EFFECTS OF COMBINED INHALATION EXPOSURE OF RATS TO $^{239}PuO_2$ AND BERYLLIUM METAL. I.

Abstract — We exposed rats acutely to achieve one of two initial lung burdens (ILBs) of $^{239}PuO_2$ alone or in combination with one of three ILBs of beryllium metal. Additional control groups of rats were sham-exposed to air. Currently, approximately 58% of all rats planned for inclusion have been exposed. This report describes procedures used for the exposure, maintenance, and evaluation of rats in this study. Most of the animals are to be held for their life span in order to quantitate cancer incidence, with other animals assigned to serial sacrifice groups for quantitation of Pu and Be retention and determination of translocation patterns. Exposure to beryllium at any of the three doses tested retarded clearance of plutonium from the lung by a factor of approximately six. Acute inflammatory responses were studied in a separate group of rats exposed to Be. Except for rats receiving the highest ILB of beryllium metal, no differences between exposed and sham-exposed control groups have yet been noted in terms of mortality, weight changes, and clinical signs.

PRINCIPAL INVESTIGATORS

G. L. Finch
J. A. Mewhinney
M. D. Hoover
P. J. Haley
R. G. Cuddihy
W. C. Griffith
B. B. Boecker

Workers fabricating parts in the nuclear industry have the potential for inhalation exposures to plutonium and other toxic agents such as beryllium metal. Although the epidemiologic evidence implicating beryllium in the induction of lung cancer in man is weak, animal studies have demonstrated the lung tumorigenic activity of beryllium, and it is classified as a suspect human carcinogen.[1] Inhaled plutonium delivers alpha-particle radiation to the lung and is a well-known carcinogen in experimental animal models.[2] The presence of one tumorigenic agent in the lung may either add to or multiply the risk of the induction of lung cancer from another agent.

Traditional methods designed to evaluate the risk of combined exposures to toxic materials involve determining the risk for each individual material and summing these risks to estimate the total risk. The reliability of this approach has not been scientifically validated. Research in this project is directed towards life-span observation of rats exposed to plutonium and/or beryllium. Information obtained will permit the formulation of mathematical models of cancer risk for the agents, both individually and in combination. This study shares its experimental design with other projects underway or planned at the Institute to characterize the cancer risk to humans of inhaled plutonium and low-LET X irradiation (this report, pp. 230-233). inhaled plutonium and cigarette smoke, and of other agents. The use of coordinated experimental designs in all of these studies will enable the development of risk models applicable to general classes of agents. We describe here a study in which rats are exposed to aerosols of $^{239}PuO_2$ and/or beryllium metal using a block design methodology.

MATERIALS AND METHODS

When exposures are completed, a total of 4280 rats will have been entered into 12 separate exposure groups (Table 1). The initial lung burdens (ILBs) of $^{239}PuO_2$ and beryllium metal were

selected to provide 0, 5, and 15% lung tumor incidences. Target ILBs for $^{239}PuO_2$ are 0, 1.5, and 4.7 nCi (0, 55, and 174 Bq), corresponding to 0, 25 and 90 rads (0, 0.25, and 0.9 Gy) lifetime radiation dose to lung, respectively. These levels were selected on the basis of results from previous studies performed at the Institute (unpublished data).

An equal number of male and female F344/N rats, 11-13 wk of age at the time of exposure, are assigned to each group. Except during exposure, animals are held in a barrier-maintained facility. Animals receive food and water ad libitum and are housed either 3 males or 4 females per filter-topped polycarbonate cage containing hardwood chip bedding.

Animals are being entered into the study in 12 blocks of either 354 or 360 animals each. As of November 1, 1988, 7 of the 12 groups (58%) have been exposed. This design ensures that animals will be entered into each exposure group at a uniform rate during the course of the study, thus reducing the influence of potential biological drift of the colony. Within each block, animals are randomized by litter for assignment to exposure groups to ensure that biological variability is distributed over all groups.

Table 1
Experimental Design for the Combined Effects of
$^{239}PuO_2$ and Beryllium Metal Inhaled by Rats

	$^{239}PuO_2$ ILB, Dose to Lung, Lung Tumor Incidence[a]			
	Sham	1.5 nCi	4.7 nCi	
Be Metal ILB	0 rad	25 rad	90 rad	Subtotal
Lung Tumor Incidence	0.015[b]	0.05	0.15	Number of Rats
Sham	320[c,d]	360[e,f]	360[d]	1040
0.015[b]	(0.015)[g]	(0.065)	(0.165)	
50 µg	360[e]	360[e]	360[e]	1080
0.05	(0.065)	(0.115)	(0.215)	
150 µg	360[d]	360[e]	360[d]	1080
0.15	(0.165)	(0.215)	(0.315)	
450 µg	360[d]	360[e]	360[d]	1080
0.45	(0.465)	(0.515)	(0.615)	
Subtotal Number of Rats	1400	1440	1440	N = 4280

[a]Activities of 1.5 and 4.7 nCi correspond to 55 and 174 Bq, and doses of 25 and 90 rad correspond to 0.25 and 0.9 Gy, respectively.

[b]Expected crude incidence (not corrected for spontaneous incidence) of lung tumors in rats from single exposures.

[c]Number of rats in each experimental group of the matrix. Equal numbers of male and female rats in each group.

[d,e]Groups with the same letter (d or e) are exposed within a single block.

[f]Forty rats from each group of exposed rats are designated for serial sacrifice to determine $^{239}PuO_2$ and beryllium retention.

[g]Number in parentheses is the expected crude incidence if tumor induction from plutonium and beryllium is strictly additive.

The aerosols used are $^{239}PuO_2$ and beryllium metal. The production of the ^{169}Yb-radiolabeled $^{239}PuO_2$ aerosols has been described (1970-71 Annual Report, LF-44, pp. 7-14). Beryllium metal aerosol is produced by passing bulk industrial beryllium metal powder (I-400, Brush Wellman Co., Elmore, OH) through stage 3 of an aerosol cyclone (1986-87 Annual Report, LMF-120, pp. 45-49). Aerosols are passed to an ITRI 96-port, small animal, nose-only exposure chamber. Both aerosols are sampled using filters, cascade impactors, and electrostatic precipitators for the determination of aerosol concentration, size, and morphology, respectively. Different $^{239}PuO_2$ ILBs are achieved by varying the exposure duration, with particle size and concentration remaining constant. For beryllium metal, the highest ILB is achieved by increasing both exposure duration and aerosol mass concentration.

Rats are exposed in groups of 60. Animals first receive $^{239}PuO_2$ exposure or room air (for the sham-Pu groups). The aerosol delivery system is then reconfigured to provide a subsequent exposure to beryllium (or to air for the sham-beryllium groups). Exposures, including aerosol line reconfiguration, last 45-90 min. Animals assigned to the sham-plutonium and sham-beryllium groups are exposed to nebulized heat-treated 0.6 N HCl (vehicle used for plutonium exposure) for 30 min in a separate, uncontaminated aerosol line.

Animals that receive $^{239}PuO_2$ are whole-body counted to detect the ^{169}Yb radiolabel incorporated into the PuO_2. Whole-body counting is done immediately following exposure and then weekly for 6 wk. A single-component negative exponential function is fitted to the 1-6 wk, whole-body counting data. Initial lung burdens (ILBs) and clearance halftimes are estimated by evaluating the fitted function for each animal at t = 0 days.

For each block of animals entered into the study, one exposure group will be serially sacrificed for determination of clearance and translocation of material. Four animals (two of each sex) are randomly assigned to sacrifice at 8, 16, 32, 64, 128, 256, 360, and 450 days after exposure. At the end of the study, a total of 440 animals (40 from each exposure group except the sham-plutonium, sham-beryllium exposure group rats) will have been sacrificed for this purpose. All lungs and about 10% of all livers and femurs from these animals are designated for assay for Pu and Be using radiochemical and atomic absorption spectroscopic techniques, respectively.

In addition to the serial sacrifice group described above, a second group will be serially sacrificed later in life to provide information on tumor incidence. Groups of rats will be sacrificed after achieving various percentages of the expected life span. Both this sacrifice series and the analytical framework for lung tumor induction were previously described (1986-87 Annual Report, LMF-120, pp. 318-322).

All animals not assigned to sacrifice groups are being held for life-span observation. Animals are inspected daily, and moribund animals are euthanitized. Animals are weighed monthly for the first 3 mo after exposure, then at 3 mo intervals thereafter. At death, a complete necropsy is performed; lungs, lesions, and other selected tissues are fixed in formalin for histopathologic analysis.

A separate group of male rats received the 450 ILB of beryllium metal for an ancillary study. Exposed animals plus controls were assigned to serial sacrifice groups at 3, 7, 10, 14, 30, 60, 120, and 180 days after exposure for examination of acute inflammatory responses and potential resolution of these responses. Endpoints included histopathologic analysis, beryllium assay, and measurements of lung lavage fluid for cytology and levels of total protein, lactate dehydrogenase, and β-glucuronidase.[3]

RESULTS

The aerosol characteristics for the exposures are shown in Table 2. A total of 2502 of the planned number of 4280 rats (58%) have been entered into the study (Table 3). A total of 120 rats have thus far been sacrificed for determination of clearance and translocation of plutonium and beryllium. The morbidity and mortality among groups other than the 450 μg ILB beryllium metal group are not different from those of controls, and are not different from the historical experience of the Institute's colony.

Exposure to the 450 μg ILB of beryllium metal has resulted in an acute mortality of approximately 37% of male rats and 49% of female rats, and in decreased weight gains in surviving rats. The deaths occurred at about 2 wk after exposure and are independent of the level of $^{239}PuO_2$ exposure. At necropsy, the lungs appeared reddened, and the lymph nodes were dark and enlarged. Histopathologic analysis of selected lungs from these animals demonstrated a marked infiltrating, hemorrhagic pneumonitis.

Table 2
Characteristics of $^{239}PuO_2$ and Beryllium Metal Aerosols
Used for Inhalation Exposures of Rats

Aerosol	Target ILB[a]	Aerodynamic Diameter[b] (μm)	σ_g[b]	Mean Exposure Duration (min)	Mean Aerosol Concentration
$^{239}PuO_2$	1.5 nCi	0.70	1.67	7	21 nCi/L[c]
	4.7 nCi	0.71	1.61	16	26
Be Metal	50 μg	1.23	1.72	10	450 μg/L
	150 μg	1.25	1.91	31	420
	450 μg	1.50	1.74	40	990

[a]ILB = Initial lung burden.

[b]Aerodynamic diameter is activity median aerodynamic diameter (AMAD) for $^{239}PuO_2$ aerosols and mass median aerodynamic diameter (MMAD) for beryllium metal aerosols; σ_g = geometric standard deviation. Aerosol size determined by cascade impaction.

[c]A concentration of 21 nCi/L corresponds to 780 Bq/L.

Table 3

Status of Rats Entered into Study of Combined Effects of $^{239}PuO_2$ and Beryllium Metal[a]

Group		Dead		Euthanitized		Scheduled Sacrifices		Removed		Alive		
PuO_2	Be Metal	M	F	M	F	M	F	M	F	M	F	Total
Sham	Sham	0	1	0	1	0	0	0	0	81	79	162
Sham	50 μg	1	0	0	0	0	0	0	0	119	120	240
Sham	150 μg	1	1	0	0	0	0	0	0	89	89	180
Sham	450 μg	39	53	0	0	0	0	1	0	50	37	180
1.5 nCi[b]	Sham	1	1	0	1	4	4	2	0	113	114	240
1.5 nCi	50 μg	2	0	0	0	14	14	0	0	104	106	240
1.5 nCi	150 μg	1	1	0	0	10	10	0	0	109	109	240
1.5 nCi	450 μg	47	47	2	1	10	10	0	0	61	62	240
4.7 nCi[b]	Sham	0	1	0	0	12	12	0	0	78	77	180
4.7 nCi	50 μg	1	0	0	1	0	0	3	0	116	119	240
4.7 nCi	150 μg	1	0	0	0	0	0	0	2	89	88	180
4.7 nCi	450 μg	25	47	1	0	10	10	0	0	54	33	180
Total		119	152	3	4	60	60	6	2	1063	1033	2502

[a]As of November 1, 1988. Animals that died spontaneously were classified as dead. Moribund rats were euthanitized. Animals classified as removed were assigned to groups, but died either before or during exposure.

[b]Activities of 1.5 and 4.7 nCi correspond to 55 and 174 Bq, respectively.

Data obtained from serial whole-body counting of $^{239}PuO_2$-exposed rats are analyzed for the determination of ^{239}Pu ILB and clearance halftime. ILBs achieved thus far are shown in Table 4; these values are slightly above the intended levels. Figure 1 illustrates the influence of beryllium exposure on the retention of ^{239}Pu. There is no difference in ^{239}Pu retention following exposure to either 1.5 or 4.7 nCi (55 or 174 Bq) doses of $^{239}PuO_2$, in the absence of beryllium; retention halftimes of 49 and 48 days are observed, respectively. However, when rats are exposed to both materials, the retention of ^{239}Pu is significantly enhanced; halftimes for the 6 groups exposed to both materials range from 285 to 410 days. Interestingly, the ILB of beryllium metal does not influence this enhanced retention, within the range of ILBs tested. Direct chemical and radiochemical analyses of beryllium and plutonium content in lung, liver, and femurs of exposed rats are underway.

Table 4

Initial Lung Burdens of $^{239}PuO_2$ (nCi) Achieved During Inhalation Exposure of Rats to $^{239}PuO_2$ and/or Beryllium Metal

Projected Dose of Beryllium Metal	Projected Dose of ^{239}Pu[a] 0 nCi	1.5 nCi	4.7 nCi
0 µg			
Actual Pu ILB[b]	-	1.68 ± 0.60	4.70 ± 1.39
N[c]		179	177
50 µg			
Actual Pu ILB	-	2.10 ± 1.48	6.50 ± 2.94
N		180	178
150 µg			
Actual Pu ILB	-	2.13 ± 0.77	5.09 ± 1.57
N		180	178
450 µg			
Actual Pu ILB	-	2.24 ± 1.07	5.60 ± 1.74
N		145	121

[a]Activities of 1.5 and 4.7 nCi correspond to 55 and 174 Bq, respectively.

[b]Numbers shown are mean ± standard deviation. Values for ILB determined by whole-body counting for the ^{169}Yb radiolabel incorporated in the $^{239}PuO_2$ particle matrix.

[c]N = Number of animals.

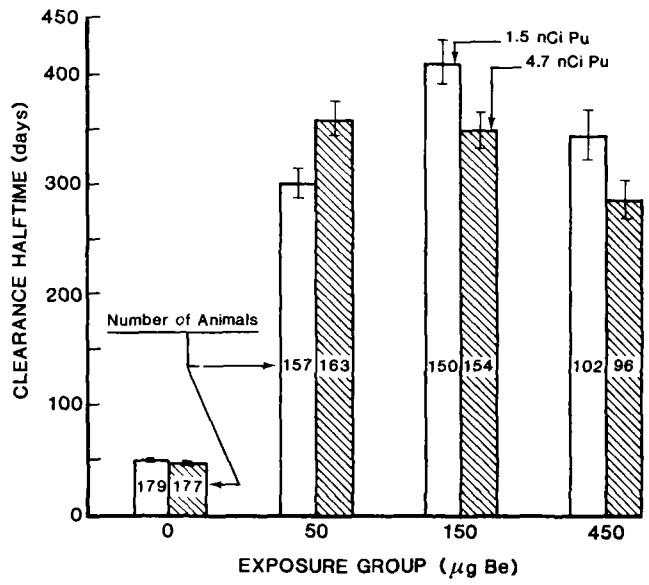

Figure 1. Plot of retention halftime of plutonium as a function of exposure to beryllium metal. Any one of the three beryllium metal ILBs increased plutonium retention halftime by a factor of 6-8. Bars are standard error of the mean, and the number of rats in each group is shown in each bar.

Inflammatory responses resulting from the 450 μg ILB of beryllium metal are shown in Figure 2. This portion of the study is still in progress. Beryllium-exposed rats exhibit an increase in total, lavageable nonepithelial cells (Fig. 2A), and an increased percentage of neutrophils, with an associated decrease in the percentage of lavageable macrophages (Fig. 2B). Increased levels of total protein (Fig. 2C) and of the enzymes, LDH and β-glucuronidase (Fig. 2D), are also evident. Responses are clearly different from those of control groups for most parameters, at nearly all of the timepoints examined thus far.

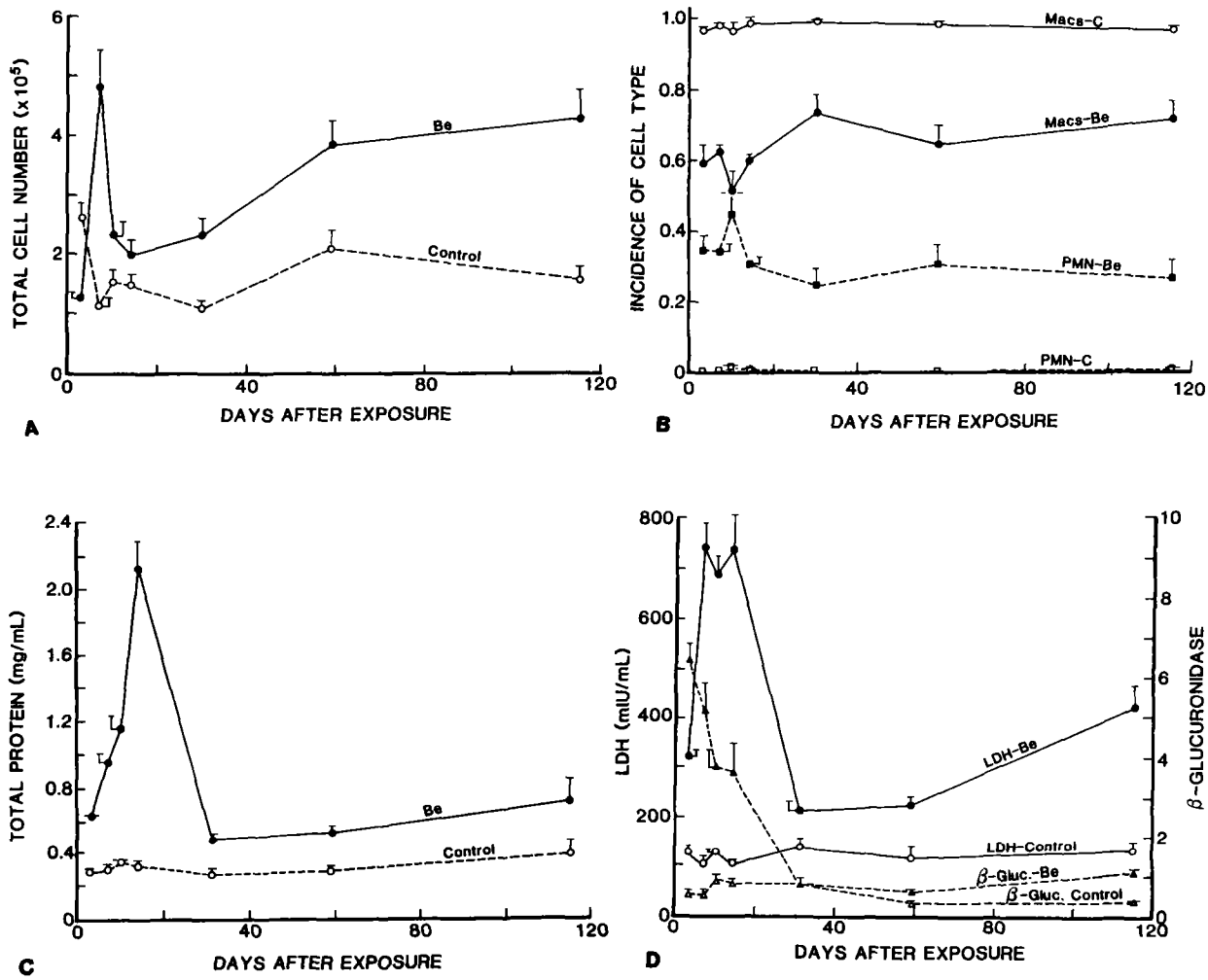

Figure 2. Lavage fluid and cytologic profile obtained from rats exposed to the 450 μg ILB of beryllium metal. A. Total number of nonepithelial cells obtained from exposed and control rats as a function of days after exposure. B. Percentage of macrophages (Macs) and polymorphonuclear neutrophils (PMN) plotted as a function of days after exposure for exposed and control animals. C. Lavage fluid content of total protein (TP) as a function of days after exposure for exposed and control animals. D. Lactate dehydrogenase (LDH) and β-glucuronidase (β-Gluc.) as a function of days after exposure for exposed and control animals. Bars are the standard error of the mean.

DISCUSSION

Rats exposed thus far to achieve ILBs of beryllium metal of 150 μg or less, regardless of $^{239}PuO_2$ exposure, have exhibited survival patterns, weight gains, and clinical signs similar to those of control animals. At the 450-μg ILB of beryllium metal, however, significant acute mortality occurs at approximately 2 wk after exposure. Lung responses observed using lavage are characteristic of a persistent inflammatory response.[3] The potential association between inflammatory responses (and the resolution of such responses) attributable to inhaled beryllium and the eventual induction of cancer is unknown; however, it has been suggested that humans who have suffered acute responses to beryllium might be at increased risk for the induction of the chronic disease.[4] Histopathological examination of lungs from the animals exposed to achieve a 450-μg ILB of beryllium metal is underway, and will be described in future reports.

Exposure of rats to beryllium metal at any of the three dose levels was found to enhance the retention of ^{239}Pu significantly, as determined by whole-body counting through 6 wk after exposure (Fig. 1). This should enhance the effects of Pu (cancer) and suggests that a synergistic interaction between Pu and Be may be observed. Enhanced retention of plutonium in the presence of beryllium has been observed by other researchers.[5,6] Because these data are limited to only 6 wk after exposure, a single component was sufficient to describe retention. These results will be investigated further by chemical analyses of the lung content of ^{239}Pu and beryllium. In addition, selected samples of livers and femurs of exposed rats will be analyzed for both materials. Because translocation from lung to these sites over time has been described for both plutonium[2] and beryllium (1986-87 Annual Report, LMF-120, pp. 146-153), these analyses are expected to provide information regarding the relative insolubility of the two aerosols. Correlations between whole-body counting for plutonium and direct analyses of plutonium and beryllium are underway, and will be described in future reports. Although initial lung burdens of $^{239}PuO_2$ (Table 4) achieved to date are slightly greater than those intended, this will not present a problem in the dose-response analysis phase of the study, because animals will be grouped for modeling purposes by actual measured dose.

In conclusion, a life-span study of the cancer risk in rats due to inhalation of aerosols of $^{239}PuO_2$ alone or in combination with beryllium metal is underway. Exposures should be completed by the end of 1989. As data become available and are analyzed, a mathematical model for the combined risk of exposure to plutonium and beryllium will be developed.

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