407	10.2	80	130	1300	170	1700	.29		1600		548		
448T	02-876	F	L	71091	413	8.3	81	120	960	140	1200	.26		1500		548		
343A	03-687	M	C	69328	400	9.3	82	110	1100	120	1100	.26		1400		1041		
405U	04-818	F	H	70252	403	6.8	83	110	720	150	1000	.24		1300		752		
334C	01-665	M	A	69269	409	8.3	84	100	850	140	1200	.24		1300		1100		
436V	04-868	F	J	71056	414	7.4	85	100	750	180	1300	.23		1200		583		
438B	02-868	M	I	71056	405	8.6	86	98	840	150	1300	.22		1200		583		
379B	02-748	M	E	70126	402	10.7	87	90	960	110	1200	.21		1100		878		
372T	04-748	F	F	70126	424	10.4	88	83	860	92	960	.18		1000		878		
340T	02-687	F	D	69328	425	10.2	89	80	810	100	1100	.18		990		1041		
333E	01-660	M	A	69265	414	9.4	C	0	0	0	0	0		0		1104		
334T	02-660	F	B	69265	405	8.5	C	0	0	0	0	0		0		1104		
349B	01-683	M	C	69321	372	12.2	C	0	0	0	0	0		0		1048		
348S	02-683	F	D	69321	372	9.0	C	0	0	0	0	0		0		1048		
378A	01-745	M	E	70121	407	11.6	C	0	0	0	0	0		0		883		
383U	02-745	F	F	70121	375	6.0	C	0	0	0	0	0		0		883		
407T	01-812	F	H	70247	398	8.0	C	0	0	0	0	0		0		757		
401A	02-812	M	G	70247	413	9.2	C	0	0	0	0	0		0		757		
441B	01-862	M	I	71050	394	8.6	C	0	0	0	0	0		0		589		
438U	02-862	F	J	71050	399	7.8	C	0	0	0	0	0		0		589		
448A	01-873	M	K	71085	407	10.0	C	0	0	0	0	0		0		554		
447W	02-873	F	L	71085	408	6.6	C	0	0	0	0	0		0		554		

* kg refers to total body weight.
**D, E, or S -- Died, Euthanized, or Sacrificed and the most prominent features associated with death.

8. ⁹¹Y Fused Clay Longevity Dogs

⁹¹Y FUSED CLAY LONGEVITY DOGS (front)

⁹¹Y FUSED CLAY LONGEVITY DOGS, cont'd. (back)

Number Tattoo Radiobiol.		Sex	Inhalation Exposure				Initial Lung Burden Rank μCi/kg* μCi		Initial Body Burden μCi/kg* μCi		Radiation Dose to Lung							Cumulative (rads)			Date of Death	91 Y Clay Inhalation		Comment***			
			Block	Date	Age (Days)	Wt (kg)					60** Days	120** Days	250** Days	On 9-30-72	At Death	To 9-30-72	Potential Infinite					To Death	9-30-72		Death		
386T	04-759	F	D	70154	400	13.5	1	360	4900	1000	14000	780	340				170	32000				57000+	45000	70267	113	D-Pul. Injury	
375A	01-722	M	A	70079	369	10.4	2	320	3300	870	9000	690	280	110			85	27000	38000			46000+	40000	70219	140	D-Pul. Injury	
384A	02-758	M	C	70153	404	12.0	3	300	3600	770	9200	650	300	140			54	27000	40000			51000+	47000	70347	194	D-Pul. Injury	
383S	01-760	F	B	70155	409	11.0	4	300	3300	840	9200	650	280	120			67	26000	38000			46000+	42000	70317	162	D-Pul. Injury	
384S	02-759	F	B	70154	405	10.9	5	300	3300	700	7700	650	280	120			40	26000	38000			47000+	44000	70356	202	D-Pul. Injury	
372A	03-724	M	A	70082	380	11.2	6	270	3100	670	7500	590	250	110			42	24000	34000			41000+	38000	70267	185	D-Pul. Injury	
384B	03-758	M	C	70153	404	10.2	7	260	2700	640	6500	560	250	110			24	23000	34000			42000+	40000	71024	236	D-Pul. Injury	
392U	01-761	F	D	70156	368	9.4	8	260	2400	470	4400	560	250	110			71	23000	33000			42000+	36000	70309	153	D-Pul. Injury	
385A	03-759	M	C	70154	401	11.0	9	230	2600	350	3900	500	220	96			47	21000	29000			37000+	33000	70327	173	D-Pul. Injury	
393S	01-758	F	B	70153	362	10.8	10	210	2300	390	4300	450	190	83			38	18000	26000			32000+	29000	70330	177	D-Pul. Injury	
374A	03-722	M	A	70079	369	10.8	11	200	2100	290	3100	420	190	85			59	17000	25000			31000+	27000	70226	147	D-Pul. Injury	
387V	02-760	F	D	70155	399	7.1	12	190	1300	400	2800	410	180	78			75	17000	24000			30000+	24000	70278	123	D-Pul. Injury	
489C	01-951	M	K	71257	382	7.6	13	190	1500	360	2800	410	180	76	12		6.1	17000	24000	28000		29000	29000	72190	298	D-Pul. Injury	
484E	01-953	M	K	71259	398	9.1	14	180	1700	310	2800	400	170	71			19	16000	23000			28000+	26000	72107	213	D-Pul. Injury	
423C	03-835	M	E	70342	391	8.9	15	170	1500	230	2000	360	160	67			38	15000	21000			26000+	23000	71137	160	D-Pul. Injury	
426S	04-834	F	F	70341	386	7.9	16	170	1300	320	2600	340	150	63			22	14000	20000			24000+	23000	71172	196	D-Pul. Injury	
491A	04-952	M	I	71258	368	9.8	17	170	1700	380	3700	370	160	66			22	15000	21000			26000+	24000	72089	196	D-Pul. Injury	
483T	04-951	F	L	71257	396	6.4	18	170	1100	270	1700	350	160	69	12		3.2	14000	21000	25000		26000	26000	72238	346	D-Pul. Injury	
484S	03-952	F	J	71258	397	7.2	19	170	1200	370	2600	360	150	65			31	14000	20000			25000+	23000	72065	172	D-Pul. Injury	
374B	01-724	M	A	70082	372	9.4	20	160	1500	320	3000	340	150	64			51	14000	20000			24000+	21000	70219	137	D-Pul. Injury	
385D	01-759	M	C	70154	401	9.4	21	160	1500	340	3200	340	150	66			28	14000	20000			25000+	23000	70335	181	D-Pul. Injury	
385S	04-758	F	B	70153	400	8.8	22	150	1300	470	4100	310	140	61	11		7.6	13000	19000	22000		23000	23000	71062	274	D-Pul. Injury	
420C	01-834	M	G	70341	401	10.9	23	150	1700	350	3800	330	150	66			43	14000	20000			25000+	21000	71128	152	D-Pul. Injury	
419V	04-835	F	H	70342	415	7.1	24	150	1100	170	1200	330	140	62			39	13000	19000			24000+	21000	71130	153	D-Pul. Injury	
491B	01-952	M	I	71258	368	9.0	25	150	1300	200	1800	320	130	57			23	13000	18000			22000+	21000	72074	181	E-Pul. Injury	
390V	02-761	F	D	70156	376	7.6	26	140	1100	450	3400	300	130	60	11		10	12000	18000			22000+	22000	71043	252	E-Pul. Injury	
492A	03-956	M	I	71264	374	11.3	27	140	1500	300	3200	290	120	47			10	11000	16000			19000+	18000	72115	216	D-Pul. Injury	
422C	02-834	M	E	70341	397	10.8	28	130	1400	200	2200	290	120	54			24	12000	17000			21000+	19000	71155	179	D-Pul. Injury	
485U	02-951	F	J	71257	394	6.2	29	130	830	220	1400	290	120	51	7.8	1.2		11000	16000	19000	20000	20000			382		
489B	01-954	M	K	71260	386	10.0	30	130	1300	240	2400	280	120	50	7.8	1.2		11000	16000	19000	19000	20000			379		
420U	01-836	F	F	70343	403	7.3	31	120	880	250	1800	260	120	52			31	11000	16000			19000+	17000	71137	159	E-Pul. Injury	
420B	01-837	M	G	70344	404	10.4	32	120	1300	210	2200	260	110	50			24	11000	15000			19000+	17000	71153	174	D-Pul. Injury	
422S	02-835	F	H	70342	398	11.3	33	120	1400	280	3200	260	110	45			21	10000	15000			18000+	16000	71150	173	D-Pul. Injury	
490T	02-952	F	J	71258	369	7.9	34	120	910	140	1100	250	110	48	8.2	1.4		10000	15000	18000	18000	18000			381		
430A	01-835	M	E	70342	372	10.8	35	110	1200	560	6500	230	110	47	8.4		4.6	9700	14000	17000		18000+	17000	71272	295	E-Pul. Injury	
425T	03-834	F	F	70341	387	8.2	36	110	940	360	2900	260	110	48	7.8	.024		10000	15000	18000	18000	18000			663		
484V	04-953	F	L	71259	398	6.0	37	110	680	180	1100	240	100	44	7.0	1.1		9600	14000	16000	17000	17000			380		

⁹¹Y Fused Clay Longevity Dogs cont'd.

												Radiation Dose to Lung																
Number		Inhalation Exposure					Initial Lung Burden		Initial Body Burden		Rate (rads/day)					Cumulative (rads)					Date of Death	91Y Clay Inhalation	Days Since					
Tattoo	Radiobiol.	Sex	Block	Date	Age (Days)	Wt (kg)	Rank	µCi/kg*	µCi	µCi/kg*	µCi	Initial	60** Days	120** Days	250** Days	On 9-30-72	At Death	60** Days	120** Days	250** Days	To 9-30-72	Potential Infinite	To Death	Death	9-30-72	Death	Comment***	
376B	02-724	M	A	70082	370	8.4	38	110	900	280	2300	230	100	44	7.1			9500	14000	16000		17000	17000	72162		810	D-Pul. Injury	
422B	03-838	M	E	70348	404	11.4	39	110	1200	160	1800	230	100	44	7.5	.029		9400	13000	16000	17000	17000			656			
428A	02-841	M	G	70351	393	9.4	40	110	1100	200	1900	240	100	45	7.2	.025		9800	14000	17000	17000	17000			653			
484B	03-951	M	I	71257	396	8.6	41	110	930	170	1500	230	96	41	6.3	.93		9200	13000	15000	16000	16000			382			
489S	02-956	F	J	71264	390	8.1	42	110	890	180	1400	240	98	41	6.2	1.0		9400	13000	16000	16000	16000			375			
387S	01-767	F	D	70162	406	7.7	43	100	800	330	2600	220	95	40	6.4	.001		9000	13000	15000	16000	16000			842			
419T	02-838	F	F	70348	421	7.8	44	100	800	250	1900	220	97	42			27	9000	13000			16000+	14000	71135	152	D-Pul. Injury		
490S	03-954	F	L	71260	372	8.1	45	100	850	190	1500	220	94	40			11	9000	13000			16000+	15000	72101	206	D-Pul. Vasc.Inj.		
390T	04-766	F	B	70161	381	8.6	46	97	830	200	1700	210	91	40	6.8	.002		8500	12000	15000	15000	15000			843			
483D	02-953	M	I	71259	398	7.7	47	94	720	140	1100	200	87	38	6.2	1.0		8200	12000	14000	14000	14000			380			
490A	02-954	M	K	71260	372	9.2	48	92	840	150	1400	200	84	35			33	7900	11000			14000+	11000	72019	124	D-Pul.Vasc.Inj.		
492S	04-956	F	J	71264	374	8.0	49	90	720	270	2300	190	74	28	3.5	.47		7500	10000	12000	12000	12000			375			
428S	03-837	F	H	70344	386	7.1	50	89	640	210	1500	200	88	40	7.1	.031		8100	12000	14000	15000	15000			660			
484D	01-956	M	I	71264	401	7.7	51	88	670	170	1300	190	80	35	5.6	.97		7500	11000	13000	13000	13000			375			
488U	03-953	F	L	71259	389	8.5	52	87	740	120	1100	190	81	35	5.7	.92		7600	11000	13000	13000	13000			380			
420A	04-841	M	E	70351	411	12.4	53	82	1000	210	2600	180	84	40	7.9		.89	7500	11000	14000		14000	14000	72047	426	D-Pul. Injury		
383C	01-766	M	C	70161	415	10.1	54	80	820	160	1600	170	75	32	5.1	.001		7100	10000	12000	12000	12000			843			
432A	04-838	M	E	70348	367	9.7	55	80	780	130	1200	170	77	35	6.2	.028		7100	10000	12000	13000	13000			656			
426A	01-838	M	G	70348	393	11.5	56	79	910	160	1900	170	75	33	5.7	.023		7000	10000	12000	13000	13000			656			
485W	04-954	F	J	71260	398	6.6	57	76	500	130	870	160	77	36	6.9	1.4		6900	10000	12000	13000	13000			379			
422T	03-841	F	F	70351	407	9.9	58	75	740	230	2300	160	73	33	6.1	.031		6600	9700	12000	12000	12000			653			
425S	04-837	F	H	70344	390	10.5	59	73	760	270	2800	160	69	30	5.2	.020		6400	9200	11000	11000	11000			660			
491S	02-958	F	J	71265	376	8.1	60	69	560	110	900	150	59	24	3.3	.51		5800	8100	9500	9700	9700			374			
426T	02-837	F	F	70344	389	7.4	61	67	490	200	1500	140	62	27	4.5	.016		5800	8300	9900	10000	10000			660			
487B	01-958	M	K	71265	396	7.2	62	59	430	140	1000	130	57	25	4.4	.82		5300	7600	9100	9400	9500+			374			
391T	02-766	F	D	70161	375	8.4	63	59	500	190	1600	130	55	24	4.0	.001		5100	7400	8800	9100	9100			843			
382B	03-766	M	C	70161	417	6.8	64	57	390	120	850	120	54	24	4.0	.001		5000	7200	8600	8900	8900			843			
431A	01-839	M	E	70349	376	10.1	65	49	500	100	1000	110	46	20	3.4	.013		4300	6200	7400	7700	7700			655			
492C	03-958	M	I	71265	375	7.2	66	47	340	92	660	100	43	18			7.3	4100	5800			7100+	6500	72083	183	D-Pul.Vasc. Inj.		
421T	02-836	F	F	70343	402	9.6	67	45	430	120	1100	95	43	19	3.4	.014		3900	5700	6900	7100	7100			661			
489T	04-958	F	L	71265	391	7.7	68	44	340	97	750	93	39	16	2.5	.41		3700	5300	6200	6400	6400			374			
396X	03-767	F	B	70162	363	7.9	69	44	340	200	1600	96	45	21	4.1	.002		4000	5900	7200	7600	7600			842			
430C	04-836	M	G	70343	373	7.9	70	42	340	90	710	89	38	16	2.4	.006		3600	5100	6000	6200	6200			661			
428T	02-840	F	H	70350	392	5.7	71	41	230	110	610	87	38	17	2.9	.012		3600	5100	6200	6400	6400			654			
488B	04-959	M	I	71266	396	8.1	72	39	310	57	460	82	37	16	2.9	.55		3400	4900	5900	6100	6100			373			
372B	02-722	M	A	70079	377	11.6	73	35	400	270	3100	73	30	12	1.7	.001		2900	4100	4700	4800	4800			925			
387U	02-767	F	B	70162	406	7.8	74	34	260	93	730	72	31	13	2.1	.001		2900	4100	4900	5000	5000			842			

⁹¹Y Fused Clay Longevity Dogs cont'd.

⁹¹Y FUSED CLAY LONGEVITY DOGS, cont'd. (front)

¹⁴⁴Ce FUSED CLAY LONGEVITY DOGS -- SERIES I (back)

Radiation Dose to Lung

Number		Inhalation Exposure					Initial Lung Burden		Initial Body Burden		Rate (rads/day)					Cumulative (rads)							Days Since 91 Y Clay Inhalation					
Tattoo	Radiobiol.	Sex	Block	Date	Age (Days)	Wt. (kg)	Initial Rank	µCi/kg*	µCi	µCi/kg*	µCi	Initial	Days	60**	120**	250**	On 9-30-72	At Death	60**	120**	250**	To 9-30-72	Potential Infinite	To Death	Date of Death	9-30-72	Death	Comment***
396S	04-767	F	D	70162	363	8.8	75	33	300	78	690	73	33	15	2.7	.001			3000	4400	5300	5500	5500				842	
489D	02-959	M	K	71266	392	9.6	76	33	320	65	630	71	29	12	1.8	.29			2800	4000	4700	4800	4800				373	
424S	03-839	F	H	70349	398	9.3	77	31	290	37	340	67	29	13	2.2	.009			2700	3900	4700	4900	4900				655	
488S	01-960	F	J	71267	397	7.3	78	31	230	76	560	67	29	12	2.0	.36			2700	3900	4600	4700	4700				372	
386A	03-763	M	C	70159	405	11.0	79	30	330	38	420	65	29	13	2.3	.001			2700	3900	4700	4800	4800				845	
376A	03-725	M	A	70084	372	8.4	80	29	240	40	340	63	27	11	1.8	.001			2500	3600	4300	4400	4400				920	
420D	03-836	M	E	70343	403	9.3	81	27	250	110	1000	57	25	11	2.0	.008			2300	3400	4100	4200	4200				661	
429S	04-839	F	F	70349	382	10.2	82	27	270	86	880	57	25	11	1.8	.007			2300	3300	4000	4100	4100				655	
484T	03-960	F	L	71267	406	6.6	83	27	180	67	440	57	23	9.4	1.3	.21			2300	3200	3700	3800	3800				372	
383V	04-763	F	D	70159	413	7.3	84	23	170	51	370	50	21	8.9	1.4				2000	2900	3400	3500	3500				845	
422A	02-839	M	G	70349	405	11.6	85	19	230	37	430	42	18	7.5	1.2	.004			1700	2400	2900	2900	2900				655	
425A	01-840	M	G	70350	396	9.1	86	19	180	57	520	41	18	8.2	1.4	.006			1700	2400	2900	3100	3100				654	
487S	04-960	F	J	71267	398	6.6	87	19	130	45	290	41	18	7.8	1.3	.24			1700	2400	2900	2900	3000+				372	
420S	04-840	F	H	70350	410	7.6	88	18	140	42	320	40	18	8.4	1.6	.009			1700	2400	2900	3100	3100				654	
382C	02-763	M	C	70159	415	7.4	89	18	130	27	200	38	17	7.2	1.2				1600	2200	2700	2700	2700				845	
487A	03-959	M	I	71266	397	8.1	90	16	130	30	240	33	15	6.5	1.1	.21			1400	2000	2400	2400	2500+				373	
492B	02-960	M	K	71267	377	8.9	91	16	140	30	270	34	14	6.1	.95	.17			1400	1900	2300	2400	2400				372	
485T	01-959	F	L	71266	404	7.4	92	16	120	27	200	32	15	6.7	1.2	.25			1300	2000	2400	2500	2500				373	
373A	02-725	M	A	70084	378	9.0	93	15	140	37	330	33	14	5.8	.90				1300	1900	2200	2300	2300				920	
383W	01-763	F	B	70159	413	7.8	94	14	110	43	340	31	13	5.2	.76				1200	1700	2000	2100	2100				845	
423U	03-840	F	F	70350	399	8.3	95	13	110	26	220	28	12	5.2	.85	.003			1100	1600	1900	2000	2000				654	
432B	01-841	M	E	70351	370	8.1	96	11	92	25	210	25	11	5.1	.92	.005			1000	1500	1800	1900	1900				653	
370A	01-725	M	A	70084	393	9.6	C	O	O	O	O																920	
385T	01-755	F	B	70147	394	8.1	C	O	O	O	O																857	
389W	02-755	F	D	70147	381	8.4	C	O	O	O	O																857	
381B	03-755	M	C	70147	414	11.2	C	O	O	O	O																857	
420T	01-833	F	H	70338	398	8.7	C	O	O	O	O																666	
424A	02-833	M	E	70338	387	9.8	C	O	O	O	O																666	
431B	03-833	M	G	70338	365	9.2	C	O	O	O	O																666	
428U	04-833	F	F	70338	380	6.3	C	O	O	O	O																666	
488T	01-950	F	L	71256	386	8.6	C	O	O	O	O																383	
483A	02-950	M	J	71256	395	9.1	C	O	O	O	O																383	
485S	03-950	F	K	71256	394	8.3	C	O	O	O	O																383	
488C	04-950	M	I	71256	386	8.2	C	O	O	O	O																383	

*kg refers to total body weight.

**Days post-inhalation exposure.

***D, E, or S -- Died, Euthanized, or Sacrificed and the most prominent features associated with death.

+ Died before potential infinite dose was received.

9. ¹⁴⁴Ce Fused Clay Longevity Dogs (Series I)

												Radiation Dose to Lung												Date of Death	Days Since ¹⁴⁴ Ce Clay Inhalation		Comment***
			Inhalation Exposure				Initial Lung Burden		Initial Body Burden		Rate (rads/day)					Cumulative (rads)					At Death						
Tattoo	Radiobiol.	Sex	Block	Date	Age (Days)	Wt (kg)	Rank	μCi/kg*	μCi	μCi/kg*	μCi	Initial	Days	Days	Days	9-30-72	Death	Days	Days	Days		9-30-72	Potential Infinite				
228B	02-490	M	C	68029	372	8.4	1	210	1700	550	4600	1000	690	570			550	50000	87000		530000+	100000	68172		143	D-Pul. Injury	
210B	01-474	M	A	67348	419	7.9	2	190	1500	430	3400	880	680	530			420	46000	82000		210000+	110000	68156		173	D-Pul. Injury	
209B	02-474	M	A	67348	421	9.1	3	190	1700	300	2800	790	610	480			370	42000	74000		190000+	100000	68164		181	D-Pul. Injury	
208B	01-478	F	B	67355	432	11.0	4	180	2000	450	5000	820	660	530			430	44000	80000		230000+	110000	68172		182	D-Pul. Injury	
211G	02-478	F	B	67355	424	7.5	5	120	890	270	2000	540	420	330			270	29000	51000		130000+	66000	68161		171	D-Pul. Injury	
226C	01-490	M	C	68029	374	7.8	6	96	740	300	2400	430	330	250			190	23000	40000		97000+	55000	68218		189	D-Pul. Injury	
217A	01-491	M	C	68030	407	8.8	7	68	600	130	1100	300	230	170			130	16000	28000		65000+	38000	68216		186	D-Pul. Injury	
211A	03-473	M	A	67347	416	8.1	8	66	540	99	800	300	230	180			98	16000	28000		69000+	46000	68239		257	D-Pul. Injury	
211E	03-477	F	B	67354	423	8.6	9	51	440	120	1100	230	170	130	52		45	12000	21000	42000		57000+	44000	69033		410	D-Pul. Injury
228A	02-491	M	C	68030	373	9.1	10	34	330	67	670	150	110	83	33		1.2	7700	13000	27000		36000	36000	71252		1318	E-Hemangiosarcoma
211D	02-473	M	A	67347	416	7.1	11	27	190	54	380	120	82	58	19		.93	6000	10000	18000		24000	23000	71071		1185	D-Hemangiosarcoma
211F	02-477	F	B	67354	423	8.7	12	19	170	37	320	86	62	47	18	.13		4400	7600	15000	20000	20000			1746		
223A	03-491	M	C	68030	382	9.8	13	15	150	34	330	70	48	34	12	.12		3500	5900	11000	15000	15000			1705		
208D	01-477	F	B	67354	431	5.9	14	15	91	26	150	70	54	42	16	.12		3700	6500	13000	17000	17000			1746		
209C	01-473	M	A	67347	420	9.0	15	11	100	27	240	50	39	30	11.5	.090		2600	4700	9400	13000	13000			1753		
208A	01-476	M	A	67353	430	8.9	C	O	O	O	O														1747		
209D	02-476	F	B	67353	426	7.9	C	O	O	O	O														1747		
220C	01-492	M	C	68032	391	10.2	C	O	O	O	O														1703		

*kg refers to total body weight.
**Days post-inhalation exposure.
***D, E, or S -- Died, Euthanized, or Sacrificed and the most prominent features associated with death.
+Died before potential infinite dose was received

10. ¹⁴⁴Ce Fused Clay Longevity Dogs (Series II)

¹⁴⁴Ce FUSED CLAY LONGEVITY DOGS
- SERIES II (front)

¹⁴⁴Ce FUSED CLAY LONGEVITY DOGS
- SERIES II, cont'd. (back)

Number		Inhalation Exposure					Initial Lung Burden		Initial Body Burden		Radiation Dose to Lung										Date of Death	144 Days Since Ce Clay Inhalation			Comment***						
		Sex	Block	Date	Age (Days)	Wt. (kg)					Rank	µCi/kg*	µCi	µCi/kg*	µCi	Rate (rads/day)						Cumulative (rads)					9-30-72	Death			
Tattoo	Radiobiol.											Initial	60** Days	120** Days	365** Days	On 9-30-72	At Death	60** Days	120** Days	365** Days	On 9-30-72	Potential Infinite	To Death								
315V	02-595	F	D	69149	398	7.2	1	66	470	170	1200	290	210	160			100	15000	26000			70000+	42000	70030		246		D-Pul. Injury			
298B	02-586	M	A	69121	399	9.1	2	65	590	130	1200	290	230	190			120	16000	28000			79000+	45000	69355		234		D-Pul. Injury			
327A	01-642	M	E	69213	387	9.4	3	56	520	120	1100	250	190	140			78	13000	23000			66000+	39000	70121		273		D-Pul. Injury			
479U	04-947	F	L	71225	379	6.8	4	54	360	180	1200	250	150	110	45	37		11000	19000	37000	39000	49000			414						
330S	02-642	F	F	69213	374	8.3	5	53	440	110	920	240	180	140			72	12000	22000			56000+	39000	70127		279		D-Pul. Injury			
297S	03-586	F	B	69121	402	10.4	6	46	470	170	1800	210	160	120	47		12	11000	19000	39000		52000	48000	71141		750		E-Hem. Sarc. Fib. Sarc.			
470A	03-947	M	K	71225	397	11.0	7	44	480	67	730	210	160	120			54	11000	19000			42000+	32000	72135		275		D-Pul. Injury			
465S	03-918	F	J	71176	382	7.9	8	41	330	100	820	190	140	100			72	9600	17000			36000+	22000	71361		185		D-Pul. Injury			
465A	04-918	M	I	71176	382	11.4	9	41	460	61	700	180	140	110			52	9700	17000			45000+	32000	72122		311		D-Pul. Injury			
330U	03-641	F	F	69212	373	6.0	10	37	220	85	510	170	120	86	34		2.5	8300	14000	28000		37000	36000	72194		1077		E-Heman. Sarc.			
315A	01-595	M	C	69149	398	10.9	11	35	380	130	1400	160	100	81	34		4.7	7500	13000	26000		36000	34000	71335		916		D-Heman. Sarc. & Bronch-Alv. Ca.			
330B	04-641	M	E	69212	373	6.3	12	34	220	72	460	150	110	85	30	1.9		8000	14000	27000	34000	35000			1157						
303A	01-586	M	A	69121	422	9.5	13	33	320	76	720	150	110	83			58	7700	13000			31000+	19000	69314		193		D-Pul. Vasc. Occlusive			
454A	03-883	M	G	71106	402	8.8	14	32	280	68	600	140	110	79	24	10		7500	13000	24000	27000	29000			533						
453S	04-883	F	H	71106	408	8.0	15	29	230	38	300	130	100	81	31	16		6900	12000	25000	29000	33000			533						
464B	01-918	M	I	71176	385	9.4	16	27	250	45	420	130	86	63	23	15		6300	11000	20000	22000	26000			463						
310T	02-594	F	D	69148	421	8.9	17	26	230	130	1100	120	88	68	26		6.2	6100	11000	21000		28000	27000	71183		765		E-Heman. Sarc.			
460S	02-918	F	J	71176	419	7.9	18	24	190	80	630	120	76	56	21	14		5600	9600	18000	20000	24000			463						
480S	02-947	F	L	71225	373	8.3	19	24	200	51	430	110	80	61	23	19		5700	9900	19000	20000	25000			414						
312B	03-594	M	C	69148	399	9.0	20	24	210	49	440	110	81	62	24	1.2		5600	9900	20000	26000	26000			1221						
298S	03-585	F	B	69120	398	10.4	21	23	240	62	650	100	76	58	23	1.1		5400	9300	18000	25000	25000			1249						
455B	01-883	M	G	71106	402	11.7	22	19	220	60	700	86	66	51	18	8.5		4500	8000	16000	18000	20000			533						
471A	01-947	M	K	71225	397	7.5	23	19	150	34	260	91	63	46	17	14		4500	7700	15000	16000	19000			414						
453T	02-883	F	H	71106	408	6.4	24	18	110	46	290	80	59	45	16	8.4		4100	7200	14000	16000	18000			533						
315U	01-594	F	D	69148	397	8.3	25	18	150	51	420	82	60	45	17	.91		4200	7400	14000	19000	19000			1221						
304S	01-585	F	B	69120	386	7.4	26	17	120	35	260	77	57	43	16	.74		4000	6900	14000	18000	18000			1249						
311B	03-593	M	C	69147	400	9.3	27	14	130	20	190	62	45	34	12	.62		3200	5500	11000	14000	14000			1222						
328T	02-641	F	F	69212	385	10.6	28	13	140	26	270	60	44	33	12	.69		3100	5300	10000	13000	13000			1157						
467A	03-916	M	I	71175	373	12.2	29	13	160	26	310	59	44	33	10	6.4		3100	5400	10000	11000	12000			464						
467T	04-946	F	L	71224	422	6.4	30	13	81	18	120	61	43	34	13	11		3000	5300	11000	11000	14000			415						
297B	02-585	M	A	69120	401	9.6	31	12	110	50	480	54	35	27	11	.46		2600	4400	8700	12000	12000			1249						
326C	01-641	M	E	69212	391	9.4	32	12	110	18	170	55	40	30	11	.70		2800	4900	9400	12000	13000			1157						
463A	02-916	M	I	71175	411	10.9	33	12	130	20	220	56	40	29	11	7.2		2800	4900	9300	10000	12000			464						
480B	03-946	M	K	71224	372	8.2	34	11	91	20	170	53	36	27	11	8.8		2600	4500	8700	9200	12000			415						
454S	04-882	F	H	71105	401	9.6	35	10	95	40	390	45	35	27	9.3	4.5		2400	4200	8300	9400	10000			534						
454E	03-882	M	G	71105	401	8.9	36	9.8	87	21	190	47	33	25	9.4	4.9		2300	4100	8000	9100	10000			534						
305V	02-584	F	B	69119	382	6.9	37	9.8	67	18	120	45	29	21	7.9	.36		2200	3600	6800	9000	9000			1250						

¹⁴⁴Ce Fused Clay Longevity Dogs (Series II) cont'd.

Number		Inhalation Exposure					Initial Lung Burden		Initial Body Burden		Rate (rads/day)					Cumulative (rads)						Date of Death	144Ce Days Since Inhalation	Comment***		
		Sex	Block	Date	Age (Days)	Wt (kg)					Rank	µCi/kg*	µCi	µCi/kg*	µCi	Initial	60** Days	120** Days	365** Days	On 9-30-72	At Death				60** Days	120** Days

460T	04-916	F	J	71175	418	7.4	38	9.5	70	30	220	44	33	25	9.6	6.6	2300	4000	7900	8700	10000		464	
327B	01-640	M	E	69211	385	9.0	39	8.0	72	16	140	36	25	19	7.6	.48	1800	3100	6100	8200	8300		1158	
323V	02-640	F	F	69211	408	7.8	40	7.8	60	12	91	35	26	20	7.2	.41	1800	3200	6200	8000	8100		1158	
303B	03-584	M	A	69119	389	6.7	41	7.6	51	17	120	34	27	21	9.1	.44	1800	3300	6800	9300	9400		1250	
310S	02-593	F	D	69147	420	9.1	42	6.3	57	9.4	86	29	20	15	6.1	.30	1500	2500	4900	6600	6600		1222	
469S	02-946	F	L	71224	397	7.2	43	5.8	42	13	91	27	20	16	6.3	5.2	1400	2500	5000	5300	6700		415	
478B	01-946	M	K	71224	379	9.6	44	5.7	54	10	99	26	20	16	6.5	5.4	1400	2500	5100	5400	6800		415	
308B	01-593	M	C	69147	402	10.3	45	5.4	55	7.4	76	24	18	14	5.6	.29	1300	2200	4400	6000	6100		1222	
454C	01-882	M	G	71105	401	9.5	46	5.4	51	14	130	24	19	15	5.2	2.5	1300	2300	4500	5200	5800		534	
464T	01-916	F	J	71175	384	7.4	47	5.0	37	12	86	23	16	12	4.2	2.8	1200	2000	3800	4100	4900		464	
455T	02-882	F	H	71105	401	10.4	48	4.9	51	16	170	23	16	12	4.0	2.0	1200	2000	3800	4200	4700		534	
313S	01-598	F	D	69160	411	7.9	49	2.4	19	8.2	64	11	7.7	5.8	2.3	.12	550	950	1900	2500	2500		1209	
296B	01-592	M	A	69135	418	10.0	50	2.1	21	3.8	38	9.5	7.0	5.4	2.2	.11	490	860	1700	2300	2400		1234	
466V	04-915	F	J	71174	380	7.9	51	2.0	15	4.5	36	8.9	6.7	5.2	1.9	1.3	470	820	1600	1800	2100		465	
313C	02-598	M	C	69160	411	9.6	52	1.8	17	3.8	37	7.9	6.2	4.9	2.0	.10	420	750	1500	2100	2100		1209	
304T	02-592	F	B	69135	401	7.8	53	1.6	12	2.5	19	7.2	5.4	4.2	1.7	.083	370	660	1300	1800	1800		1234	
324V	03-638	F	F	69210	402	7.2	54	1.5	11	3.9	28	6.9	5.2	4.0	1.6	.10	360	630	1300	1700	1700		1159	
331A	04-638	M	E	69210	370	9.0	55	1.3	11	2.2	20	5.9	4.6	3.6	1.5	.087	310	560	1100	1500	1600		1159	
461B	03-915	M	I	71174	417	11.1	56	1.2	13	3.0	33	5.3	4.2	3.3	1.2	.80	280	510	1000	1100	1300		465	
467U	04-945	F	L	71223	421	6.6	57	1.2	7.5	2.7	18	7.5	4.1	3.1	1.0	.83	300	510	970	1000	1200		416	
477A	03-945	M	K	71223	380	11.0	58	1.1	12	1.8	30	5.1	3.9	3.0	1.0	.84	270	470	920	970	1200		416	
329C	03-642	M	E	69213	386	8.3	59	.71	5.9	1.2	9.9	3.2	2.4	1.8	.75	.050	170	290	590	790	800		1156	
453B	03-881	M	G	71104	406	8.1	60	.63	5.1	1.4	12	2.9	2.2	1.7	.66	.35	150	270	530	620	710		535	
463S	02-915	F	J	71174	410	10.4	61	.53	5.5	.77	8.0	2.9	1.8	1.4	.53	.35	130	220	440	490	580		465	
452U	04-881	F	H	71104	416	8.2	62	.52	4.2	4.7	38	2.4	1.9	1.5	.56	.29	130	230	460	520	600		535	
314S	04-597	F	D	69157	407	9.7	63	.45	4.4	.82	7.9	2.1	1.4	1.0	.40	.019	100	170	330	440	440		1212	
296U	02-591	F	B	69134	417	8.4	64	.44	3.7	.81	6.8	1.8	1.4	1.1	.41	.012	97	170	340	440	440		1235	
313B	03-597	M	C	69157	408	10.1	65	.37	3.7	1.1	11	1.7	1.2	.90	.33	.016	85	150	280	370	370		1212	
461A	01-915	M	I	71174	417	12.0	66	.35	4.3	.49	5.9	1.7	1.2	.94	.37	.25	84	150	300	330	390		465	
322V	02-638	F	F	69210	409	6.4	67	.32	2.0	.70	4.4	1.5	1.1	.85	.35	.022	76	130	270	360	370		1159	
476C	01-945	M	K	71223	387	9.0	68	.30	2.7	.58	5.2	1.4	1.1	.89	.37	.30	74	130	280	300	380		416	
471S	02-945	F	L	71223	395	6.3	69	.25	1.6	.90	5.7	1.1	.89	.69	.25	.21	60	110	210	230	280		416	
297A	01-591	M	A	69134	415	11.0	70	.18	2.0	.38	4.2	.83	.65	.51	.19	.006	44	79	160	210	210		1235	
453U	02-881	F	H	71104	406	5.8	71	.18	1.1	.42	2.4	.85	.68	.54	.22	.12	45	82	170	200	230		535	
457B	01-881	M	G	71104	374	8.3	72	.17	1.4	.37	3.1	.89	.61	.49	.20	.10	41	74	150	180	210		535	
472W	02-942	F	L	71222	390	8.0	73	.16	1.3	.36	2.9	.74	.58	.46	.17	.13	39	70	140	150	180		417	
298U	02-590	F	B	69129	407	9.4	74	.12	1.1	.36	3.4	.56	.44	.34	.12	.002	29	53	110	140	140		1240	

¹⁴⁴Ce Fused Clay Longevity Dogs (Series II) cont'd.

¹⁴⁴Ce FUSED CLAY LONGEVITY DOGS
- SERIES II, cont'd. (front)

¹⁴⁴Ce FUSED CLAY SACRIFICE DOGS
- SERIES II, III and IV (back)

Number		Sex		Inhalation Exposure			Initial Lung Burden		Initial Body Burden		Radiation Dose to Lung						Cumulative (rads)						Date of Death	Days Since ¹⁴⁴ Ce Clay Inhalation		Comment***	
				Block	Date	Age (Days)					Wt (kg)	Rate (rads/day)					Potential					To Death		9-30-72	Death		
												Rank	µCi/kg*	µCi	µCi/kg*	µCi	Initial	60**	120**	365**	On						At
Tattoo	Radiobiol.												Days	Days	Days	Days	9-30-72	Death	Days	Days	Days	Days	9-30-72				
462C	02-914	M	I	71173	409	9.0	75	.086	.78	.16	1.4	.40	.31	.25	.089	.058			21	38	75	83	98			466	
476B	01-942	M	K	71222	386	8.5	76	.080	.68	.11	.96	.37	.29	.23	.083	.65			20	35	70	74	91			417	
303S	02-589	F	B	69128	398	8.9	77	.077	.68	.23	2.1	.36	.28	.22	.080	.0015			19	34	67	87	88			1241	
308U	01-597	F	D	69157	412	10.1	78	.073	.74	.13	1.3	.34	.27	.21	.076	.0021			18	32	64	82	83			1212	
464S	01-914	F	J	71173	382	8.1	79	.064	.52	.12	1.0	.30	.23	.18	.067	.044			16	28	56	62	73			466	
451T	04-880	F	H	71103	415	8.0	80	.053	.42	.088	.70	.25	.19	.15	.055	.028			13	23	46	54	60			536	
310A	02-597	M	C	69157	430	11.5	81	.050	.58	.21	2.4	.23	.18	.14	.052	.0015			12	22	44	57	57			1212	
304A	01-590	M	A	69129	395	11.3	82	.044	.50	.15	1.7	.20	.16	.13	.046	.0008			11	19	39	50	50			1240	
310U	03-596	F	D	69156	429	8.0	83	.041	.33	.30	2.4	.19	.15	.12	.043	.0012			10	18	36	46	47			1213	
323T	05-636	F	F	69209	386	8.4	84	.039	.34	.15	1.2	.18	.14	.11	.041	.0015			9.6	17	34	44	44			1160	
306A	01-589	M	A	69128	389	9.5	85	.033	.31	.19	1.7	.15	.12	.094	.034	.0006			8.1	15	29	37	38			1241	
312A	04-596	M	C	69156	406	11.0	86	.025	.27	.050	.53	.12	.091	.072	.026	.0007			6.1	11	22	28	28			1213	
472U	02-941	F	L	71221	389	8.5	87	.020	.17	.036	.30	.093	.073	.057	.021	.016			4.9	8.8	18	19	23			418	
465B	01-912	M	I	71172	378	11.2	88	.018	.20	.040	.45	.084	.066	.051	.019	.012			4.4	7.9	16	17	20			467	
450D	03-880	M	G	71103	419	11.1	89	.016	.18	.067	.74	.074	.058	.046	.017	.0083			3.9	7.0	14	16	18			536	
327D	06-636	M	E	69209	383	8.7	90	.015	.13	.078	.68	.070	.055	.043	.016	.0006			3.7	6.6	13	17	17			1160	
462S	02-912	F	J	71172	408	8.1	91	.014	.11	.040	.32	.065	.051	.040	.15	.0095			3.4	6.2	12	14	16			467	
327C	03-636	M	E	69209	383	9.4	92	.0096	.090	.061	.58	.045	.035	.027	.0099	.0003			2.4	4.2	8.4	11	11			1160	
478C	01-941	M	K	71221	376	8.9	93	.0092	.081	.025	.22	.043	.033	.026	.0095	.0075			2.3	4.0	8.1	8.6	10			418	
324T	04-636	F	F	69209	401	10.8	94	.0063	.068	.053	.57	.029	.023	.018	.0065	.0002			1.5	2.8	5.5	7.1	7.2			1160	
453A	01-880	M	G	71103	405	9.0	95	.0030	.027	.0051	.046	.014	.011	.0085	.0031	.016			.74	1.3	2.6	3.0	3.4			536	
452T	02-880	F	H	71103	415	9.4	96	.0024	.023	.019	.18	.011	.0087	.0068	.0024	.0012			.59	1.1	2.1	2.4	2.7			536	
306D	02-588	M	A	69127	388	9.4	C	O	O	O	O															1242	
303V	01-588	F	B	69127	397	7.5	C	O	O	O	O															1242	
310B	01-596	M	C	69156	429	11.0	C	O	O	O	O															1213	
308T	02-596	F	D	69156	411	9.3	C	O	O	O	O															1213	
324B	01-636	M	E	69209	401	8.8	C	O	O	O	O															1160	
322U	02-636	F	F	69209	408	6.8	C	O	O	O	O															1160	
450A	01-878	M	G	71099	415	11.8	C	O	O	O	O															540	
452S	02-878	F	H	71099	411	10.2	C	O	O	O	O															540	
467B	01-911	M	I	71169	367	6.9	C	O	O	O	O															470	
464U	02-911	F	J	71169	378	8.9	C	O	O	O	O															470	
477B	01-940	M	K	71218	375	8.7	C	O	O	O	O															421	
479T	02-940	F	L	71218	372	8.0	C	O	O	O	O															421	

*kg refers to total body weight.

**Days post-inhalation exposure.

***D, E, or S -- Died, Euthanized, or Sacrificed and the most prominent features associated with death.

+ Died before potential infinite dose was received.

11. ¹⁴⁴Ce Fused Clay Sacrifice Dogs (Series II, III and IV)

Number		Sex	Inhalation Exposure		Age		Wt		Initial Lung Burden		Initial Body Burden		Radiation Dose to Lung								Date of Death	144 Days Since		Comment***		
			Series	Date									Rank	μCi/kg*	μCi	Rate (rads/day)			Cumulative (rads)				9-30-72		Ce Clay Inhalation	
																Initial	60**	120**	To Death	60**		120**				Potential Infinite
Tattoo	Radiobiol.				(Days)	(kg)							Initial	Days	Days		Days	Days	Days			Death				
541S	01-999	F	II	72103	395	7.9	1	71	560	140	1100	320	240	180		180	16000	29000	70000+	30000	72231	128	S-Pul. Injury			
520A	02-998	M	II	72102	427	11.0	2	66	720	130	1400										172					
530C	04-1007	M	II	72115	417	9.2	3	64	590	140	1300										159					
530B	01-1002	M	II	72109	411	9.5	4	60	560	91	860	270	170		150	13000		38000+	16000	72189	80	S-Pul. Injury				
525W	01-1004	F	II	72111	428	7.3	5	58	420	110	810										163					
527A	03-1007	M	II	72115	418	9.2	6	54	500	78	720										159					
521T	03-998	F	II	72102	426	8.7	7	52	450	93	810	250	180	140	130	13000	22000	51000+	23000	72230	128	S-Pul. Injury				
526A	01-1001	M	II	72108	416	7.5	8	52	390	97	730	250	150		130	12000		32000+	15000	72189	81	S-Pul. Injury				
526B	02-1000	M	II	72104	412	5.8	9	52	300	120	680										170					
526S	01-1007	F	II	72115	423	6.6	10	51	330	100	670	230	160		150	12000		42000+	15000	72196	81	S-Pul. Injury				
525T	02-1003	F	II	72110	427	8.8	11	48	420	110	990	230	170	130	120	12000	20000	47000+	22000	72241	131	S-Pul. Injury				
522T	03-1003	F	II	72110	432	8.0	12	48	380	140	1100										164					
525U	02-1004	F	II	72111	428	9.1	13	46	420	89	810										163					
539A	03-997	M	II	72101	394	9.3	14	41	380	120	1100										173					
530A	04-998	M	II	72102	404	11.5	15	39	450	83	950										172					
541U	01-1000	F	II	72104	396	7.9	16	35	280	62	490										170					
535C	03-1000	M	II	72104	399	7.7	17	34	260	70	540										170					
539D	04-1000	M	II	72104	397	7.9	18	33	260	52	410										170					
522U	01-998	F	II	72102	424	7.8	19	33	250	92	720	150	110	76	73	7600	13000	27000+	14000	72230	128	S-Pul. Injury				
526C	02-997	M	II	72101	409	6.9	20	33	230	68	470										173					
519S	04-1004	F	II	72111	439	8.3	21	32	270	73	610										163					
524S	02-1001	F	II	72108	425	6.7	22	32	210	55	370										166					
522S	04-997	F	II	72101	423	8.7	23	31	270	78	680										173					
527B	02-1002	M	II	72109	412	9.3	24	31	290	56	520										165					
532U	02-1007	F	II	72115	413	7.8	25	31	240	42	330										159					
521B	03-999	M	II	72103	427	7.6	26	30	230	84	640										171					
536T	03-1001	F	II	72108	403	7.6	27	29	220	66	500										166					
519T	03-1004	F	II	72111	439	8.7	28	28	240	91	790	130	96	71	67	6700	12000	26000+	12000	72241	130	S-Pul. Injury				
527D	01-997	M	II	72101	404	7.9	29	27	220	47	370										173					
519A	03-1002	M	II	72109	437	8.6	30	27	230	110	940										165					
520B	04-1003	M	II	72110	435	11.9	31	26	310	180	2100										164					
523T	02-1008	F	II	72116	435	6.0	32	26	150	47	280										158					
543C	04-1002	M	II	72109	399	7.6	33	26	190	42	320										165					
520S	01-1003	F	II	72110	435	6.7	34	24	160	67	450										164					
526D	02-999	M	II	72103	411	5.5	35	16	86	51	280										171					
541A	05-1000	M	II	72104	396	8.3	36	16	140	28	230										170					
538S	04-1001	F	II	72108	402	6.7	37	14	95	24	160										166					

¹⁴⁴Ce Fused Clay Sacrifice Dogs (Series II, III and IV) cont'd.

¹⁴⁴Ce FUSED CLAY SACRIFICE DOGS
- SERIES II, III and IV, cont'd. (front)

¹⁴⁴Ce FUSED CLAY IMMATURE LONGEVITY DOGS (back)

Number		Sex	Inhalation Exposure			Initial Lung Burden		Initial Body Burden		Radiation Dose to Lung				Date of Death	144 Days Since		Comment***
			Series	Date	Age (Days)	Wt (kg)	Rank	µCi/kg*	µCi	Rate (rads/day)		Cumulative (rads)			9-30-72	Death	
Tattoo	Radiobiol.									Initial	Days	Days	Death				
533T	01-1008	F	II	72116	413	5.9	38	14	81	20	120					158	
523S	01-995	F	II	72097	416	8.8	C	O	O	O	O					177	
538B	02-995	M	II	72097	391	9.3	C	O	O	O	O					177	
533A	03-995	M	II	72097	394	8.3	C	O	O	O	O			72224		127	S-Normal
542S	04-995	F	II	72097	388	8.2	C	O	O	O	O					177	
540T	05-995	F	II	72097	389	5.7	C	O	O	O	O					177	
542A	06-995	M	II	72097	388	9.1	C	O	O	O	O					177	
522V	01-996	F	II	72098	420	7.8	C	O	O	O	O					176	
547B	02-996	M	II	72098	378	10.4	C	O	O	O	O					176	
522A	03-996	M	II	72098	420	8.7	C	O	O	O	O					176	
540B	04-996	M	II	72098	390	8.0	C	O	O	O	O					176	
530S	05-996	F	II	72098	400	8.6	C	O	O	O	O					176	
521S	06-996	F	II	72098	422	8.6	C	O	O	O	O					176	
538A	03-1016	M	III	72137	428	9.1	1	69	630	95	860					137	
540U	01-1013	F	III	72132	424	6.6	2	59	390	150	990					142	
535T	01-1015	F	III	72136	431	6.3	3	50	320	250	1600					138	
535B	03-1019	M	III	72144	438	7.8	4	50	390	140	1100					130	
540S	04-1019	F	III	72144	435	6.5	5	50	320	220	1400					130	
539B	02-1014	M	III	72133	426	8.6	6	48	410	95	820					141	
542C	03-1014	M	III	72133	424	7.8	7	48	380	130	1000					141	
547C	02-1015	M	III	72136	416	9.9	8	35	340	77	761					138	
535A	01-1019	M	III	72144	438	7.9	9	34	270	100	810					130	
547T	04-1014	F	III	72133	413	7.1	10	32	230	85	600					141	
544T	02-1019	F	III	72144	432	7.4	11	32	230	100	760					130	
530T	04-1016	F	III	72137	439	10.2	12	29	300	73	740					137	
547D	01-1017	M	III	72140	420	7.3	C	O	O	O	O					134	
541W	04-1017	F	III	72140	432	8.7	C	O	O	O	O					134	
544U	05-1017	F	III	72140	429	7.0	C	O	O	O	O					134	
527C	06-1017	M	III	72140	443	10.2	C	O	O	O	O					134	
539C	01-1018	M	III	72143	436	8.5	C	O	O	O	O					131	
541T	02-1018	F	III	72143	435	8.4	C	O	O	O	O					131	
539T	03-1013	F	IV	72132	425	8.6	1	41	350	120	990					142	
543A	04-1013	M	IV	72132	422	10.8	2	33	350	85	920					142	
541V	02-1016	F	IV	72137	429	7.8	3	33	260	69	540					137	
542B	01-1016	M	IV	72137	428	9.7	4	32	320	110	1100					137	
543B	01-1014	M	IV	72133	423	9.2	5	31	290	44	410					141	
543S	02-1013	F	IV	72132	422	9.2	6	29	270	100	950					142	
539S	02-1017	F	IV	72140	433	7.6	C	O	O	O	O					134	
530U	03-1017	F	IV	72140	442	8.5	C	O	O	O	O					134	
538C	07-1017	M	IV	72140	434	7.5	C	O	O	O	O					134	

*kg refers to total body weight.
**Days post-inhalation exposure.
***D, E or S -- Died, Euthanized, or Sacrificed and the most prominent features associated with death.
+ Died before potential infinite dose was received.

12. ¹⁴⁴Ce Fused Clay Immature Longevity Dogs

Number		Sex	Inhalation Exposure				Initial Lung Burden			Initial Body Burden		Radiation Dose to Lung				Days Since ¹⁴⁴ Ce Clay Inhalation		Comment***
			Block	Date	Age (Days)	Wt (kg)						Rate (rads/day)		Cuml. (rads)				
Rank	μCi/kg*						μCi	μCi/kg*	μCi	60**	60**	Potential						
										Initial	Days	Days	Infinite	9-30-72	Death			
629A	01-1055	M	A	72221	92	2.75	1	36	100	320	880	170	68	6200	18000	53		
627B	03-1054	M	A	72220	94	3.50	2	24	83	61	210	110	54	4500	17000	54		
630B	02-1054	M	A	72220	88	2.80	3	9.5	27	82	230	44	16	1500	4800	54		
630A	01-1054	M	A	72220	88	3.75	4	5.7	21	15	56	26	13	1100	3800	54		
624D	04-1048	M	A	72209	90	2.65	5	3.0	8.0	8.2	22	14	5.9	510	2300	65		
623A	03-1048	M	A	72209	91	4.00	6	.28	1.1	1.6	6.5	1.3	.63	53	190	65		
624C	02-1048	M	A	72209	90	2.90	7	.061	.18	1.2	3.6	.28	.095	9.4	26	65		
624A	01-1048	M	A	72209	90	3.95	8	.014	.054	.81	3.2	.063	.031	3.2	12	65		
623B	01-1046	M	A	72208	90	3.50	C	O	O	O	O					66		

*kg refers to total body weight.

**Days post-inhalation exposure.

***D, E or S -- Died, Euthanized, or Sacrificed and the most prominent features associated with death.

13. ¹⁴⁴Ce Fused Clay Aged Longevity Dogs

¹⁴⁴Ce FUSED CLAY AGED LONGEVITY DOGS (front)

⁹⁰Sr FUSED CLAY LONGEVITY DOGS (back)

Number		Sex	Inhalation Exposure		Age (Days)	Wt (kg)	Initial Lung Burden			Initial Body Burden		Radiation Dose to Lung			Cumulative (rads)		Date of Death	Days Since ¹⁴⁴ Ce fused clay Inhalation		Comment***
			Block	Date			Rank	µCi/kg*	µCi	µCi/kg*	µCi	Rate (rads/day)	60**	Death	60**	Potential Infinite	Death	9-30-72	Death	
FD-49	02-982	F	B	72040	3748	9.5	1	75	710	170	1600	350	240	120	17000	62000+	41000	72237	197	D-Pulmonary Injury
FD-40	02-991	F	E	72055	3565	12	2	66	800	170	2000	310	230		13000	60000		219		
FD-108	03-987	F	D	72046	3537	12	3	59	690	160	1900	270	210		14000	60000		228		
FD-98	01-987	F	C	72046	3686	11	4	56	610	150	1700	260	190		13000	58000		228		
FD-118	01-990	F	F	72054	3318	6.7	5	50	330	179	1200	230	160		12000	42000		220		
FD-145	03-982	F	A	72040	3840	11	6	42	440	81	890	200	150	76	10000	43000+	26000	72249	209	D-Pulmonary Injury
FD-12	02-987	F	C	72046	3714	15	7	28	400	120	1800	130	92	42	6500	24000+	16000	72260	214	D-Pulmonary Injury
FD-7	01-991	F	E	72055	3511	10	8	24	240	130	1300	110	84		5700	26000		219		
FD-31	04-982	F	A	72040	3859	11	9	23	250	44	480	110	70		5200	16000		234		
FD-94	03-990	F	D	72054	3461	6.7	10	23	150	61	410	110	77		5400	22000		220		
FD-121	02-990	F	F	72054	3119	16	11	22	360	88	1400	100	76		5300	22000		220		
FD-100	01-983	F	B	72041	3841	8.8	12	22	190	63	550	100	75		5300	22000		233		
FD-103	01-982	F	B	72040	3705	9.4	13	20	190	37	350	93	68		4800	19000		234		
FD-32	03-984	F	D	72045	3542	7.8	14	14	110	28	220	65	48		3300	13000		229		
FD-47	02-984	F	C	72045	3585	8.4	15	14	110	29	240	63	45		3200	11000		229		
FD-30	02-983	F	A	72041	3877	11	16	13	150	43	470	62	46		3200	13000		233		
FD-15	01-989	F	F	72053	3273	12	17	13	160	71	850	59	42		3000	11000		221		
FD-153	02-989	F	E	72053	3320	8.6	18	11	95	45	390	52	37		2600	10000		221		
FD-95	01-984	F	C	72045	3563	7.4	19	8.8	65	18	130	41	29		2100	7600		229		
FD-154	04-989	F	F	72053	3313	11	20	8.8	100	27	300	41	31		2100	9300		221		
FD-48	04-984	F	D	72045	3544	12	21	8.3	100	26	310	38	27		1900	6500		229		
FD-38	03-983	F	B	72041	3826	8.7	22	7.7	67	23	200	36	26		1800	6700		233		
FD-104	04-983	F	A	72041	3931	12	23	6.8	83	16	190	31	21		1500	4600		233		
FD-150	03-980	F	E	72053	3320	9.9	24	5.6	55	23	230	26	19		1300	5500		221		
FD-101	05-981	F	A	72039	3842	9.9	C											235		
FD-6	06-981	F	B	72039	3815	11	C											235		
FD-117	01-981	F	C	72039	3679	6.2	C											235		
FD-147	02-981	F	D	72039	3525	8.9	C											235		
FD-4	03-981	F	E	72039	3499	15	C											235		
FD-149	04-981	F	F	72039	3261	8.2	C											235		

*kg refers to total body weight.

**Days post-inhalation exposure.

***D, E, or S -- Died, Euthanized, or Sacrificed and the most prominent features associated with death.

14. ⁹⁰Sr Fused Clay Longevity Dogs

		Radiation Dose to Lung																				Date of Death	90 Days Since		Comment***	
		Inhalation Exposure					Initial Lung Burden		Initial Body Burden		Rate (rads/day)					Cumulative (rads)					90 Sr Clay Inhalation		Days Since 9-30-72 Death			
Number	Tattoo Radiobiol.	Sex	Block	Date	Age (Days)	Wt (kg)	Rank	µCi/kg*	µCi	µCi/kg*	µCi	Initial	60** Days	120** Days	365** Days	On 9-30-72	At Death	60** Days	120** Days	365** Days	To 9-30-72	Potential to 5000 days	At Death	9-30-72	Death	
417A	04-828	M	H	70288	379	10.6	1	94	1000	250	2700	390	360	340	140		310	23000	44000			320000+	68000	71118	195	D-Pul. Injury
415T	01-828	F	I	70288	396	9.2	2	88	810	120	1100	360	310	260			200	20000	37000			130000+	60000	71143	220	D-Pul. Injury
403A	03-809	M	E	70238	396	7.0	3	81	570	140	970	340	250	210			140	17000	31000	72000		190000+	75000	71259	386	D-Pul. Injury
393A	01-792	M	C	70218	427	10.3	4	81	830	100	1000	330	260	210			180	18000	32000			87000+	40000	71012	159	D-Pul. Injury
397T	04-792	F	D	70218	409	8.6	5	78	670	150	1300	320	220	180			140	16000	28000			99000+	43000	71071	218	D-Pul. Injury
416B	02-828	M	H	70288	382	11.6	6	77	890	210	2400	320	290	260			230	18000	34000			190000+	50000	71107	184	D-Pul. Injury
435A	01-856	M	J	71032	391	11.5	7	73	840	140	1700	300	270	250			200	17000	33000			170000+	57000	71263	231	E-Pul. Injury
417T	03-828	F	I	70288	379	9.5	8	72	680	300	2800	300	240	210			160	16000	29000			160000+	56000	71190	267	E-Pul. Injury
500B	04-964	M	L	71300	369	8.6	9	70	600	150	1300	290	230	200	140		170	15000	28000			200000+	52000	72190	255	D-Pul. Injury
398U	02-792	F	D	70218	393	6.8	10	68	460	94	640	280	210	190			150	14000	26000			210000+	62000	71182	329	D-Pul. Injury
432T	01-855	F	K	71029	413	8.2	11	67	550	190	1600	280	210	190			150	14000	26000			140000+	51000	71297	268	D-Pul. Injury
403U	01-809	F	F	70238	396	6.8	12	66	450	150	1000	270	240	210			160	15000	29000			130000+	55000	71131	258	D-Pul. Injury
355S	01-703	F	B	70036	424	8.6	13	63	540	150	1300	260	210	190			140	14000	26000			160000+	62000	71013	342	D-Pul. Injury
405X	01-824	F	G	70266	416	8.6	14	63	540	130	1100	260	220	190			140	14000	27000			110000+	47000	71144	243	D-Pul. Injury
355B	02-703	M	A	70036	424	9.4	15	62	580	100	940	260	210	190			140	14000	26000	65000		200000+	66000	71044	373	D-Pul. Injury
433A	02-854	M	J	71028	411	11.0	16	61	670	110	1300	250	230	210			170	14000	27000			150000+	52000	71280	252	E-Pul. Injury
408T	02-824	F	G	70266	408	8.6	17	58	500	100	890	240	210	180	90		130	13000	25000			99000+	43000	71139	238	D-Pul. Injury
398A	03-792	M	C	70218	393	11.4	18	57	650	81	930	240	210	190			140	13000	25000			130000+	53000	71136	283	D-Pul. Injury
418S	03-827	F	I	70286	365	10.0	19	56	560	90	900	230	190	160			120	13000	23000			71000+	34000	71121	200	D-Pul. Injury
402D	02-809	M	E	70238	397	6.7	20	55	370	190	1300	230	180	160			130	12000	22000			290000+	48000	71173	300	D-Pul. Injury
357A	01-702	M	A	70035	421	9.8	21	54	530	150	1400	220	180	160			140	12000	22000			410000+	54000	71011	341	D-Pul. Injury
361T	03-701	F	B	70034	415	8.4	22	54	450	90	750	220	200	180			140	13000	24000			120000+	46000	70299	265	D-Pul. Injury
494A	03-964	M	L	71300	404	9.4	23	53	500	100	970	220	150	130			110	11000	19000			160000+	42000	72245	310	D-Pul. Injury
431U	02-855	F	K	71029	421	7.2	24	49	350	240	1800	200	170	150			130	11000	21000			140000+	36000	71255	226	D-Pul. Injury
403S	04-809	F	F	70238	396	7.0	25	53	370	140	970	220	190	170	70		140	12000	23000			110000+	41000	71105	232	D-Pul. Injury
437D	03-855	M	J	71029	382	8.3	26	47	390	100	860	190	160	140			88	10000	19000	47000		98000+	48000	72040	376	E-Pul. Injury
411A	01-827	M	H	70286	421	14.2	27	44	630	260	3600	180	170	150			130	10000	20000			100000+	31000	71122	201	D-Pul. Injury
400S	04-808	F	F	70237	404	9.1	28	42	380	84	770	170	150	140			97	9700	18000			85000+	37000	71158	286	D-Pul. Injury
402A	01-808	M	E	70237	396	9.5	29	42	400	56	530	170	120	110			78	8600	15000			95000+	35000	71212	340	D-Pul. Injury
433S	04-854	F	K	71028	411	9.0	30	39	350	62	560	160	150	140			110	9300	18000			120000+	37000	71307	279	D-Pul. Injury
411T	03-824	F	G	70266	400	7.6	31	39	300	69	530	160	140	120			89	9100	17000			70000+	31000	71160	259	D-Pul. Injury
398D	02-790	M	C	70216	391	9.5	32	36	340	51	490	150	110	98			52	7800	14000	34000		100000+	50000	72130	644	E-Hemangiosarcoma
497B	02-964	M	L	71300	375	8.8	33	35	310	49	430	150	110	95		59		7300	13000		30000	56000		339		
396T	03-790	F	D	70216	417	8.6	34	34	290	80	680	140	110	91	58		35	7400	13000	31000		73000+	47000	72204	718	D-Angiosarcoma
354W	02-702	F	B	70035	425	7.5	35	29	220	61	460	120	100	92	68		59	6600	12000	32000		90000+	39000	71147	477	D-Pul. Injury
355A	04-701	M	A	70034	422	10.6	36	25	270	98	1000	110	83	75	52		31	5500	10000	25000		60000+	40000	72019	715	D-Hemangiosarcoma
433C	01-854	M	J	71028	411	9.8	37	24	240	29	280	100	92	83	60	46		5800	11000	28000	41000	96000		611		

⁹⁰Sr Fused Clay Longevity Dogs cont'd.

⁹⁰Sr FUSED CLAY LONGEVITY DOGS, cont'd. (front)

⁹⁰Sr FUSED CLAY LONGEVITY DOGS, cont'd. (back)

Radiation Dose to Lung																											
Inhalation Exposure										Radiation Dose to Lung																	
Number		Sex	Block	Date	Age (Days)	Wt (kg)	Initial Lung Burden			Initial Body Burden		Rate (rads/day)					Cumulative (rads)					At Death	Date of Death	Days Since		Comment***	
Tattoo	Radiobiol.						Rank	µCi/kg*	µCi	µCi/kg*	µCi	Initial	60** Days	120** Days	365** Days	On 9-30-72	At Death	60** Days	120** Days	365** Days	To 9-30-72			Potential to 5000 days	9-30-72		Death
414T	04-827	F	I	70286	397	7.0	38	23	160	53	370	95	75	67	52	37		5000	9100	23000	39000	79000			718		
416C	02-827	M	H	70286	380	10.7	39	22	240	42	450	93	70	61	42	24		4700	8600	21000	32000	48000			718		
430S	03-854	F	K	71028	423	7.7	40	22	170	30	230	91	74	64	45	34		4900	9100	22000	32000	64000			611		
398B	01-790	M	C	70216	391	11.2	41	20	220	28	320	81	69	60	41	26		4500	8300	20000	34000	58000			788		
399U	03-808	F	F	70237	407	7.2	42	19	140	53	380	80	66	58	42	26		4300	8100	20000	34000	56000			767		
401B	02-808	M	E	70237	403	9.0	43	19	170	50	450	78	67	61	46	29		4300	8100	21000	36000	62000			767		
404S	01-823	F	G	70265	438	8.6	44	19	160	24	200	77	58	50	34	21		4000	7200	17000	27000	43000			739		
495C	01-964	M	L	71300	385	8.0	45	18	140	24	190	72	50	44		26		3400	6200		14000	25000			339		
397S	04-790	F	D	70216	407	8.3	46	16	130	26	220	65	55	48	30	17		3600	6700	16000	25000	43000			788		
354A	01-701	M	A	70034	424	10.4	47	15	160	49	510	64	54	47	31	14		3500	6500	16000	29000	39000			970		
362T	02-701	F	B	70034	413	6.8	48	15	100	36	240	61	53	47	33	17		3400	6400	16000	31000	45000			970		
413B	02-826	M	H	70285	409	10.9	49	15	160	23	250	61	38	24	3.5	20		2900	4700	7300	7700	7700			719		
415V	01-826	F	I	70285	393	7.5	50	15	110	23	180	61	48	42	26	13		3100	5800	14000	21000	27000			719		
405A	02-806	M	E	70236	387	9.7	51	11	110	21	210	47	32	26	14	6.3		2300	4000	8700	13000	16000			768		
358T	01-704	F	B	70037	422	9.0	52	10	90	24	210	41	38	35	26	14		2400	4500	12000	24000	37000			967		
438A	04-853	M	J	71027	376	9.9	53	9.8	97	20	200	41	29	25	17	11		2000	3600	8600	12000	19000			612		
413C	03-826	M	H	70285	409	12.2	54	9.0	110	12	140	37	32	28	19	12		2100	3900	9500	15000	23000			719		
411S	03-823	F	G	70265	400	8.1	55	8.9	72	23	190	37	33	30	19	9.9		2100	4000	9900	15000	21000			739		
399T	01-806	F	F	70236	406	8.0	56	8.9	71	13	110	37	30	26	19	12		2000	3700	9000	15000	28000			768		
393T	03-789	F	D	70215	424	6.6	57	8.0	53	15	86	33	27	23	15	8.1		1800	3300	7700	12000	18000			789		
393C	02-789	M	C	70215	424	8.2	58	8.0	66	11	92	33	26	23	17	9.7		1700	3200	7900	13000	21000			789		
367B	04-700	M	A	70033	385	8.6	59	7.9	68	27	230	33	29	26	19	9.2		1800	3500	8800	17000	25000			971		
494D	01-963	M	L	71299	403	8.0	60	7.5	60	12	97	31	28	26		18		1800	3400		8100	19000			340		
430V	02-853	F	K	71027	422	7.2	61	7.2	52	22	160	30	27	25	17	11		1700	3300	8300	12000	19000			612		
405S	02-823	F	G	70265	416	9.9	62	6.7	66	14	140	28	21	17	9.2	5.2		1400	2500	5500	8100	12000			739		
413S	04-826	F	I	70285	409	10.4	63	6.0	62	16	170	25	22	20	14	7.8		1400	2700	6800	11000	15000			719		
352B	02-704	M	A	70037	433	7.9	64	4.9	39	8.5	67	20	19	18	14	9.0		1200	2300	6000	13000	27000			967		
398S	04-789	F	D	70215	390	9.6	65	4.9	47	9.6	92	20	14	13	9.1	5.2		980	1800	4400	7400	11000			789		
494B	02-963	M	L	71299	403	9.4	66	4.9	46	11.0	100	20	16	14		7.8		1100	2000		4300	7800			340		
431T	01-853	F	K	71027	419	6.6	67	4.7	31	9.9	65	19	18	17	12	8.6		1100	2200	5600	8100	15000			612		
399S	04-806	F	F	70236	406	9.7	68	4.3	42	8.6	83	18	16	14	11	7.1		990	1900	5000	8500	15000			768		
403B	03-806	M	E	70236	394	7.3	69	4.2	31	7.0	51	18	16	14	9.1	4.3		1000	1900	4600	7300	9700			768		
393D	01-789	M	C	70215	424	10.5	70	4.2	44	7.4	78	17	13	12	9.7	6.7		890	1600	4300	7800	15000			789		
360T	02-700	F	B	70033	414	7.9	71	4.1	32	25	200	17	15	13	7.2	1.8		940	1800	4100	6500	7200			971		
435C	03-853	M	J	71027	386	9.0	72	3.7	33	6.8	61	15	14	13	9.6	7.0		880	1700	4400	6500	12000			612		
361B	01-699	M	A	70027	408	12.0	C	O	O	O	O															977	

⁹⁰Sr Fused Clay Longevity Dogs cont'd.

												Radiation Dose to Lung										Date of Death	Days Since			Comment**
Number		Inhalation Exposure					Initial Body Burden		Rate (rads/day)				Cumulative (rads)						90 Sr Clay Inhalation							
Tattoo	Radibiol.	Sex	Block	Date	Age (Days)	Wt. (kg)	Initial Rank	Lung Burden µCi/kg*	µCi	µCi/kg*	µCi	Initial	60** Days	120** Days	365** Days	On 9-30-72	At Death	60** Days		120** Days	365** Days		To 9-30-72	Potential to 5000 days	At Death	
354S	02-699	F	B	70027	417	7.8	C	O	O	O	O														977	
397U	01-788	F	D	70212	403	7.5	C	O	O	O	O														792	
399B	02-788	M	C	70212	382	10.9	C	O	O	O	O														792	
401S	01-811	F	F	70240	406	8.5	C	O	O	O	O														764	
402B	02-811	M	E	70240	399	11.1	C	O	O	O	O														764	
405W	01-816	F	G	70247	398	6.8	C	O	O	O	O														757	
413U	01-830	F	I	70289	413	9.4	C	O	O	O	O														715	
418C	02-830	M	H	70289	368	11.4	C	O	O	O	O														715	
437A	01-851	M	J	71025	378	10.9	C	O	O	O	O														614	
431S	02-851	F	K	71025	417	7.4	C	O	O	O	O														614	
497A	01-962	M	L	71299	374	11.1	C	O	O	O	O														340	

*kg refers to total body weight.
**Days post-inhalation exposure.
***D, E or S -- Died, Euthanized, or Sacrificed and the most prominent features associated with death.
+ Died before potential infinite dose was received.

APPENDIX B

Publication of Technical Reports

1. McClellan, R. O. and F. C. Rupprecht (editors), "Fission Product Inhalation Program -- Annual Report -- 1970-1971," AEC Research and Development Report, LF-44, AEC Technical Information Service Extension, Oak Ridge, Tenn., November 1971.

APPENDIX C

Publications in the Open Literature

1. Barnes, J. E.,¹ R. O. McClellan, C. H. Hobbs and G. M. Kanapilly, "Toxicity in the Dog of Inhaled ^{90}Y in Fused Clay Particles. Distribution, Retention Kinetics and Dosimetry," Radiat. Res. **49**: 416-429, 1972.
2. Barnes, J. E.¹ and L. A. Osborn,² "Radiation Dose Distribution in the Beagle Dog During Radiographic Exposure," Amer. J. Vet. Res. **33**: 131-136, January 1972.
3. Benjamin, S. A., A. L. Brooks and R. O. McClellan, "Toxicity of ^{90}Sr Citrate in Chinese Hamsters: Late Effects," Radiat. Res. **51**: 477, August 1972 (abstract).
4. Benjamin, S. A., B. B. Boecker, T. L. Chiffelle, F. F. Hahn, C. H. Hobbs, R. K. Jones, R. O. McClellan, J. A. Pickrell and H. C. Redman, "Neoplasia in Beagle Dogs after Inhalation of $^{144}\text{CeCl}_3$," Proceedings of Hanford Symposium on Radionuclide Carcinogenesis, Richland, Wash., May 11-13, 1972 (in press).
5. Bielfelt, S. W., H. C. Redman and R. O. McClellan, "Sire- and Sex-Related Differences in Rates of Epileptiform Seizures in a Purebred Beagle Dog Colony," Amer. J. Vet. Res. **32**: 2039-2048, December 1971.
6. Boecker, B. B., "Toxicity of $^{137}\text{CsCl}$ in the Beagle Dog: Metabolism and Dosimetry," Radiat. Res. **50**: 556-573, June 1972.
7. Brooks, A. L., Diane K. Mead and R. F. Peters, "Effect of Chronic Exposure to ^{60}Co on the Frequency of Metaphase Chromosome Aberrations in the Liver Cells of the Chinese Hamster (*In Vivo*)," Int. J. Radiat. Biol. **20**: 599-604, 1971.
8. Brooks, A. L., J. A. Mewhinney and R. O. McClellan, "The *in vivo* Cytogenetic Effects of ^{252}Cf on Liver and Bone Marrow of the Chinese Hamster," Health Phys. **22**: 701-706, June 1972.
9. Brooks, A. L. and R. O. McClellan, "Cytogenetic Effects of ^{144}Ce on the Metaphase Chromosome of the Chinese Hamster Liver Cells *in vivo*," Radiat. Res. **51**: 511-512, August 1972 (abstract).
10. Brooks, A. L., R. O. McClellan and S. A. Benjamin, "The Effects of ^{144}Ce - ^{144}Pr on the Metaphase Chromosomes of the Chinese Hamster Liver Cells *in vivo*," Radiat. Res. (in press).
11. Cuddihy, R. G. and W. C. Griffith, "Tissue Distribution and Retention of ^{140}Ba and ^{140}La in Beagle Dogs after Inhalation of BaCl_2 - LaCl_3 ," Health Phys. **21**: 16, December 1971 (abstract).
12. Cuddihy, R. G., R. G. Thomas and R. P. Hall,² "Comparison of the Lung Retention of $^{133}\text{BaCl}_2$, $^{133}\text{BaSO}_4$ and ^{133}Ba -Fused Clay Aerosols with *in vitro* and *in vivo* Dissolution of the Aerosol Particle Suspensions," Health Phys. **23**: 401, September 1972 (abstract).
13. Cuddihy, R. G. and W. C. Griffith, "A Biological Model Describing Tissue Distribution and Whole-Body Retention of Barium and Lanthanum in Beagle Dogs after Inhalation and Gavage," Health Phys. **23**: 621-634, November 1972.
14. Cuddihy, R. G., D. G. Brownstein,² O. G. Raabe and G. M. Kanapilly, "Respiratory Tract Deposition of Inhaled Polydisperse Aerosols in Beagle Dogs," Aerosol Sci. (in press).
15. Cuddihy, R. G. and Jennie A. Ozog,² "Nasal Absorption of CsCl , SrCl_2 , BaCl_2 and CeCl_3 in Syrian Hamsters," (submitted for publication).
16. Cuddihy, R. G. and B. B. Boecker, "Controlled Administration of Respiratory Tract Burdens of Inhaled Radioactive Aerosols in Dogs," Toxicol. Appl. Pharmacol. (in press).
17. Dubin, S. E.,³ A. J. Wilson⁴ and R. C. Pfleger, "Changes in Respiratory Function of Beagle Dogs Given Pulmonary Lavage," Amer. J. Vet. Res. **32**: 2033-2038, December 1971.

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APPENDIX D

Presentations Before¹

Regional or National Scientific Meetings

and

Educational and Scientific Seminars

1. Barnes, J. E. and A. J. Hulbert, "Relative Nonhomogeneities of Radiation Dose to the Respiratory Tract from Inhaled Radionuclides," 6th Annual Health Physics Midyear Topical Symposium, Richland, Wash., November 2-5, 1971.
2. Benjamin, S. A., A. L. Brooks and R. O. McClellan, "Myeloproliferative Disorders and Other Pathological Changes in Chinese Hamsters Injected with ^{90}Sr - ^{90}Y ," American Association for Laboratory Animal Science, New York, N. Y., October 11-15, 1971.
3. Benjamin, S. A., "The Toxicity of ^{144}Ce Chloride in Beagle Dogs," American College of Veterinary Pathologists Annual Meeting, Toxicology Subsection, Denver, Colo., December 4, 1971.
4. Benjamin, S. A., B. B. Boecker, T. L. Chiffelle, F. F. Hahn, C. H. Hobbs, R. K. Jones, R. O. McClellan and H. C. Redman, "Neoplasia in Beagle Dogs after Inhalation of $^{144}\text{CeCl}_3$," 12th Hanford Symposium on Radionuclide Carcinogenesis, Richland, Wash., May 11-13, 1972.
5. Benjamin, S. A., A. L. Brooks and R. O. McClellan, "Toxicity of ^{90}Sr Citrate in Chinese Hamsters: Late Effects," 20th Annual Meeting of the Radiation Research Society, Portland, Oreg., May 14-18, 1972.
6. Brooks, A. L., "The Use of Metaphase Liver Chromosomes to Evaluate Biological Damage from Protracted Exposure to Internally Deposited Radioactive Materials," Environmental Mutagen Society Annual Meeting, Cherry Hill, N. J., March 26-29, 1972.
7. Brooks, A. L., S. A. Benjamin, R. K. Jones and R. O. McClellan, "Hepatic Neoplasms and Chromosomal Aberrations in Chinese Hamsters Following Internally Deposited ^{144}Ce - ^{144}Pr and Partial Hepatectomy," 12th Hanford Symposium on Radionuclide Carcinogenesis, Richland, Wash., May 11-13, 1972.
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9. Cuddihy, R. G., R. G. Thomas and R. P. Hall,² "Comparison of the Lung Retention of $^{133}\text{BaSO}_4$ and ^{133}Ba -Fused Clay Aerosols with *In Vitro* and *In Vivo* Dissolution of the Aerosol Particle Suspensions," 17th Annual Meeting of the Health Physics Society, Las Vegas, Nev., June 12-16, 1972.
10. Engen, R. L.,³ Rogene F. Henderson and R. C. Pflieger, "Influence of Calcium, Sialic Acid and pH on the Surface Tension Measurements of Lung Surfactant," Pennsylvania State University, University Park, Pa., August 29, 1972.
11. Hahn, F. F., "Mast Cell Tumor in a Beagle Dog," University of New Mexico Continuing Education Pathology Seminar, Albuquerque, N. Mex., March 1, 1972.
12. Hahn, F. F., "Pulmonary Neoplasia in a Beagle Dog Following Inhalation of ^{144}Ce Fused Clay Particles," University of New Mexico Continuing Education Pathology Seminar, Albuquerque, N. Mex., May 2, 1972.
13. Hahn, F. F., S. A. Benjamin, B. B. Boecker, T. L. Chiffelle, C. H. Hobbs, R. K. Jones, R. O. McClellan and H. C. Redman, "Induction of Pulmonary Neoplasia in Beagle Dogs by Inhaled ^{144}Ce Fused Clay Particles," 12th Hanford Symposium on Radionuclide Carcinogenesis, Richland, Wash., May 11-13, 1972.

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21. Lundgren, D. L., F. F. Hahn, A. Sanchez, Randi L. Thomas and R. O. McClellan, "Toxicity of Inhaled ^{90}Y in Fused Clay Particles and its Effect on Pulmonary Clearance of Staphylococcus aureus in Mice," 72nd Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., April 23-28, 1972.
22. McClellan, R. O., "Plutonium Inhalation Toxicology," Telelecture, Brooks Air Force Base, San Antonio, Tex., October 21, 1971.
23. McClellan, R. O., "Inhalation Toxicology" and "Radiation Biology," Lectures given at the Veterinary Toxicology Training and Review Workshop, Iowa State University, Ames, Iowa, February 24, 1972.
24. McClellan, R. O., S. A. Benjamin, B. B. Boecker, T. L. Chiffelle, C. H. Hobbs, R. K. Jones, J. A. Pickrell and H. C. Redman, "Neoplasms in Dogs that Inhaled $^{90}\text{SrCl}_2$," 12th Hanford Symposium on Radionuclide Carcinogenesis, Richland, Wash., May 11-13, 1972.
25. McClellan, R. O., "Experimental Studies with Inhaled Radionuclides," Late Effects Group Meeting, Portland, Oreg., May 14, 1972.
26. McClellan, R. O., "Lavage - A New Approach to Treatment of Lung Contamination," Panel Discussion, Seminar on Medical Planning and Care in Radiation Accidents, Oak Ridge, Tenn., May 21-24, 1972.
27. McClellan, R. O., H. A. Boyd, S. A. Benjamin, R. G. Cuddihy, F. F. Hahn, R. K. Jones, J. L. Mauderly, J. A. Mewhinney, B. A. Muggenburg and R. C. Pfleger, "Recovery of ^{239}Pu Following Bronchopulmonary Lavage and DTPA Treatment of an Accidental Inhalation Exposure Case," 17th Annual Meeting of the Health Physics Society, Las Vegas, Nev., June 12-16, 1972.
28. Mauderly, J. L., "Pulmonary Function Testing of Unanesthetized Beagle Dogs," National Conference on Research Animals in Medicine, Washington, D. C., January 28-30, 1972.
29. Mewhinney, J. A. and M. D. Harris, III,² "Retention and Distribution of ^{252}Cf : An Inter-species Comparison," 17th Annual Meeting of the Health Physics Society, Las Vegas, Nev., June 12-16, 1972.
30. Muggenburg, B. A., J. L. Mauderly, J. A. Pickrell, T. L. Chiffelle, R. K. Jones, R. O. McClellan, U. C. Luft and R. C. Pfleger, "Risk Considerations in the Use of Bronchopulmonary Lavage for Removing Inhaled Radionuclides," 17th Annual Meeting of the Health Physics Society, Las Vegas, Nev., June 12-16, 1972.

31. Newton, G. J., C. H. Hobbs and R. O. McClellan, "The Intraorgan Distribution in the Beagle Dog of ^{91}Y Inhaled as the Oxide, Phosphate and in Fused Clay," 17th Annual Meeting of the Health Physics Society, Las Vegas, Nev., June 12-16, 1972.
32. Pfleger, R. C., H. C. Redman and S. A. Benjamin, "Lipid Changes in Beagle Dog Lungs after Inhalation of ^{144}Ce Fused Montmorillonite Clay Aerosols," American Oil Chemists' Society Meeting, Atlantic City, N. J., October 3-6, 1971.
33. Pfleger, R. C. and Rogene F. Henderson, "Pulmonary Surfactant Phospholipids and Surface-Tension Characteristics," 11th Congress of the International Society for Fat Research, Göteborg, Sweden, June 18-22, 1972.
34. Raabe, O. G., "Instruments and Methods for Characterizing Radioactive Aerosols," Institute of Electrical and Electronic Engineers (IEEE) Nuclear Science Symposium, San Francisco, Calif., November 3-5, 1971.
35. Raabe, O. G., "Standardization of Aerosol Sampling," Workshop of the Environmental Protection Agency, Las Vegas, Nev., February 29, 1972.
36. Raabe, O. G., H. A. Boyd and C. J. Wilkinson, "Production of Monodisperse Aerosols of Respirable Particles of $^{238}\text{PuO}_2$," 17th Annual Meeting of the Health Physics Society, Las Vegas, Nev., June 12-16, 1972.
37. Redman, H. C., "Use of the EEG and Provocative Testing Techniques in Diagnosis of Brain Lesions in the Canine," Conference on the EEG in Disorders of the Central Nervous System of Animals and Man, Colorado State University, Fort Collins, Colo., October 16, 1971.
38. Thomas, Randi L. and R. O. McClellan, "Retention, Distribution and Dosimetry of ^{144}Ce in Syrian Hamsters Following Inhalation of $^{144}\text{CeO}_2$," 17th Annual Meeting of the Health Physics Society, Las Vegas, Nev., June 12-16, 1972.
39. Thomas, R. G., "Physical and Chemical Factors that Affect Aerosol Particles," and "Retention Characteristics of Inhaled Particles," Class on Environmental Problems, Highlands University, Las Vegas, N. Mex., November 11, 1971.
40. Thomas, R. G., "The Toxicology of Plutonium," Telelecture, Brooks Air Force Base, San Antonio, Tex., February 17, 1972, May 24, 1972 and September 27, 1972.
41. Thomas, R. G. and T. L. Chiffelle, "Experimental Alteration of Rat Lung Clearance Patterns Following Inhalation," 17th Annual Meeting of the Health Physics Society, Las Vegas, Nev., June 12-16, 1972.
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¹ If multiple-authored papers are presented by other than the senior author, the name of the individual who presented the paper is underlined.

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³ Sabbatical leave from Iowa State University.

⁴ Research performed at Purdue University.

APPENDIX E

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