BIOLOGICAL RESPONSE OF BEAGLE DOGS TO INHALED MONODISPERSE AEROSOLS OF $^{239}PuO_2$

R. A. Guilmette, B. A. Muggenburg, F. F. Hahn, J. H. Diel, J. A. Mewhinney, B. B. Boecker and R. O. McClellan
Lovelace Inhalation Toxicology Research Institute
P. O. Box 5890, Albuquerque, New Mexico 87185, U.S.A.

Inhalation has proven to be a likely route for human exposure to ^{239}Pu aerosols. Such exposures have occurred in occupational accidents involving fugitive releases of aerosols, and to the general population as a result of inhaling fallout ^{239}Pu. The studies reported here, in which dogs received single brief inhalation exposures to monodisperse $^{239}PuO_2$ aerosols, are part of a group of studies in which the role of local alpha dose rate from individual particles, initial average dose rate to lung, total average dose, and fractional lung irradiation are being evaluated with respect to the production of biological effects, primarily in the lung. Results from parallel studies in which dogs that inhaled $^{239}PuO_2$ were serially sacrificed have shown that less than 0.1% of the alpha radiation dose was delivered to tissues other than the lung and thoracic lymph nodes by two years after exposure (1).

MATERIALS AND METHODS

A total of 216 Beagle dogs from the Institute's colony have inhaled aerosols of monodisperse particles of $^{239}PuO_2$. Forty-eight dogs were exposed in a nose-only exposure system to 0.75 μm AMAD particles, 96 to 1.5 μm AMAD particles and 72 to 3.0 μm particles. Thirty-six dogs were exposed to the aerosol vehicle, a dilute ammonium hydroxide solution, to serve as controls. Each study had four or more desired activity levels, expressed as initial pulmonary burdens: 0.37, 1.1, 2.6, 5.2 kBq/kg body weight, and with 12 dogs per activity level. Two additional levels were used in the study with 3.0 μm AMAD $^{239}PuO_2$, 10.4 and 20.7 kBq/kg, and four additional levels were used in the 1.5 μm AMAD $^{239}PuO_2$ study, 0.0092, 0.085, 10.4 and 20.7 kBq/kg. Some physical and dosimetric details for this experimental design are given in Table 1. Methods for the preparation of the aerosols and the exposure of the dogs have been previously described (2,3). In addition to pre-exposure clinical evaluation, each dog received daily observation, annual physical and radiographic examination, and semi-annual blood cell counts and serum chemistry tests. Sick dogs were examined and tested to establish a diagnosis. A few dogs died from their illness but most were euthanized when moribund. Necropsies were done on all dogs, and tissues were evaluated histologically. Both tissues and collected excreta samples were analyzed radiochemically for ^{239}Pu content by alpha liquid scintillation counting.

TABLE 1. Design for the Study of the Effects of Inhaled $^{239}PuO_2$ in Dogs

Parameter	0.75 μm (AMAD)	1.5 μm (AMAD)	3.0 μm (AMAD)
Physical size, μm	0.18	0.44	0.96
MBq per particle	0.048	0.74	7.4
Local dose rate, mGray/day	0.65	9.6	99
Number of particles, range	$6x10^7 - 2x10^9$	$6x10^5 - 6x10^8$	$4x10^5 - 1x10^8$
Fraction of lung irradiated	1.0	0.03 – 1.0	0.02 – 1.0
Initial pulmonary burden, kBq	3 – 74	0.3 – 330	2 – 630
Average initial dose rate to lung, Gray/day	0.002 – 0.07	0.003 – 0.3	0.002 – 0.5

Research conducted under U.S. Department of Energy Contract No. DE-AC04-76EV01013 and in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

RESULTS

Results from the study in which dogs were serially sacrificed subsequent to inhaling monodisperse $^{239}PuO_2$ aerosols have shown that up to two years after exposure, 99% of the burden measured at sacrifice was in lung and thoracic lymph nodes. The largest fraction of the initial pulmonary burden (65-90% IPB) was being retained in the lung with half times of 700-1800 days. Greater retention times were noted with increasing particle size. Of the ^{239}Pu leaving the lung, ≈ 75% was cleared mechanically via the bronchi and trachea to be swallowed and excreted in the feces; the remaining 25% accumulated approximately linearly with time in the thoracic lymph nodes. As an example, the tissue retention curves for lung, thoracic lymph nodes, skeleton, and liver are shown for the 1.5 μm AMAD group (Figure 1) (1). These patterns were markedly different from those of $^{238}PuO_2$, where accelerated clearance from lung and increased solubilization *in vivo* was noted beginning about 100 days after exposure (3). The accumulation of radiation dose in the four organ systems of concern (Figure 2) indicate a range of 10^5 in absorbed dose between bone and thoracic lymph nodes. By 2 years, the lung has accumulated a dose of 0.1 Gray/kBq of initial pulmonary burden.

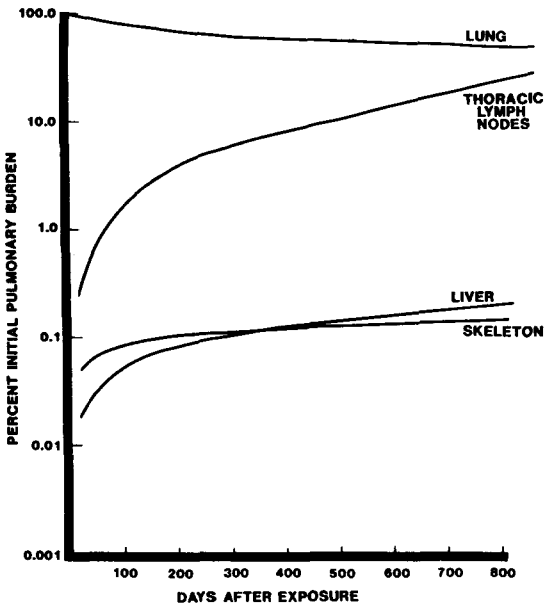

FIGURE 1. Retention of ^{239}Pu in tissues of Beagle dogs exposed to 1.5 μm AMAD monodisperse PuO_2 aerosols.

As of September 30, 1983, all surviving dogs had been on study at least 4.5 years. The biological effects to date have been radiation pneumonitis with or without pulmonary fibrosis, and pulmonary carcinoma (Table 2). The earliest death from radiation pneumonitis occurred 105 days after exposure; dogs are continuing to die at and beyond 4.5 years with pneumonitis and pulmonary fibrosis. Most of these dogs have accumulated alpha radiation doses to lung of 10-80 Gray. The earliest appearance of pulmonary carcinoma at death was 1108 days after exposure. However, the incidence of carcinoma did not increase appreciably until about 1500 days. Of the 18 dogs that have died since 1300 days after exposure, 16 had pulmonary carcinoma. The mean (± SD) dose to lung at death for those dogs dying with pulmonary tumors were 18 (± 7) Gray for the 0.75-μm group, 27 (± 10) Gray for the 1.5-μm group, and 49 (± 24) Gray for the 3.0-μm group.

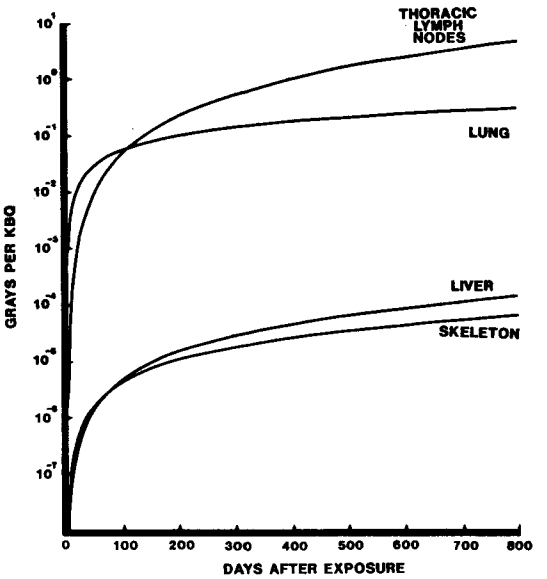

FIGURE 2. Cumulative radiation dose to the tissues of a 10 kg Beagle dog that inhaled 1.5 μm AMAD $^{239}PuO_2$ aerosol.

TABLE 2. Biological Effects from Inhaled $^{239}PuO_2$ as of September 30, 1983*

Study	Dogs Originally on Study	Radiation Pneumonitis/ Pulmonary Fibrosis	Pulmonary Carcinoma	Age Range of Surviving Dogs (days after exposure)
0.75	48	9	4	1681-2458
1.5	96	35	3	1646-2452
3.0	72	35	8	1674-2460
All	216	79	15	1646-2460

*Dogs which had both pneumonitis/fibrosis and carcinoma were counted twice.

DISCUSSION

The initial pulmonary burdens of ^{239}Pu in these studies represent a continuum of activity levels from very low, i.e., equivalent to a "maximum permissible lung burden" in man, to very high, i.e., levels capable of producing death from radiation pneumonitis within a few hundred days. The dogs were exposed 1600-2500 days ago, and thus far only dogs with high pulmonary burdens have shown biological response.

Lymphopenia, the first biological effect observed, was seen as early as 180 days after exposure. Those dogs with severe lymphopenia usually developed clinical signs of radiation pneumonitis. Dogs with only a moderately depressed lymphocyte count did not always develop clinical disease, and in some dogs lymphocyte counts returned to near normal three to four years after exposure. No depression of neutrophil counts have been observed, in contrast to those seen in dogs inhaling $^{238}PuO_2$.

Radiation pneumonitis was the earliest cause of death, as it was for dogs inhaling monodisperse $^{238}PuO_2$ (4). At four to seven years after exposure, pneumonitis and fibrosis continue to be significant findings at death. Given the relative insolubility of the $^{239}PuO_2$ particles used in these studies, and the long retention times of the particles in lung, it is possible that pneumonitis and fibrosis may continue to be important biological effects, perhaps for the entire duration of the study. This cannot be stated with certainty, however, because a dose or dose rate threshold might exist, below which the radiation pneumonitis and pulmonary fibrosis either do not occur, or if they occur, are either subclinical or are repairable.

Several differences in the development of radiation pneumonitis/pulmonary fibrosis have been noted for the dogs inhaling $^{239}PuO_2$ vs $^{238}PuO_2$. The distribution of fibrosis for the former case has been more uniform and widespread than for the latter. This most likely reflects the relatively larger fraction of lung irradiated for equal lung burdens of $^{239}PuO_2$ vs $^{238}PuO_2$. Additionally, beyond 1000 days after exposure, very few cases of pneumonitis/fibrosis have been observed for the dogs with ^{238}Pu. Their major finding at death has been bone cancer.

Three years after exposure, pulmonary tumors began to be found as incidental findings at necropsy, similar to the findings with $^{238}PuO_2$. At later times, the cancers caused death and the incidence rate appears to be increasing. However, it is too early to predict the ultimate pattern of tumor incidence with respect to time or alpha radiation dose. It must be remembered that the tumor incidence observed thus far encompassed dogs that have died between 4.5 and 7.0 years after exposure. Likewise, the surviving dogs have a similar range of times after exposure. Therefore, the number of tumors observed to September 30, 1983, cannot be used as is to determine cancer incidence rates. Presently the average lung doses to death for those dogs dying with lung carcinomas increased with increasing particle size. If this pattern continues in time, these studies may provide key information in evaluating the role of nonuniformity of alpha radiation dose, and of "wasted" radiation on the induction of lung cancer by inhaled insoluble particles containing alpha-emitting radionuclides.

In summary, studies of the biological effects in Beagle dogs from inhaling widely differing activity levels of insoluble monodisperse $^{239}PuO_2$ aerosols are continuing. Presently very few dogs with initial pulmonary burdens greater than 3 kBq/kg are alive. Of the survivors, the projected range of lifetime doses to lung are 0.2 to 70 Gray. These developing results will provide important insights into the role of nonuniformity of alpha radiation in lung (and extra-pulmonary tissues as well) and of the risk of biological effects from inhaling insoluble alpha-emitting aerosols in dogs and ultimately in man.

Acknowledgments:

The authors gratefully acknowledge the contributions of the professional and technical staff of the ITRI, in particular those of the aerosol science and exposure groups, animal care, analytical radiochemistry, pathology, and radiobiology groups.

REFERENCES

1. Guilmette, R. A., Diel, J. H., Muggenburg, B. A., Mewhinney, J. A., Boecker, B. B., and McClellan, R. O., 1983, *Int. J. Rad. Biol.* (submitted).
2. Raabe, O. G., Boyd, H. A., Kanapilly, G. M., Wilkinson, C. J., and Newton, G. J., 1975, *Health Phys.* 28, 655.
3. Mewhinney, J. A. and Diel, J. H., 1983, *Health Phys.*, 45, 39.
4. Muggenburg, B. A., Mewhinney, J. A., Merickel, B. S., Boecker, B. B., Hahn, F. F., Guilmette, R. A., Mauderly, J. L., and McClellan, R. O., 1980, Proceedings of the Vth ICRP Congress, Vol. II, pp 115-118.