RADIATION RESEARCH 93, 85–92 (1983)

Alveolar Deposition of Sized Particles of $^{239}PuO_2$ in the Mouse

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MORGAN, A., BLACK, A., MOORES, S. R., PRITCHARD, J. N., WALSH, M., AND LAMBERT, B. E. Alveolar Deposition of Sized Particles of $^{239}PuO_2$ in the Mouse. *Radiat. Res.* **93,** 85–92 (1983).

Data on the alveolar deposition of $^{239}PuO_2$ particles in the mouse are presented. It is shown that alveolar deposition falls with increasing activity median aerodynamic diameter (AMAD) over the range 0.6 to 2.4 μm. In the mouse, any peak in alveolar deposition must occur at an AMAD of less than 1 μm. Information is also provided on the relative lobar concentrations (RLCs) of $^{239}PuO_2$. The RLC is greatest in the right apical lobe and increases quite sharply with AMAD. The RLCs of the other lobes of the right lung decline with AMAD. The RLC in the left lobe is relatively unaffected by AMAD.

INTRODUCTION

In a joint program involving the Atomic Energy Research Establishment, Harwell, and the Department of Radiobiology of the Medical College, St. Bartholomew's Hospital, both early and late effects of inhaled $^{239}PuO_2$ on the mouse lung are being studied. Of particular interest is the effect of particle size on carcinogenicity (the "hot-particle" problem), and, to this end, discrete-sized fractions of $^{239}PuO_2$ have been prepared by water sedimentation and administered to mice by inhalation. In other experiments, the effect of particle size on the fibrogenicity of $^{239}PuO_2$ is being investigated. To enable radiological doses to the lung to be assessed over appropriate periods, additional groups of mice have been exposed to aerosols of sized $^{239}PuO_2$ to give a range of initial alveolar depositions (IADs) corresponding to those used in the studies of radiation-induced lung cancer and fibrosis. These animals are being sacrificed serially to enable the retention of ^{239}Pu in the lung and its translocation to other organs to be determined. In this paper the results have been analyzed in terms of the effect of particle size and aerosol concentration on alveolar deposition of $^{239}PuO_2$. Data on the interlobar distribution of $^{239}PuO_2$ are included.

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0033-7587/83/010085-08$03.00/0

MATERIALS AND METHODS

Mice

Random-bred male and female SAS 4 mice from a closed colony were used in these experiments. They were about 6 weeks old at the time of exposure when their weights ranged from 18 to 26 g, and they were given MRC 41B diet and water *ad libitum.*

Preparation of Sized $^{239}PuO_2$

The $^{239}PuO_2$ used was prepared by calcination of the oxalate at 550°C. The particles produced in this manner were separated into discrete small-, medium-, and large-sized fractions, with nominal activity median aerodynamic diameters (AMADs) of 0.8, 1.5, and 2.2 μm, respectively, using a water sedimentation technique described by Black *et al.* (*1*). Geometric standard deviations (σ_g) were in the range 1.2 to 1.3.

Administration of Sized $^{239}PuO_2$ Particles

The system used for the administration of sized particles of $^{239}PuO_2$ to mice by inhalation has been described by Walsh *et al.* (*2*) and permits the simultaneous nose-only exposure of up to 60 mice. Both aerosol generation and exposure systems were contained in a glove-box suite. Exposed mice were normally maintained for 1 week in an air-conditioned glove box which allowed time for radioactive material deposited on the pelt to be removed by preening.

In the experiments described, the aerosol was generated from an aqueous suspension of sized $^{239}PuO_2$ using a Retec nebulizer (Cavitron Corp., Van Nuys, CA). The suspension was stabilized with dilute ammonia (*3*) since ionic and nonionic surfactants could have been cytotoxic. The nebulizer was operated with medical-grade nitrogen to eliminate changes in the pH of the suspension which would have resulted from the absorption by ammonia of CO_2 present in compressed air. To minimize stress, mice were generally exposed for 30 min only and the tubes in which they were restrained were cooled so that their internal temperatures normally did not exceed 30°C. Only for the highest IADs was it necessary to increase exposure times to about 90 min. During exposure, the aerosol was sampled on two precalibrated in-line filters to assess its concentration and also on a Mercer seven-stage cascade impactor (*4*) to measure its AMAD.

Analysis of Lungs for ^{239}Pu

The IAD for each group of exposed mice was measured by killing a number of animals 2 days following exposure. By this time, it was considered that the clearance, by mucociliary action, of particles deposited in the conducting airways would have been essentially complete. As reported by Stirling and Patrick (*5*) there is evidence that mucociliary clearance does not remove all the material deposited in the conducting airways of the lung but the amount remaining represents only a small fraction of that deposited.

To determine ^{239}Pu, lungs were wet ashed with 8 *M* nitric acid and the residue was heated at 500°C. The ash was treated with nitric/hydrofluoric acids to dissolve

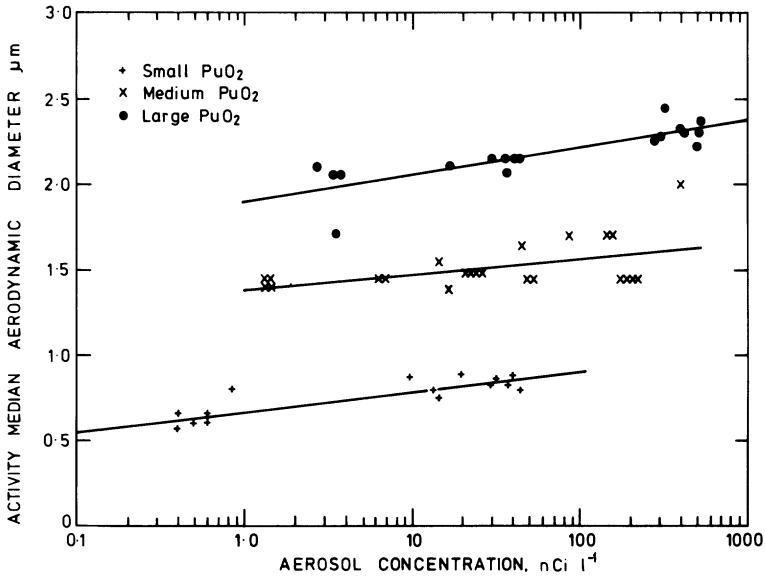

FIG. 1. Correlation between the activity median aerodynamic diameter of $^{239}PuO_2$ aerosols and aerosol concentration.

the PuO_2. The ^{239}Pu activity was measured by a solvent extraction and liquid scintillation counting technique based on that described by Keough and Powers (*6*).

RESULTS

The experiments were planned to give IADs ranging from 0.1 nCi (4 Bq) to 25 nCi (925 Bq). This range was selected because, on the basis of published evidence for ^{239}Pu-induced lung cancer in other rodents (*7*), it was anticipated that the maximum tumor incidence would occur with IADs in the range 1 to 10 nCi. The other factor which limited the range of useful IADs was the likelihood of increased early mortality at IADs greater than 25 nCi (925 Bq) due to radiation pneumonitis. The desired range of IADs was achieved by varying the concentration of sized $^{239}PuO_2$ in the nebulizer.

TABLE I

Summary of Data Relating to Alveolar Deposition of Sized $^{239}PuO_2$

Nominal size of $^{239}PuO_2$	*AMAD (µm)*		*No. of mice killed at 2 days*	*Nominal IAD[a] (nCi)*
	Range	*Mean ± SE*		
Small	0.57–0.88	0.76 ± 0.03	121	0.551 ± 0.049
Medium	1.40–2.00	1.52 ± 0.03	241	0.318 ± 0.016
Large	1.76–2.45	2.18 ± 0.04	197	0.099 ± 0.005

[a] Normalized to an aerosol dosage of 100 nCi liter^{-1} min.

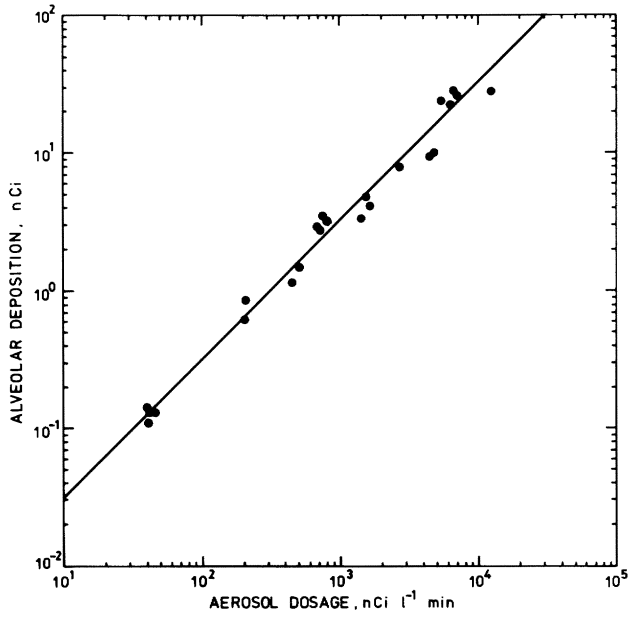

FIG. 2. Correlation between alveolar deposition of medium-sized $^{239}PuO_2$ and aerosol dosage ($r = 0.99$; $P < 0.001$).

When AMAD was plotted against log aerosol concentration (see Fig. 1) it was apparent that there was a tendency for it to increase with aerosol concentration. Correlation coefficients of 0.91, 0.52, and 0.82 were obtained for the small, medium, and large particles, respectively. The changes were significant ($P < 0.01$) in all cases and were attributed to the necessity of having relatively high concentrations of $^{239}PuO_2$ in the nebulizer in order to achieve high IADs. Under these circumstances,

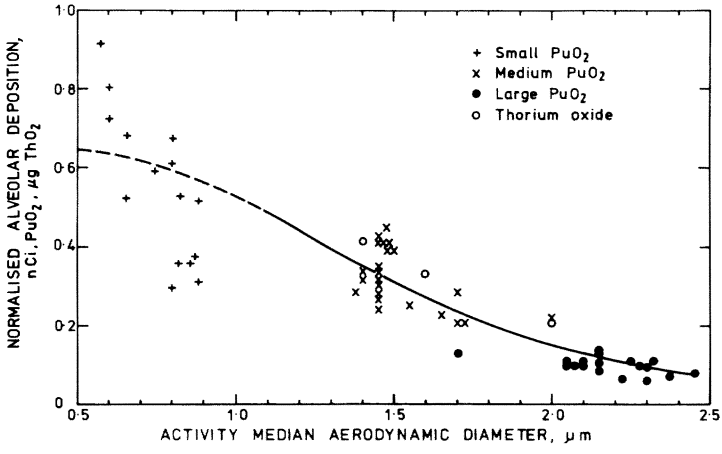

FIG. 3. Effect of activity median aerodynamic diameter on alveolar deposition, normalized to an aerosol dosage of 100 nCi liter^{-1} min for $^{239}PuO_2$ and 100 μg liter^{-1} min for ThO_2.

TABLE II

Distribution of $^{239}PuO_2$ among Various Lobes 2 Days Postexposure; Mean ± SE

Nominal size of $^{239}PuO_2$	*Range of AMADs (μm)*	*No. of mice*	*Distribution (%)*				
			R. apical	*R. diaphragmatic*	*R. cardiac*	*R. azygous*	*Left*
Small	0.60–0.88	19	20.9 ± 0.4	24.3 ± 0.3	10.4 ± 0.3	9.7 ± 0.2	34.7 ± 0.4
Medium	1.40–1.48	71	22.3 ± 0.4	22.5 ± 0.4	10.9 ± 0.3	9.9 ± 0.2	34.4 ± 0.6
Large	2.10–2.45	63	24.7 ± 0.5	20.5 ± 0.3	10.2 ± 0.2	8.9 ± 0.2	35.7 ± 0.5

the probability of generating droplets containing more than one particle was increased as was the probability of airborne particles coagulating. A similar effect has been reported by Craig *et al.* (*8*) when generating aerosols of $^{239}PuO_2$ for animal inhalation experiments. The AMAD of the small-sized $^{239}PuO_2$ increased by 36% as its concentration increased over two orders of magnitude. The corresponding value for the medium and large particles was about 15%. Despite these variations there was essentially no overlap in the ranges of AMADs for the different particle sizes. The ranges and overall mean values for the AMADs of small-, medium-, and large-sized $^{239}PuO_2$ are given in Table I.

For each experiment the aerosol dosage (the product of aerosol concentration and exposure time) was calculated. For the medium- and large-sized $^{239}PuO_2$ there was a satisfactory linear relationship between IAD and aerosol dosage. For the small particles, however, there was a departure from linearity at high aerosol dosages due presumably to the greater variation in AMAD associated with this material. In Fig. 2 alveolar deposition of the medium-sized $^{239}PuO_2$ has been plotted against aerosol dosage in log–log form, and it is clear that the relationship is essentially linear.

In Fig. 3 IADs achieved in the various experiments, normalized to an aerosol dosage of 100 nCi liter^{-1} min, have been plotted against the AMADs of the corresponding aerosols. This shows that the normalized IADs declined with increasing AMAD and that the data for each particle size were reasonably discrete.

Information on the distribution of $^{239}PuO_2$ among the five lobes of the mouse lung 2 days postexposure is given in Table II. This shows that most deposition occurred in the left lobe and the least in the azygous lobe. There were changes in the ranking of the right apical and diaphragmatic lobes with increasing AMAD.

TABLE III

Relative Lobar Weights Based on Data from 153 Mice; Mean ± SE

Lobe	*Relative lobar weight (%)*
R. apical	18.3 ± 0.2
R. diaphragmatic	26.2 ± 0.2
R. cardiac	12.4 ± 0.1
R. azygous	9.8 ± 0.1
Left	33.3 ± 0.2

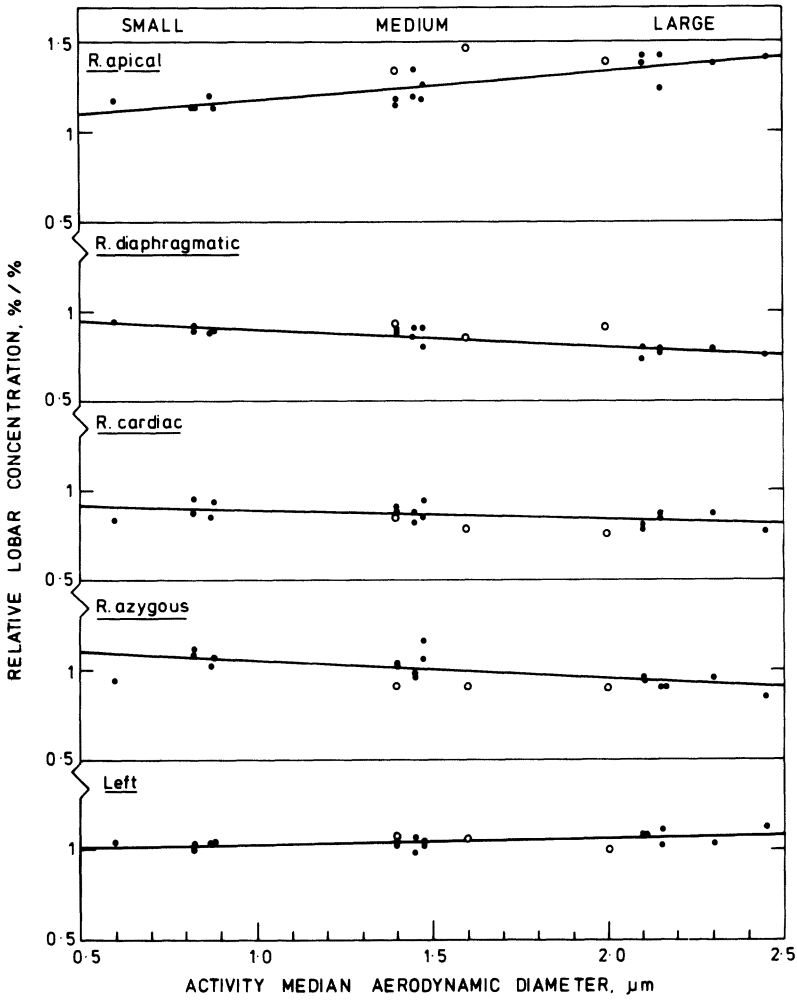

FIG. 4. Effect of activity median aerodynamic diameter on the relative lobar concentrations of $^{239}PuO_2$ in the mouse lung. Results for $^{239}PuO_2$ (●) and for ThO_2 (O).

DISCUSSION

Deposition of $^{239}PuO_2$

Figure 3 shows that, for a given aerosol dosage, IAD declined with increasing AMAD. Included in Fig. 3 are results obtained by Moores *et al.* (*9*) for the alveolar deposition of sized thorium oxide in the mouse lung normalized on a mass rather than an activity basis. The only other relevant data on the alveolar deposition of inhaled particles in the mouse have been reported by Lundgren *et al.* (*10*) who used $^{144}CeO_2$. With much greater aerosol concentrations than those in the present study they obtained alveolar depositions, normalized on the same basis, of about 0.2 nCi for aerosols with AMADs in the range 1.6 to 1.8 μm. This is not dissimilar to that obtained for $^{239}PuO_2$ particles with AMADs in the same range.

In man, alveolar deposition peaks at an aerodynamic diameter of about 3 μm (*11*), while in the rat, the corresponding value is about 2 μm (*12*). It appears from Fig. 3 that the aerodynamic diameter at which maximum alveolar deposition occurs in the mouse is probably less than 1 μm. Variations in the optimum aerodynamic diameter for deposition in the nonciliated alveolar region of the lung are probably related to differences in the morphology of the airways of the upper respiratory tract in different species.

Interlobar Distribution of $^{239}PuO_2$

In considering the dosimetry of $^{239}PuO_2$ in the lung, it is important to establish how uniformly the radioactive material is distributed both initially and at later times. It is clear from Table II that the initial distribution of deposited $^{239}PuO_2$ between lobes was affected by the particle size of the aerosol. This effect was particularly marked for the right apical and diaphragmatic lobes. In order to determine the effect of these changes on the concentration of ^{239}Pu in the various lobes, relative lobar concentrations (RLCs) were calculated as described by Raabe *et al.* (*13*). RLCs were obtained by dividing the proportion of the total mass (or activity) of aerosol found in a particular lobe by the proportion of the total lung weight represented by that lobe. If material is distributed uniformly throughout the lung, the RLC for each lobe would have a value of 1. Mean lobar weights, expressed as a percentage of the total wet weight of mouse lung, are given in Table III.

RLCs obtained in the present study are shown in Fig. 4. Data obtained in earlier experiments using thorium oxide (*9*) are included for each lobe. In that case, RLCs were calculated from relative dry lobar weights which, however, were only significantly different from relative wet weights for the right cardiac lobe. Even in this case, the difference (<7%) would not have affected the overall trend.

It is clear from Fig. 4 that there was a change in RLCs with AMAD. However, the effect was more marked in some lobes than in others. For example, there was a marked increase with AMAD in the right apical lobe and a marked decrease in the right diaphragmatic and azygous lobes. The left lung and the right cardiac lobe were less affected by AMAD. These changes, which were significant ($P < 0.05$) for all lobes, follow a similar pattern to those observed by Raabe *et al.* (*13*) in the Syrian hamster and the rat.

ACKNOWLEDGMENTS

This program was supported in part by the Commission of the European Community (Euratom Contract 252-77-1 BIO UK). The authors would like to acknowledge gratefully the technical assistance of D. R. Belcher and F. Orpwood of the Environmental and Medical Sciences Division, J. Wilkins of the Chemistry Division, AERE, Harwell, and M. L. Phipps of the Department of Radiobiology, The Medical College, St. Bartholomew's Hospital, London.

RECEIVED: January 11, 1982; REVISED: May 21, 1982

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