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Relationships Between the Size Distribution of $^{239}\text{PuO}_2$ Aerosols,
Aerosol Concentration and Nebulizer Suspension Concentration*

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ABSTRACT

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In a study of low-level effects of inhaled $^{239}\text{PuO}_2$ in beagle dogs, alveolar burdens over the 1500-fold range from 2 nCi to 3 μCi were deposited in unanesthetized dogs by aerosol inhalation. Aerosols were generated by nebulizing magnetically-stirred suspensions of $^{239}\text{PuO}_2$ particles in water; the aerosol concentration (CONC) being varied, primarily, by changing the Pu concentration of the suspension (SSA).

If the mass concentration of the suspension was kept below about 10 mg (540 μCi) PuO_2/ml , a good correlation ($R = 0.827$ for $n = 64$) was observed between CONC and SSA with constant air flow through the exposure chamber. SSA values from 1 to 600 $\mu\text{Ci}/\text{ml}$ gave CONC values in the range 1.5 to about 3000 nCi/l. However, the aerodynamic equivalent size distribution also varied significantly with CONC. The activity median aerodynamic diameter (AMAD) increased from about 1.5 to 3 μm as CONC increased ($R = 0.786$ for $n = 64$), while the geometric standard deviation (GSD) decreased ($R = 0.576$ versus AMAD).

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These observations help to explain the large variation observed in the percentage alveolar deposition of inhaled $^{239}\text{PuO}_2$ aerosols from less than 1% to nearly 50% in 64 dogs for which the data are thus far available. A change in AMAD from $1.54\text{ }\mu\text{m}$ (GSD 2.61) to $2.77\text{ }\mu\text{m}$ (GSD 1.61) decreased the percentage of ^{239}Pu activity in particles having aerodynamic equivalent diameters less than $1\text{ }\mu\text{m}$ from 32.63% to 1.62%. This effect on particle size is the major factor determining percentage alveolar deposition.

Introduction

In a study of the low-level effects of inhaled $^{239}\text{PuO}_2$ in beagle dogs, alveolar burdens over the 1500-fold range from 2 nCi to 3 μCi were deposited in unanesthetized dogs by aerosol inhalation. Since these were acute rather than chronic exposure studies, repeated exposures were not considered desirable as a method of achieving deposition over such a large range.

The time for which a dog can be subjected to nose-only exposure to a toxic aerosol is limited by practical considerations involving dog training time, the comfort of the dog and the animal-handling technicians, the length of time over which a constant concentration of a suitable aerosol can be generated, and the time required to obtain representative samples for characterization of the exposure conditions. The range applicable to the present experiment was from 2 to 40 minutes, a factor of 20. The volume of air inhaled by a dog was measured but is a variable over which we have no control. The 16 to 20 month old beagles involved had minute volumes of between 1 and 5 l/min during their exposure.

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The volume of air inhaled (l) times the aerosol concentration ($\mu\text{g/l}$ or $\mu\text{Ci/l}$) determines the quantity of material that is inhaled by the dog during its exposure. In the absence of other variables, it is this quantity that will determine how much of the material is deposited in the alveoli of the dog. Clearly, if it is desired to deposit alveolar burdens over a large range in a group of animals, the only parameter that can conveniently be changed by several orders of magnitude is the aerosol concentration. In the present experiment, the mean concentration of $^{239}\text{PuO}_2$ varied from 0.4 $\mu\text{Ci/l}$ to 3.86 $\mu\text{Ci/l}$.

It is reasonable to expect that the particle size distribution of the inhaled material will play an important part in determining its fractional deposition in various regions of the dog's respiratory tract. It therefore seemed important to investigate in detail the effect, if any, that changes in the aerosol mass concentration over nearly four orders of magnitude had on the particle size distribution of the $^{239}\text{PuO}_2$ aerosols to which the dogs were exposed.

Methods.

The exposure chambers, equipment and techniques used for the dog inhalation experiments have been described elsewhere.^(1,2) Plutonium-239 aerosols were generated by nebulizing magnetically stirred suspensions of particles in water with Lovelace nebulizers.⁽³⁾ The PuO_2 was prepared by calcining ^{239}Pu oxalate for 2 hours at 750°C and making a suspension of mass concentration 20 mg/ml of triple distilled water. This contained

many large particles which were not broken up by ultrasonic agitation. To eliminate large particles, the suspension was shaken vigorously by hand for 2 minutes and, after allowing it to stand for 10 minutes, the top 3.5 cm of supernatant was withdrawn. Spheres of diameter $3.34\ \mu\text{m}$ and density $10.6\ \text{g/cm}^3$ (Stokes' diameter for PuO_2) will settle this distance through water in 10 minutes. The Stokes' diameter distribution of particles in the supernatant was log-normal at the time of preparation, with a mass median diameter (MMD) of $1.10\ \mu\text{m}$ and a geometric standard deviation (GSD) of 1.96. This stock suspension had an activity concentration of about $100\ \mu\text{Ci/ml}$ and was either diluted or concentrated prior to nebulizing it, depending upon the dose group of the dogs being exposed. No dispersing agent was used in any of the suspensions because of the limited information available on the inhalation toxicity of these agents, but the containers were "dry filmed" with dimethyldichlorosilane to prevent Pu sticking to them.

The PuO_2 suspension to be used for a particular exposure was calibrated to determine its activity concentration (SSA) and immediately prior to transferring it to the nebulizer bowl, it was ultrasonically agitated. A magnetic stirring bar was included in each suspension and the nebulizer was mounted on a magnetic stirrer during operation (Figure 1). Compressed air, usually at 20 p.s.i.g., was supplied to the nebulizer. Under these conditions, the outputs of the nebulizers used were in the range 45 to $100\ \mu\text{l}$ liquid/min in approximately 1.6 l air/min under standard conditions, the exact flow depending upon the upstream air pressure and the diameter of the nebulizer jet. The output of the nebulizer was determined by dividing the

difference in liquid volume before and after operation by the time of operation.

The nebulizer output was passed up a half-inch ID copper tube around which was wound a heating tape. Power was supplied to heat the air passing through the tube to between 125 and 150°C, the intention being to evaporate the water droplets containing the PuO_2 particles. The aerosol was mixed with diluting air at the top of the aerosol exposure chamber, the air being exhausted through two 47 mm diameter membrane filters at 10 l/min ($T = 70^\circ\text{F}$, $P = 29.92$ inHg), except for 3 cases in which 20 l/min was used.

Aerosol concentration measurements were made by counting the activity of each of two 25 mm diameter membrane filters, through which air was drawn at a fixed rate for the duration of the dog exposure. Results from the two samples, expressed in nCi/l, were usually within a few percent of each other and their mean was taken as the chamber aerosol concentration. Point-to-plane electrostatic precipitator (ESP)⁽⁴⁾ and Walkenhorst thermal precipitator (TP)⁽⁵⁾ samples were taken under appropriate conditions to yield samples of the PuO_2 particles on electron microscope grids. These were shadowed with chromium at an angle of 26° and then examined in an electron microscope (EM). Figures 2 and 3 are micrographs of the PuO_2 aerosols, one having a low and the other a high concentration.

The difficulties involved in obtaining meaningful particle size distributions from EM samples have been discussed elsewhere.⁽⁶⁾ Samples for the aerodynamic equivalent size classification of the aerosols were taken with Mercer cascade impactors,⁽⁷⁾ the sample duration being the same as the dog exposure. The filter papers and the silicone-coated glass slides

from the impactor were sandwiched between two strips of cellophane adhesive tape and their activity determined by counting the ^{239}Pu 17 keV X-rays under appropriate conditions. Mercer's effective cutoff diameter (ECD) method⁽⁸⁾ was used in conjunction with a computer program to calculate the activity median aerodynamic diameter (AMAD) and the GSD of the PuO_2 aerosols, assumed to be log-normally distributed. (The aerodynamic equivalent diameter of a particle is the diameter of the unit density sphere which has the same final settling velocity as the particle. For a homogeneous material like PuO_2 , the AMAD and mass median aerodynamic diameter are the same and are related to the MMD by the square root of the particle density and a slip correction.)

The quantity of plutonium inhaled by a dog was calculated by multiplying the mean aerosol concentration in nCi/l (CONC) by the volume of air inspired by the dog.⁽¹⁾ The amount of alveolar deposited plutonium was estimated by averaging the 14- and 28-day postexposure, 17 keV X-ray, thorax counts that were made for each dog.⁽⁹⁾ Plutonium cleared before this was assumed not to have been deposited in the alveoli.* This amount was divided by the quantity inhaled to obtain the percentage alveolar deposition (DEP) for each dog.

Some of the variables, measured as described above, during the exposure of 64 dogs, are given in Table 1. The CONC values have been ordered from lowest (0.42 nCi/l) to highest (3962 nCi/l) for convenience. Linear correlation

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* Dogs sacrificed 14 days postexposure had 99% of their ^{239}Pu in the lung.

analyses were conducted between each relevant set of variables to seek significant relationships, the correlation coefficients (R) obtained being tested for significance at the $p < 0.05$ and the $p < 0.01$ levels. Where it appeared desirable, linear regression analyses were conducted to quantify the relationship between variables.

Results

Some of the results obtained from the correlation analyses between variables are given in Table 2. The mean aerosol concentration in the chamber under fixed flow conditions (10 l/min) was significantly correlated with the nebulizer suspension specific activity ($R = 0.827$). CONC has been plotted against SSA on log x log paper in Figure 4. The correlation coefficient between $\log_{10}$ CONC and $\log_{10}$ SSA is 0.976, which is excellent considering the fact that the results presented were obtained during a series of dog exposures over a five-month period. A number of different Lovelace nebulizers, having outputs ranging from 44 to 108 $\mu\text{l}/\text{min}$ with a mean of 76 $\mu\text{l}/\text{min}$, were involved. No adjustment to the CONC values was made on this account since only the mean output for each combination of generator and suspension, involving from 1 to 8 CONC values, was available. However, with only a couple of exceptions, points that fell far from the regression line in Figure 4 corresponded to nebulizer outputs that differed considerably from the mean value.

Both SSA and CONC are significantly correlated with the AMAD of the $^{239}\text{PuO}_2$ aerosol, the correlation coefficient between AMAD and CONC being

0.777. These variables have been plotted against each other in Figure 5 and a regression equation between AMAD and $\log_{10}$ CONC was computed. This gave a multiple correlation coefficient of 0.872, a highly significant value ($p < 0.01$). At the same time, GSD was observed to be negatively correlated with AMAD ($R = -0.576$) and, therefore, also with SSA and CONC. GSD has been plotted against AMAD in Figure 6, from which it appears that the above observation may be largely due to the few distributions having GSD values greater than 2.2. However, eliminating these values and recomputing the regression equation still gives coefficients that are significantly different from zero at the $p < 0.01$ level of significance.

The distribution parameters AMAD and GSD were calculated from the Mercer cascade impactor data, it being assumed that the activity was normally distributed with respect to the logarithm of the aerodynamic equivalent size. Since AMAD increased with CONC while GSD decreased, the fraction of activity on particles in various size ranges changes as the aerosol concentration changed.

The linear correlation coefficients between the percentage alveolar deposition (DEP) for each dog, and a number of the variables of Table 1, are listed in Table 2. Ten values for which the alveolar burden was not significantly different from zero at $p < 0.05$ and two values for which the concentration was less than 1 nCi/l, too low to reliably determine the quantity of activity inhaled by those dogs, have been excluded. This gives $N = 52$ instead of $N = 64$. DEP is clearly well-correlated with all the parameters listed.

Discussion

The relationships observed between SSA, CONC and the particle size distribution of the aerosol can be explained in terms of the probability of there being more than one PuO_2 particle per droplet escaping from the nebulizer. If we assume that the particles are uniformly distributed in nebulizer suspension and that their concentration is directly proportional to SSA, this probability can be calculated if we know something about the distribution of droplet sizes escaping from the nebulizer. The results of some rough calculations along these lines are given in Table 3 for SSA values ranging from 1 $\mu\text{Ci/ml}$ to 1000 $\mu\text{Ci/ml}$.

Assuming that the Stokes' diameter distribution of particles in suspension remains the same as SSA changes, i.e. at $\text{MMD} = 1.10 \mu\text{m}$ and $\text{GSD} = 1.96$, the number of PuO_2 particles per unit volume increases from 2.2×10^7 to $2.2 \times 10^{10}/\text{ml}$ over the above SSA range. Mercer⁽³⁾ gives the approximate mass distribution of droplets escaping from the nebulizer as $\text{MMD} = 5.4 \mu\text{m}$, $\text{GSD} = 1.9$, from which we can calculate the distribution of droplets with respect to volume. The product of a droplet volume and the particle concentration yields the average number of particles that droplets of that size will contain.

Obviously, if this average is much less than unity, there is little chance that nebulizing a uniform suspension of particles will result in a size distribution that differs from that of the particles in the suspension. However, if we compute the relative number of droplets likely to have more than one particle, we will obtain an idea as to the number of aggregates

there will be in the aerosol. The values given in Table 3 are based on a nebulizer output of 76 $\mu\text{l}/\text{min}$, the droplets being log-normally distributed with an MMD of 5.4 μm and a GSD of 1.9. Since the values are intended only to indicate order of magnitude effects, they have been rounded off to two significant figures and should not be taken too literally. A far more sophisticated analysis, taking into account both the particle and the droplet distributions and the droplets not escaping from the nebulizer, would be necessary to derive the expected aerosol size distribution as a function of SSA and CONC, which also depends upon the total air flow through the exposure chamber. The values in the last column of Table 3 provide an explanation of the differences between the aerosols shown in Figure 2 for a concentration of about 10 nCi/l and in Figure 3 for a concentration of about 500 nCi/l. The reason for the decrease in GSD as AMAD and CONC increase is believed to be that, since small particles are relatively more numerous than large ones (for MMD = 1.1 μm and GSD = 2.0, the CMD = 0.26 μm), they are more likely to be involved in aggregation. Aggregates of large particles are also heavier and more likely to be lost by sedimentation (in the horizontal section of the intake line) and impaction (in the mixer). The combined effect of these mechanisms is to decrease the range of particle sizes, hence the GSD of the distribution.

The fact that pulmonary deposition of aerosols is a function of the particle size distribution of the inhaled aerosol is, of course, well established. However, the published curves relating percentage deposition to particle size (for man) (10) span three orders of magnitude in particle

diameters. Under fixed respiration conditions, the percentage deposition changes by less than a factor of 2 as the particle diameter increases from 0.4 μm to 4 μm . For the 52 dogs included in the above analyses, DEP varied from 1.4 to 31.2% while the size distribution parameters AMAD and GSD varied from 1.54 to 3.07 μm , and 2.61 to 1.58, respectively. The effect that these variations have on the percentage of the $^{239}\text{PuO}_2$ on particles of aerodynamic equivalent diameter less than 1 μm can be seen in Table 1, viz. 1.62% to 32.63% for calculated values. They lead to a correlation between DEP and CLS-1.0 of 0.642, which is significant at the $p < 0.01$ level.

Conclusions

A highly significant correlation has been shown ($p < 0.01$) between the concentration of plutonium in magnetically-stirred suspensions (SSA) and the aerosol concentration (CONC) obtained by nebulizing the suspensions under constant conditions. However, significant correlations ($p < 0.01$) have also been obtained between both SSA and CONC and the aerodynamic equivalent size distribution of the aerosol. An explanation of this phenomenon based on aggregate formation due to the greater likelihood of there being more than one PuO_2 particle per nebulizer droplet at the higher SSA values has been advanced.

The importance of the relationship between CONC and the aerosol size distribution lies in the effect of the latter on DEP. The correlation between the percentage alveolar deposition and the percentage of $^{239}\text{PuO}_2$ on aerodynamic equivalent diameters less than 1 μm (CLS-1.0) is significant at the $p < 0.01$ level. For 52 dogs, DEP varied from 1.4 to 31.3% and

CLS-1.0 varied from 1.62 to 32.62%. Although this excludes 12 dogs for which DEP could not be accurately determined, their inclusion would not have altered the above conclusions.

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Figures

- Figure 1. Photograph of nebulizer in position on magnetic stirrer. Also shown is the coupling to the compressed air line and the heating tape wound around the aerosol inlet tube.
- Figure 2. Electron micrograph of $^{239}\text{PuO}_2$ aerosol, shadowed at 26° . TP sample, concentration ~ 10 nCi/l. X 10,000
- Figure 3. Electron micrograph of $^{239}\text{PuO}_2$ aerosol, shadowed at 26° . ESP sample, concentration ~ 500 nCi/l. X 10,000
- Figure 4. Graph of mean $^{239}\text{PuO}_2$ aerosol concentration as a function of nebulizer suspension specific activity.
- Figure 5. Graph of activity median aerodynamic diameter as a function of $^{239}\text{PuO}_2$ concentration.
- Figure 6. Geometric standard deviation as a function of activity median aerodynamic diameter of $^{239}\text{PuO}_2$ aerosols.

Table 1

Aerosol Size Distribution and Alveolar Deposition
Data for Various Aerosol and Suspension Concentrations

Aerosol CONC nCi/l	Suspension Spec. Act. μCi/ml	Aerosol Size Distribution Data			Alveolar Deposition %
		Parameters		Calculated % <1.0 μm	
		AMAD (μm)	G.S.D.		
0.42	0.767	2.11	2.48	20.55	100
0.63	0.767	1.66	2.25	26.60	48.5
1.00	0.767	1.81	1.88	17.36	2.6*
1.64	0.906	1.99	1.79	11.86	12.4*
1.69	0.906	2.01	1.86	13.03	0 *
2.03	0.906	1.54	2.61	32.63	27.0
2.30	0.908	1.89	2.03	18.43	16.2
2.33	0.908	2.34	1.83	7.97	16.5*
2.54	0.767	1.66	2.27	26.82	28.3
2.58	0.906	1.77	2.08	21.78	0 *
3.13	0.906	1.65	2.23	26.62	15.5
4.51	1.42	1.58	2.23	28.42	0 *
4.85	1.42	2.08	2.46	20.79	0 *
5.52	1.42	1.72	2.32	25.97	0 *
5.30	4.45	2.13	1.76	9.05	13.3
5.89	1.42	1.68	2.34	27.09	8.7*
5.91	1.42	1.83	2.43	24.81	13.7
6.35	4.23	2.03	1.82	11.85	9.5
6.74	4.45	1.90	1.72	11.83	8.2
7.06	4.23	2.06	1.95	13.96	1.4*
7.16	4.23	1.84	1.93	17.69	14.2
7.43	4.23	2.10	1.86	11.59	20.1
8.04	3.32	1.83	2.51	25.57	3.6
8.08	4.23	1.84	1.93	17.69	11.3
10.4	16.5	1.58	1.83	22.45	12.2
11.3	4.45	1.91	1.65	9.81	31.3
13.6	3.32	1.81	1.74	14.20	7.5
13.7	5.20	2.22	2.03	13.00	1.6
14.1	5.20	1.90	1.87	15.26	3.4
15.1	20.3	1.97	1.76	11.52	3.3
71.9	20.1	2.19	1.95	12.02	11.3
181	146	2.31	1.58	3.36	4.3

*Thorax X-ray count not significantly different from zero at $p < 0.05$.

Table 1 (Continued)

Aerosol Size Distribution and Alveolar Deposition
Data for Various Aerosol and Suspension Concentrations

Aerosol CONC nCi/l	Suspension Spec. Act. μCi/l	Aerosol Size Distribution Data			Alveolar Deposition %
		Parameters		Calculated % <1.0 μm	
		AMAD (μm)	G.S.D.		
196	81.8	2.31	1.77	7.13	10.3
202	81.8	2.01	1.65	8.16	2.4
220	81.8	2.12	1.76	9.19	7.8
228	81.8	2.12	1.75	8.97	6.9
229	495	2.60	1.66	2.97	3.7
251	146	2.77	1.61	1.62	5.7
271	107	2.43	1.78	6.18	3.5
288	146	2.51	1.62	2.82	4.4
317	495	2.60	1.60	2.10	4.1
345	146	2.64	1.68	3.07	7.6
388	102	2.11	1.80	10.20	6.8
398	102	2.22	1.77	8.12	7.2
504	102	2.18	1.74	7.97	5.5
665	495	2.85	1.76	3.20	3.5
689	643	2.76	1.80	4.21	3.4
1142	478	2.43	1.78	6.18	3.5
1143	415	2.76	1.71	2.92	2.6
1158	478	2.43	1.72	5.08	3.8
1293	415	2.79	1.68	2.40	1.8
1310	415	2.63	1.73	3.89	3.6
1907	643	2.78	1.78	3.81	4.6
2121	489	2.91	1.83	3.86	2.6
2257	489	2.75	1.83	4.71	1.4
2432	489	2.97	1.78	2.95	2.2
2477	614	2.81	1.73	2.97	7.9
2520	643	2.74	1.81	4.47	5.4
2569	489	2.86	1.79	3.55	2.1
2592	489	3.07	1.78	2.59	2.6
2690	489	2.84	1.84	4.35	1.8
2725	614	2.93	1.77	2.99	10.5
3759	489	2.85	1.76	3.20	2.2
3862	489	2.80	1.77	3.57	3.6

Table 2
Correlations Between Some Measured Parameters

Variable		Linear Correlation Coefficient R (N)	Significance Level*	
X	Y		p < 0.05	p < 0.01
SSA	CONC	0.827 (64)	Yes	Yes
SSA	AMAD	0.872 (64)	Yes	Yes
SSA	GSD	-0.443 (64)	Yes	Yes
CONC	AMAD	0.777 (64)	Yes	Yes
CONC	GSD	-0.310 (64)	Yes	No
AMAD	GSD	-0.576 (64)	Yes	Yes
DEP	SSA	-0.521 (52)	Yes	Yes
DEP	CONC	-0.423 (52)	Yes	Yes
DEP	AMAD	-0.625 (52)	Yes	Yes
DEP	GSD	0.461 (52)	Yes	Yes
DEP	CLS-1.0	0.642 (52)	Yes	Yes

*For N = 64, correlation coefficient is significant at
p < 0.05 if R > 0.246 and at p < 0.01 if R > 0.320

For N = 52, correlation coefficient is significant at
p < 0.05 if R > 0.273 and at p < 0.01 if R > 0.354

DEP - Percentage of inhaled material deposited in alveoli
of dogs

SSA - Suspension specific activity in $\mu\text{Ci/ml}$

CONC - Aerosol concentration in nCi/l

AMAD - Activity median aerodynamic diameter (μm)

GSD - Geometric standard deviation

CLS-1.0 - Calculated fraction of activity on particles
less than 1.0

Table 3

The Probability of Aggregate Formation in
The Nebulization of Suspensions of Particles

Suspension Concentration (SSA) $\mu\text{Ci/ml}$	$^{239}\text{PuO}_2$ Particle Concentration #/ml	Droplet Emission Rate #/min	Droplets with Probability of Having More Than One Particle	Percentage Aggregates Formed %
1	2.2×10^7	5.9×10^9	1.5×10^4	2.5×10^{-4}
5	1.1×10^8	5.9×10^9	7.1×10^4	1.2×10^{-3}
10	2.2×10^8	5.9×10^9	3.4×10^5	5.7×10^{-3}
50	1.1×10^9	5.9×10^9	8.0×10^6	1.3×10^{-2}
100	2.2×10^9	5.9×10^9	2.8×10^7	4.7×10^{-1}
500	1.1×10^{10}	5.9×10^9	4.2×10^8	7.1
1000	2.2×10^{10}	5.9×10^9	1.1×10^9	19

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