Retention, Excretion and Translocation of $^{239}$Pu in Rats Following Inhalation of $^{239}$PuO$_2$ Calcined at 1150 and 400°C

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Wistar rats inhaled $^{239}$PuO$_2$ particles prepared by the calcination of $^{239}$Pu hydroxide at 1150 and 400°C. Lung retention, fecal and urinary excretion, and translocation of $^{239}$Pu were compared between the two calcination temperatures. The clearance of $^{239}$Pu from the lungs was significantly faster in the rats exposed to $^{239}$PuO$_2$ calcined at 400°C (low-temperature group) than those exposed to $^{239}$PuO$_2$ calcined at 1150°C (high-temperature group). Both the fecal excretion of $^{239}$Pu and the ratio of fecal excretion to urinary excretion was greater in the low-temperature group than in high-temperature group. The amounts of $^{239}$Pu translocated from the lungs to the other organs were very small. Even in the liver, which accumulated the largest amount of $^{239}$Pu except for the lungs, only 0.13–0.20% of the initial lung burden was retained 1 year after inhalation. The amount of $^{239}$Pu deposited in the liver was greater in the high-temperature group than in the low-temperature group both at 1 month and 1 year after the inhalation. These findings clearly suggest that the lung retention of $^{239}$Pu in rats is significantly affected by the calcination temperature of $^{239}$PuO$_2$.

INTRODUCTION

The potential risk of an accidental exposure of workers to plutonium (Pu) has increased in Japan because of a government policy to promote the nuclear-fuel-cycle where Pu is used as a fuel for fast reactors. Our institute launched a research project on the inhalation toxicology of...
$^{239}\text{Pu}$, and a few reports have already been published by some of the authors. Ishigure et al have determined the lung retention of $^{239}\text{Pu}$ by whole body counting in rats inhaling $^{239}\text{PuO}_2$ aerosols$^{1,2}$. Oghiso et al have demonstrated the induction of lung tumors in the same experimental animals$^{3,4}$. 

The primary purpose of the present study is to supply some supplementary $^{239}\text{Pu}$ metabolic data to the above studies. We describe here lung retention, redistribution to other organs, and fecal and urinary excretion of $^{239}\text{Pu}$ in rats which were administered $^{239}\text{PuO}_2$ by inhalation under the same experimental conditions used in the above studies.

An additional purpose of the present study is to determine whether the metabolic parameters of $^{239}\text{Pu}$ following inhalation of $^{239}\text{PuO}_2$ aerosol are affected by the calcination temperature of the aerosol. Occupational exposure to $\text{Pu}$ may occur during the fabrication of fuels, especially for fast reactors, and during the reprocessing of irradiated fuel. Since the release of $\text{Pu}$ into the working place has accidentally occurred by fire or explosion, the chemical form of $\text{Pu}$ released is mainly $\text{PuO}_2$ fired at different temperatures depending on the type of accident. It is assumed that the fired temperature may affect the solubility, chemical composition, and specific surface area of $\text{PuO}_2$ particles$^{5-7}$. There is a discrepancy in the effects of calcination temperature on lung retention and translocation of $^{239}\text{Pu}$ after the inhalation of $^{239}\text{PuO}_2$. Mewhinney et al demonstrated that the solubility of inhaled $^{239}\text{PuO}_2$ in the lungs of beagle dogs depended upon the calcination temperature during its preparation$^5$. In contrast, Morgan et al observed that the lung retention of $^{239}\text{PuO}_2$ in the mouse lung after inhalation was unaffected by calcination temperature in the range between 550–1250°C$^6,7$. In the present study, therefore, we compared the lung retention, fecal and urinary excretion, and translocation of $^{239}\text{Pu}$ in the rats after inhalation of $^{239}\text{PuO}_2$ which was calcined at 400°C (low temperature) and 1150°C (high temperature).

**MATERIALS AND METHODS**

**Preparation of $^{239}\text{PuO}_2$**

The details of the experimental procedure for the preparation and inhalation of $^{239}\text{PuO}_2$ have been reported elsewhere$^{2,8}$. In brief, a colloidal suspension of $^{239}\text{Pu}($OH)$_2$ prepared from a stock solution of $^{239}\text{Pu(NO}_3)_2$ by neutralization with NH$_4$OH was nebulized, dried at 300°C, and calcined by passing through a furnace maintained at 400 or 1150°C to change the chemical form from hydroxide to dioxide. The activity median aerodynamic diameters (AMADs) of the resultant $^{239}\text{PuO}_2$ aerosols were 0.29 and 0.38 μm with the geometric standard deviations ($\sigma_g$) of 2.1 and 1.7 for the aerosols prepared at 400 and 1150°C, respectively.

**Inhalation of $^{239}\text{PuO}_2$**

Animals used were female Wistar rats, 10-weeks-old and weighing 180–220 g at the time of $^{239}\text{PuO}_2$ inhalation. They were purchased from a domestic breeder (SLC, Shizuoka, Japan) at 4–5-weeks-old, housed in a controlled environment, and given pelleted diet and water *ad libitum*. At the administration, they were individually restrained in small plastic boxes and exposed to the $^{239}\text{PuO}_2$ aerosols for about 60 min by “nose only” inhalation using a special exposure system, as
described previously\(^1\)\(^2\)\(^3\)\(^4\). Twenty animals were administered simultaneously, five of which were then individually housed in metabolic cages. From these five rats, feces and urine were collected at scheduled times after exposure. The lung depositions of \(^{239}\)Pu in all rats were determined periodically after inhalation exposure by the in vivo counting method using a whole-body counting system\(^1\).

The initial alveolar deposition (IAD) defined the lung retention of Pu on the 2nd day after inhalation, according to the previous literature\(^2\)\(^3\)\(^4\). The average IADs of rats exposed to high- and low-temperature \(^{239}\)PuO\(_2\) were 1.4 and 2.0 kBq, respectively.

**Measurement of radioactivity**

The rats were killed by cutting the abdominal aorta under anesthesia at 1 month and 1 year after \(^{239}\)PuO\(_2\) administration. The lung, kidney, spleen and right femur were dissected. The tissue samples were acid-digested with conc. HNO\(_3\), evaporated to dryness at 500°C for 24 hr, and dissolved with 7M HNO\(_3\) containing a small amount of hydrogen fluoride. The feces were homogenized at first, and a known amount of the homogenate was treated by the same procedure as used for the tissues samples. The \(^{239}\)Pu activities in these samples were measured by a liquid scintillation counter (LS 1214, LKB, Japan). The urine was filtered with a 0.22 \(\mu\)m Millipore Filter and measured directly by a liquid scintillation counter.

**RESULTS**

Figure 1 shows the retention of \(^{239}\)Pu in the lungs after exposure to high- and low-temperature \(^{239}\)PuO\(_2\). The retentions of \(^{239}\)Pu were 79 ± 6.8 and 17 ± 2.9% IAD (mean ± s.d.) in the rats exposed to high-temperature \(^{239}\)PuO\(_2\) 1 month and 1 year after exposure, respectively. In the rats exposed to low-temperature \(^{239}\)PuO\(_2\), the \(^{239}\)Pu retentions were 58 ± 4.0 and 8.9 ± 1.8% IAD 1 month and 1 year after exposure, respectively. The clearance of \(^{239}\)Pu from the lungs was faster in the low-temperature than in the high-temperature group. Statistically significant differences between both groups were observed at 1 week, 1 month, and 1 year after exposure.

The fecal and urinary excretions of \(^{239}\)Pu, expressed as % IAD per day, in the rats exposed to \(^{239}\)PuO\(_2\) fired at different temperatures are shown in Fig. 2. In both groups, the fecal excretion of \(^{239}\)Pu was larger soon after exposure and decreased gradually thereafter. On the other hand, the urinary excretions were relatively constant throughout the experimental period. The total amount of \(^{239}\)Pu excreted is shown in Table 1. In the high-temperature group, 20 ± 1.5 and 52 ± 2.2% IAD were excreted into the feces 1 month and 1 year after exposure, respectively. In the low-temperature group, they were 32 ± 2.6 and 68 ± 1.4% IAD, respectively, which were significantly higher than those in the high-temperature group at a given period. Total excretions into the urine were 31 ± 5.9 and 23 ± 2.6% IAD in the high- and low-temperature groups, respectively, 1 year after exposure.

Table 2 shows the redistributions of \(^{239}\)Pu to the major organs other than the lungs 1 month and 1 year after exposure. The amounts of \(^{239}\)Pu redistributed from the lungs to the other organs were very small. Even in the liver, which showed the highest \(^{239}\)Pu accumulation in organs other
Fig. 1. Lung retention of Pu following the inhalation of PuO₂ calcined at different temperatures. The vertical bar denotes the standard deviation, and an asterisk indicates a significant difference between two groups (P < 0.05).

Fig. 2. Daily fecal and urinary excretion of Pu following inhalation of PuO₂ calcined at different temperatures. Each point is the mean of five rats.
than the lungs, the retentions of $^{239}$Pu (% IAD per organ) were 0.13 ± 0.02 and 0.20 ± 0.12% at 1 year in the high- and low-temperature groups, respectively.

A significant accumulation of $^{239}$Pu, i.e., a significant increase in the concentrations of $^{239}$Pu between 1 month and 1 year, was observed in the liver and femur of the high-temperature group, and in the spleen of the low-temperature group. The effect of calcination temperature on the amount of $^{239}$Pu was significant in the kidney at 1 month after inhalation, and in the kidney and femur at 1 year.

**DISCUSSION**

In the metabolic parameters determined here, lung retention has already been investigated in previous studies in our series$^2)$. Two other metabolic parameters, i.e., fecal and urinary excretion, and redistribution to the other organs, were determined in the present study, since these parameters are important and essential for estimating the radiation doses and for evaluating the biological effect of inhaled $^{239}$PuO$_2$. The lung retention of $^{239}$Pu (Fig. 1) was in good agreement with our previous data$^3)$. The lung retention of inhaled $^{239}$Pu in rats has also been reported by
many other authors9–13), where the clearance was somewhat quicker than that observed in this as well as in our previous study1,2). A possible reason for this slow clearance observed in our series of experiments has been discussed elsewhere in detail2). The excretion patterns of $^{239}$Pu into feces and urine observed in the present study are in good agreement with those previously reported by other authors5,9). In the early period after inhalation, the amount of $^{239}$Pu excreted into the feces was much higher than that into the urine (Fig. 2), suggesting that the clearance of $^{239}$Pu from lungs to gastro-intestinal tract via upper airways was still dominant in this period. In contrast, the $^{239}$Pu excretion into urine was relatively constant regardless of the time after inhalation. As has been well demonstrated in various types of particles10), the fraction excreted into urine is considered to be cleared from the lungs after dissolution. Therefore, the present result may indicate that the dissolution of $^{239}$PuO$_2$ particles occurred at a relatively constant rate in the lungs.

Regarding the effect of firing temperature on the clearance of $^{239}$Pu, two experiments have been carried out by Morgan et al6,7) and Mewhinney et al5), as described in our Introduction. Experimental conditions were different between these studies in respect to animal species, IAD and the preparation procedure of Pu oxide. Using beagle dogs, Mewhinney et al demonstrated that the solubility of inhaled $^{239}$PuO$_2$ in the lungs depended upon the temperature at which it was fired during its preparation5). They used polydisperse aerosols of $^{239}$Pu nebulized from a solution of $^{239}$Pu chloride fired at 325 to 1150°C. AMAD of both types of aerosols was 1.9 µm with $\sigma_g$ of 1.2, and IADs ranged from 21 to 72 kBq per dog. On the other hand, Morgan et al showed that the lung retention of $^{239}$Pu in mice after $^{239}$PuO$_2$ inhalation was not significantly affected by calcination temperatures in the range from 550 to 1250°C6,7). In their study, $^{239}$PuO$_2$ was prepared by firing the oxalates at 550°C, and the resulting materials were recalcined at temperatures of 750, 1000, and 1250°C. AMAD of the aerosols ranged from 1.4 to 1.6 µm with $\sigma_g$ of 1.3, and IADs ranged from 0.1 to 0.23 kBq per mouse. Although the preparation procedures were different, the physicochemical characteristics of $^{239}$PuO$_2$ as well as IADs seemed to be similar in both studies. The only apparently different factor between their studies was the difference in animal species. Since the movement and dissolution of radioactive particles in the lungs differed greatly between the animal species14), that one factor may be responsible, at least in part, for the different effects of calcination temperature on the lung retention of $^{239}$PuO$_2$ between mice and dogs. In the present study, the calcination temperatures and IADs (per gram tissues) were similar to those reported by Mewhinney et al5) and Morgan et al6,7). However, the size (AMAD) of $^{239}$PuO$_2$ particles used here was much smaller, i.e., 0.29–0.38 µm in the present study vs. 1.4–1.9 µm in the previous ones. Therefore, it is difficult to compare the metabolic parameters in detail between the present experiment and their experiments. However, it is noteworthy that the lung retention of $^{239}$Pu was affected by calcination temperature in both rats and dogs, although further investigations are necessary to elucidate the mechanism involved and the reason why the temperature effect was only observed in those two species.

In addition to $^{239}$Pu retention in the lungs, some other metabolic parameters were different between the low- and high-temperature groups. The cumulative fecal excretion for 1 year after exposure in the high-temperature group was 52% IAD, and the cumulative urinary excretion was 31% (Table 1). The ratio of fecal to urinary excretion was 1.7. In the low-temperature group, the cumulative fecal and urinary excretion was 68 and 23% IAD, respectively, and the ratio of fecal
to urinary excretion was 3.1. The accumulation of $^{239}\text{Pu}$ in some organs was also influenced by the calcination temperature. The amounts of $^{239}\text{Pu}$ in the liver and spleen seemed to be higher in the low- than in the high-temperature group at 1 year after exposure (Table 2). In turn, the amounts in the kidney and femur were lower in the low-temperature group at 1 month and 1 year. As a result, the ratio of $^{239}\text{Pu}$ amount in the liver to that in the femur or kidney was consistently higher in the low-temperature group at 1 month and 1 year after administration. These two findings, i.e., the higher fecal/urinary excretion ratio and the higher liver/femur accumulation ratio in the low-temperature group, are clearly consistent with each other in terms of the general concept of $^{239}\text{Pu}$ metabolism. The higher fecal/urinary excretion ratio in the low-temperature group suggests that some $^{239}\text{Pu}$ derived from $^{239}\text{PuO}_2$ calcined at 400°C was distributed from lungs to circulating blood or lymph systems in a colloidal or particulate form. In other words, the amount of plutonium which will be bound to transferrin and removed to urine was relatively smaller in the low-temperature group. The higher liver/femur accumulation ratio in the low-temperature group also indicated that a colloidal or particulate form of $^{239}\text{Pu}$, which tended to be trapped by reticuloendothelial tissues, might be abundant in the circulating blood of the rats in the low-temperature group.

As for risk estimation, the present data also indicate that the lungs are the major target organs for inhaled $^{239}\text{Pu}$ under the experimental conditions used here. The retention of $^{239}\text{Pu}$ in the other organs was very small compared to that in the lungs, regardless of the firing temperature. This supports the findings by Oghiso et al, which showed that tumor incidence increased in the lungs, but not in other organs of rats inhaling PuO$_2$ under the same experimental conditions used here. In conclusion, we presented here the metabolic data of $^{239}\text{Pu}$ in rats inhaling $^{239}\text{PuO}_2$ particles. These metabolic data, including lung retention, fecal and urinary excretion, and translocation to other organs, seemed to be affected by the calcination temperature of the $^{239}\text{PuO}_2$ particles. Clearance from the lungs and redistribution to other organs was faster in the rats inhaling $^{239}\text{PuO}_2$ calcined at a lower temperature.

REFERENCES