RADIATION-INDUCED MESOTHELIOMAS IN RATS DE91 017485

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Mesotheliomas have been reported in rats that inhaled plutonium, but have not been extensively studied (Dagle and Sanders, 1984). Rats injected intraperitoneally with 100 kBq of $^{239}\text{PuO}_2$ developed mesotheliomas around focal, high concentrations of plutonium "hot spots" localized in the omentum (Sanders and Jackson, 1972). In this study, 27% of the rats developed peritoneal mesotheliomas (epithelial in morphology) and 38% developed sarcomas (sarcomatous mesotheliomas). The tumor incidence was dose dependent and a greater dose was required for the induction of epithelial mesotheliomas than for the sarcomas. When 3,4-benzo-a-pyrene or chrysotile asbestos fibers were injected with the $^{239}\text{PuO}_2$, the resultant tumor incidence was additive (Sanders, 1973). The morphogenesis of the mesotheliomas from intraperitoneally injected $^{239}\text{PuO}_2$ and chrysotile asbestos was similar.

Radiation-associated mesotheliomas have also been reported in human patients several years after therapeutic external radiation for Wilms tumor, seminoma, thyroid ablation, teratocarcinoma, infiltrating breast carcinoma, or Hodgkin's disease (Peterson *et al.*, 1984). Thorium dioxide, a radioactive, radiographic contrast media, has also been implicated in mesothelioma induction. Two cases have been described in which contamination of the peritoneal or pleural cavity was associated with mesothelioma appearing 25 and 36 years later.

To investigate a possible role for inhaled radionuclides in the induction of mesotheliomas, we reviewed four life-span studies conducted at ITRI in which rats had inhaled radioactive materials. A total of 3076 F344 rats were exposed by inhalation to aerosols of $^{239}\text{PuO}_2$, mixed uranium-plutonium oxide, or $^{144}\text{CeO}_2$. Results showed that a low incidence of pleural mesotheliomas was induced by either alpha- or beta-emitting radionuclides deposited and retained in the lung.

The first study reviewed involved 716 rats exposed once or repeatedly (7 times over one year) to monodisperse aerosols of $^{239}\text{PuO}_2$ (Lundgren *et al.*, 1990). The aerosols were about 1 μm AMAD with a geometric standard deviation (σ_g) of about 1.2. The initial lung burdens (ILB's) ranged from 0.11-1.5 kBq. Most of the plutonium

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particles were retained in the lung with a half-time of about 30 days (Fig. 1a). Nine to 11 percent of the initial lung burden was retained with a half-time of 790-1050 days.

The second study reviewed involved 461 rats exposed to aerosols of plutonium dioxide (69% $^{239/240}\text{Pu}$, 17% ^{238}Pu , 15% ^{241}Am), or uranium-plutonium oxides (43% $^{239/240}\text{Pu}$, 35% ^{238}Pu , 22% ^{241}Am) obtained from glove boxes in a nuclear reactor fuel fabrication facility (Mewhinney *et al.*, 1987). The aerosols were generated from dry powders and had an AMAD of about $3.5\text{ }\mu\text{m}$ ($\sigma_g = 1.4$) for PuO_2 and an AMAD of about $2.1\text{ }\mu\text{m}$ ($\sigma_g 1.5$) for the mixed uranium plutonium oxide. Most of the PuO_2 was retained with a half-time of about 41 days (Fig. 1b). Two percent of the ILB of PuO_2 was retained with an 810 day half-time. Most of the uranium-plutonium oxide material was retained with a half-time of about 15 days. Four percent of the ILB was retained with a half-time of 1700 days.

The third study reviewed involved 329 rats exposed once or repeatedly (7 times over one year) to aerosols of $^{144}\text{CeO}_2$ (Lundgren *et al.*, 1990). The aerosols had $0.9\text{-}1.4\text{ }\mu\text{m}$ AMAD ($\sigma_g 1.4\text{-}2.0$). The ILB's ranged from 13 to 40 kBq. For the rats exposed once, 82% of the cerium was retained in the lung with a half-time of 12 days and 18% with a half-time of 70 days (Fig. 1c). Repeated exposure prolonged the retention resulting in 48% being retained with a half-time of 15 days and 52% with a half-time of 100 days.

The fourth study reviewed involved 1570 rats exposed to relatively low doses of $^{144}\text{CeO}_2$ (Lundgren *et al.*, 1990). The aerosols were $0.9\text{-}1.4\text{ }\mu\text{m}$ ($\sigma_g 1.4\text{-}2.0$). The ILB's ranged from 13-160 kBq. Most of the cerium was retained with a half-time of 29 days (Fig. 1d). Nine percent was retained with a half-time of 160 days.

A number of features were similar in all four studies. In all the exposures, aerosol particles were basically spherical in shape and respirable in size (Fig. 2). No fibers were present in the aerosols. The rats were exposed briefly (10-40 min) per nasum, to the aerosols. Approximately equal numbers of male and female F344 rats, raised in the Institute colony, were used in each study. The rats were observed twice daily until they died or were euthanized.

At death, each rat was necropsied and all major organ systems were examined. Routine paraffin-embedded, hematoxylin- and eosin-stained sections were obtained from the lung and from lesions in the thoracic cavity. Sections of other organs were taken as required for diagnosis of lung lesions.

Over the range of doses used in the four studies, only in the highest dose groups of two of the studies ($^{239}\text{PuO}_2$ single exposure and $^{144}\text{CeO}_2$ repeated exposure) was the life span significantly reduced. These life spans were 86% and 80% of their respective control groups. This long survival indicates that competing risks were not an important factor in the tumor incidence analysis.

The morphologic patterns of the 28 pleural mesotheliomas found in the 3076 rats exposed to radionuclides are noted in Table 1. Epithelial mesotheliomas with a papillary pattern were the most frequently seen (23/28 cases) (Fig. 3). Tumor tissue was found spread over much of the mesothelial surface of the lung and caused thickening of the mediastinum. Most of these tumors were considered either fatal or contributory to death, based on the extensive growth of the tumors. Two of these tumors occurred in rats that also had squamous cell carcinomas of the lung. Four of the mesotheliomas occurred in lungs where focal fibrosis was prominent. This association of mesotheliomas in rats with other lesions occurred in rats receiving the highest radiation doses (> 17 Gy alpha or > 190 Gy beta).

The sarcomatous mesotheliomas noted (2/28 cases) were characterized by marked proliferation of spindle-shaped cells with large accumulations of mature collagen (Fig. 4). The tumors appeared to arise in the hilus of the lung, invade the mediastinum and heart, and spread along the pleural surface of the lung. Other primary lung tumors were noted in the rats with sarcomatous mesotheliomas.

The three mixed mesotheliomas had both papillary epithelial elements and sarcomatous elements (Fig. 5). Two of the three rats with mixed mesotheliomas also had other primary lung tumors.

In none of the mesotheliomas was anaplasia marked or the mitotic rate more than minimally increased. Tumors were considered malignant if they invaded normal tissue, implanted along pleural surfaces, and were fatal or contributed to death.

The incidence of malignant mesotheliomas is shown in Figure 6. The incidence for the combined control animals (4 mesotheliomas/1641 control rats) was 0.24%. The incidence ranged from 0.8 to 13.8% for rats that inhaled alpha-emitting radionuclides and from 2 to 5% for rats that inhaled beta-emitting radionuclides. The interrelationship of the incidence and dose to lung was tested with linear regression. For alpha-emitting radionuclides, the relationship was described by $Y = (0.95)X + 0.029$ with a correlation coefficient of 0.72. For beta-emitting radionuclides, the relationship was $Y = (0.025)X + 0.20$ with a correlation coefficient of 0.87.

The incidence rate was only moderately correlated with increased radiation dose. The incidence of other primary lung tumors, however, was markedly increased by increased dose to lung and was much greater than the mesothelioma incidence (Fig. 7).

The chronic alpha irradiation was more effective than the chronic beta irradiation in causing mesotheliomas. The risk of a malignant mesothelioma for various groups of rats was determined using the formula:

$$\text{Tumors/Gy} = \frac{\text{number of mesotheliomas}}{\text{average dose} \times \text{number in group}}$$

Such a calculation assumes a linear dose response. Figure 6 illustrates that a linear dose response is reasonable in this situation. Using this approach, the risk of mesothelioma for alpha-emitting radionuclides deposited in the lung was 90 tumors/ 10^4 Gy. For beta-emitting radionuclides, it was 6 tumors/ 10^4 Gy. The relative effectiveness ratio was:

$$\frac{\text{Risk for } \alpha\text{-emitting radionuclides}}{\text{Risk for } \beta\text{-emitting radionuclides}} = \frac{90 \text{ tumors}/10^4 \text{ Gy}}{6 \text{ tumors}/10^4 \text{ Gy}} = 15$$

The results of these four studies of rats with radionuclides deposited in the lung show that pleural mesotheliomas can be induced with either inhaled alpha- or beta-emitting radionuclides. Pulmonary fibrosis does not appear to be a prerequisite for these radiation-induced mesotheliomas as it appears to be for asbestos-induced mesotheliomas. The incidence of these tumors is dose dependent, but much less frequent than other radiation-induced lung tumors. Chronic alpha irradiation was more effective per unit

dose in producing mesotheliomas than chronic beta irradiation of the lung by a factor of 15.

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Table 1

Characteristics of Pleural Mesotheliomas in Rats
with Lung Burdens of $^{144}\text{CeO}_2$, $^{239}\text{PuO}_2$, PuO_2 or $(\text{U}, \text{Pu})\text{O}$

Morphologic Pattern	Total ^a Number	Number of Mesotheliomas				
		Fatal	Contributory to Death	Incidental to Death	With Focal Fibrosis	With Other Lung Tumors
Epithelial, Papillary, Focal	2	0	0	2	0	0
Epithelial, Papillary, Diffuse	21	13	7	1	4	2
Sarcomatous	2	1	1	0	1	2
Mixed	3	2	1	0	1	2

^aIn 3076 rats from four studies, including 716 rats exposed to $^{239}\text{PuO}_2$ once or repeatedly, 461 rats exposed to PuO_2 or $(\text{U}, \text{Pu})\text{O}$, 329 rats exposed to $^{144}\text{CeO}_2$ once or repeatedly, and 1570 rats exposed to low doses of $^{144}\text{CeO}_2$.

FIGURE LEGENDS

Figure 1. Retention patterns for radionuclides inhaled by F344 rats. a) $^{239}\text{PuO}_2$; b) PuO_2 or $(\text{U}, \text{Pu})\text{O}_2$; c) $^{144}\text{CeO}_2$; d) $^{144}\text{CeO}_2$ low-dose exposure.

Figure 2. Exposure aerosol particles. a) Laboratory-produced monodisperse $^{239}\text{PuO}_2$ particles - AMAD = $1.0\ \mu\text{m}$, σ_g 1.2. b) Laboratory-produced polydisperse $^{144}\text{CeO}_2$ particles - AMAD = $1.4\ \mu\text{m}$, σ_g 1.7. c) Mixed $(\text{U}, \text{Pu})\text{O}_2$ particles from fuel fabrication facility - AMAD = $3.5\ \mu\text{m}$, σ_g 1.4. Bar = $1\ \mu\text{m}$.

Figure 3. Mesothelioma, papillary epithelial pattern. Bar = $100\ \mu\text{m}$.

Figure 4. Mesothelioma, sarcomatous pattern. Bar = $100\ \mu\text{m}$.

Figure 5. Mesothelioma, mixed pattern. Bar = $100\ \mu\text{m}$.

Figure 6. Dose response for mesothelioma after inhalation of alpha- or beta-emitting radionuclides. Mesothelioma incidence a linear function of dose according to the equation $Y = (0.95)X + 0.029$ for alpha emitters (correlation coefficient = 0.72) and $Y = (0.025)X + 0.20$ for beta emitters (correlation coefficient = 0.87).

Figure 7. Incidence of malignant mesotheliomas compared with incidence of other lung cancers in rats that inhaled a) alpha-emitting radionuclides or b) beta-emitting radionuclides. Control incidence = 4 mesotheliomas/1641 rats = 0.24%.

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FIGURE 2

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Figure 6

FIGURE 7