• Inhaled Plutonium Nitrate in Dogs

Principal Investigator: G. E. Dagle

Other Investigators: A. C. Case, J. F. McShane, G. J. Powers, H. A. Ragan, S. E. Rowe, R. E. Schirmer, D. L. Stevens, R. E. Weller, and E. L. Wierman

Technical Assistance: W. J. Chandon, E. T. Edmerson, R. F. Flores, F. M. Gordon, D. E. Hunter, A. J. Kopriva, R. G. Moore, and M. C. Perkins

The major objective of this project is to determine dose-effect relationships of inhaled plutonium nitrate in dogs to aid in the prediction of health effects of accidental exposure in man. For lifespan dose-effect studies, beagle dogs were given a single inhalation exposure to $^{239}Pu(NO_3)_4$, in 1976 and 1977. The earliest biological effect was on the hematopoietic system; as described in previous Annual Reports, lymphopenia and neutropenia occurred at the two highest dose levels. We have also observed radiation pneumonitis, lung cancer, and bone cancer at the highest dose levels.

The skeleton and liver are generally considered the critical tissues after inhalation of "soluble" plutonium (as in the case of plutonium nitrate), on the assumption that the plutonium will be rapidly translocated from the lung to skeleton and liver. In several rodent studies, however, inhalation of "soluble" plutonium has resulted in lung tumors as well as skeletal tumors. Lifespan studies are necessary to evaluate the complex interactions between tissues and organ systems directly or indirectly impaired by lower levels of exposure. Beagle dogs were chosen to correlate relative risks, determined in other studies, with different forms and routes of exposure to plutonium.

Six dose groups (105 dogs) were exposed, in 1976 and 1977, to aerosols of $^{239}Pu(NO_3)_4$ for lifespan observations (Table 1). In addition, 20 dogs were exposed to nitric acid aerosols as vehicle controls, 25 dogs were exposed to aerosols of $^{239}Pu(NO_3)_4$ for periodic sacrifice to study plutonium metabolism and the pathogenesis of developing lesions, seven dogs were selected as controls for periodic sacrifice, and 20 dogs were selected as untreated controls for lifespan observations. The dogs were exposed in aerosol chambers, using techniques described in previous reports. The Appendix (following the entire Annual Report) shows the current status of each dog on these experiments.

The initial deposition and early clearance of inhaled $^{239}Pu(NO_3)_4$ aerosols were discussed in previous Annual Reports. The fraction of plutonium in the lung decreased to approximately 2% of the final body burden in dogs surviving 4 yr or more (Table 2). There was early translocation to the liver and skeleton, with an average of 34% and 61%, respectively, of final body burden present in these tissues in dogs surviving 4 yr or more. Only minimal amounts were translocated to thoracic or abdominal lymph nodes. This was in contrast to dogs that inhaled $^{239}PuO_2$, in which a considerable amount translocated to the thoracic lymph nodes, but only minimal amounts translocated to liver or skeleton at these time periods. In a pilot study reported previously (Annual

TABLE 1. Lifespan Dose-Effect Studies with Inhaled $^{239}Pu(NO_3)_4$ in Beagles[a].

Dose Level Group	Number of Dogs		Initial Alveolar Deposition[b]	
	Male	Female	nCi[c]	nCi/g Lung[c]
Control	10	10	0	0
Vehicle	10	10	0	0
1	10	10	2 ± 2	0.02 ± 0.02
2	10	10	8 ± 4	0.06 ± 0.04
3	10	10	56 ± 17	0.5 ± 0.2
4	10	10	295 ± 67	2 ± 0.8
5	10	10	1709 ± 639	14 ± 6
6	3	2	5445 ± 1841	47 ± 17

[a] Exposed in 1976 and 1977
[b] Estimated from external thoracic counts at 2 weeks post-exposure and estimated lung weights (0.011 x body weight)
[c] Mean ± standard deviation

TABLE 2. Tissue Distribution of Plutonium in Beagles After Inhalation of $^{239}Pu(NO_3)_4$.

Dog Number	Time After Exposure, mo	Final Body Burden, μCi	Percent of Final Body Burden					Cause of Death
			Lungs	Thoracic Lymph Nodes[a]	Abdominal Lymph Nodes[b]	Liver	Skeleton	
1359M	0.1	0.080	90.50	0.15	0.06	2.46	3.20	Sacrifice
1375F	0.1	0.073	89.61	0.14	0.01	0.97	4.68	Sacrifice
1407F	0.1	0.092	51.87	0.41	0.13	10.99	18.70	Sacrifice
1389M	0.5	0.053	24.07	0.38	0.08	41.28	26.21	Sacrifice
1390M	0.5	0.051	24.62	0.32	0.11	20.05	44.45	Sacrifice
1445F	0.5	0.057	26.42	0.32	0.11	21.28	44.73	Sacrifice
1329F	1	0.485	70.05	0.16	0.04	8.28	18.79	Sacrifice
1346M	1	0.902	76.81	0.32	0.03	10.45	10.30	Sacrifice
1347F	1	0.699	71.71	0.36	0.08	9.33	14.09	Sacrifice
1336M	1	0.032	71.38	0.22	0.05	5.72	19.73	Sacrifice
1341F	1	0.022	64.43	0.29	0.10	12.92	18.63	Sacrifice
1344F	1	0.052	58.68	0.25	0.04	21.87	16.09	Sacrifice
1335M	1	0.003	19.52	0.07	0.06	6.68	25.04	Sacrifice
1339F	1	0.001	19.08	0.13	0.08	20.92	45.47	Sacrifice
1351M	1	0.002	40.68	1.22	0.09	17.09	28.89	Sacrifice
1522F	3	0.059	54.68	0.57	0.10	11.52	28.24	Sacrifice
1529F	3	0.049	51.68	0.40	0.07	18.48	23.74	Sacrifice
1539M	3	0.072	52.45	0.31	0.05	18.58	25.03	Sacrifice
1564F	12	0.037	18.00	1.27	0.11	33.53	42.63	Sacrifice
1571F	12	0.053	22.37	1.47	0.11	28.76	42.91	Sacrifice
1588M	12	0.053	13.14	0.40	0.12	35.85	46.18	Sacrifice
1424M	14	4.625	33.10	1.43	0.16	26.49	36.88	Radiation Pneumonitis
1518F	16	4.025	18.99	0.94	0.18	29.51	47.88	Radiation Pneumonitis
1510F	17	4.048	22.00	1.15	0.05	20.71	52.00	Radiation Pneumonitis
1420M	25	1.616	16.51	0.86	0.20	7.77	70.06	Radiation Pneumonitis
1471M	34	1.375	9.25	0.73	0.12	26.92	58.34	Radiation Pneumonitis
1518M	42	1.880	6.87	0.24	0.07	21.34	67.51	Radiation Pneumonitis + Lung Tumor
1512M	42	2.136	4.31	0.60	0.08	49.93	42.66	Bone Tumor
1508M	43	1.730	3.24	0.62	0.08	41.53	52.70	Bone Tumor
1459F	51	1.567	4.40	0.15	0.12	30.86	61.41	Radiation Pneumonitis + Lung Tumor
1492F	52	1.202	2.81	0.20	0.17	27.02	66.38	Bone Tumor
1502F	54	3.113	0.80	0.39	0.09	33.33	62.51	Bone Tumor, Lung Tumor
1485F	55	1.052	0.82	0.35	0.07	31.13	63.94	Bone Tumor
1387F	55	0.167	1.41	0.22	0.12	45.48	49.10	Bone Tumor
1429M	59	--	--	--	--	--	--	Bone Tumor, Lung Tumor

[a]Includes tracheobronchial, mediastinal and sternal lymph nodes
[b]Includes hepatic, splenic and mesenteric lymph nodes

Report, 1979), $^{238}Pu(NO_3)_4$ translocated more rapidly to liver and skeleton than did $^{239}Pu(NO_3)_4$, but both reached a similar plateau at 1 yr after exposure.

The earliest observed biological effect was on the hematopoietic system: lymphopenia occurred at the two highest dose levels at 4 wk after exposure to $^{239}Pu(NO_3)_4$. The results of these continuing evaluations through 54 mo postexposure are shown in Figure 1. Total leukocyte concentrations were reduced significantly in the two highest dose groups, i.e., Group 5

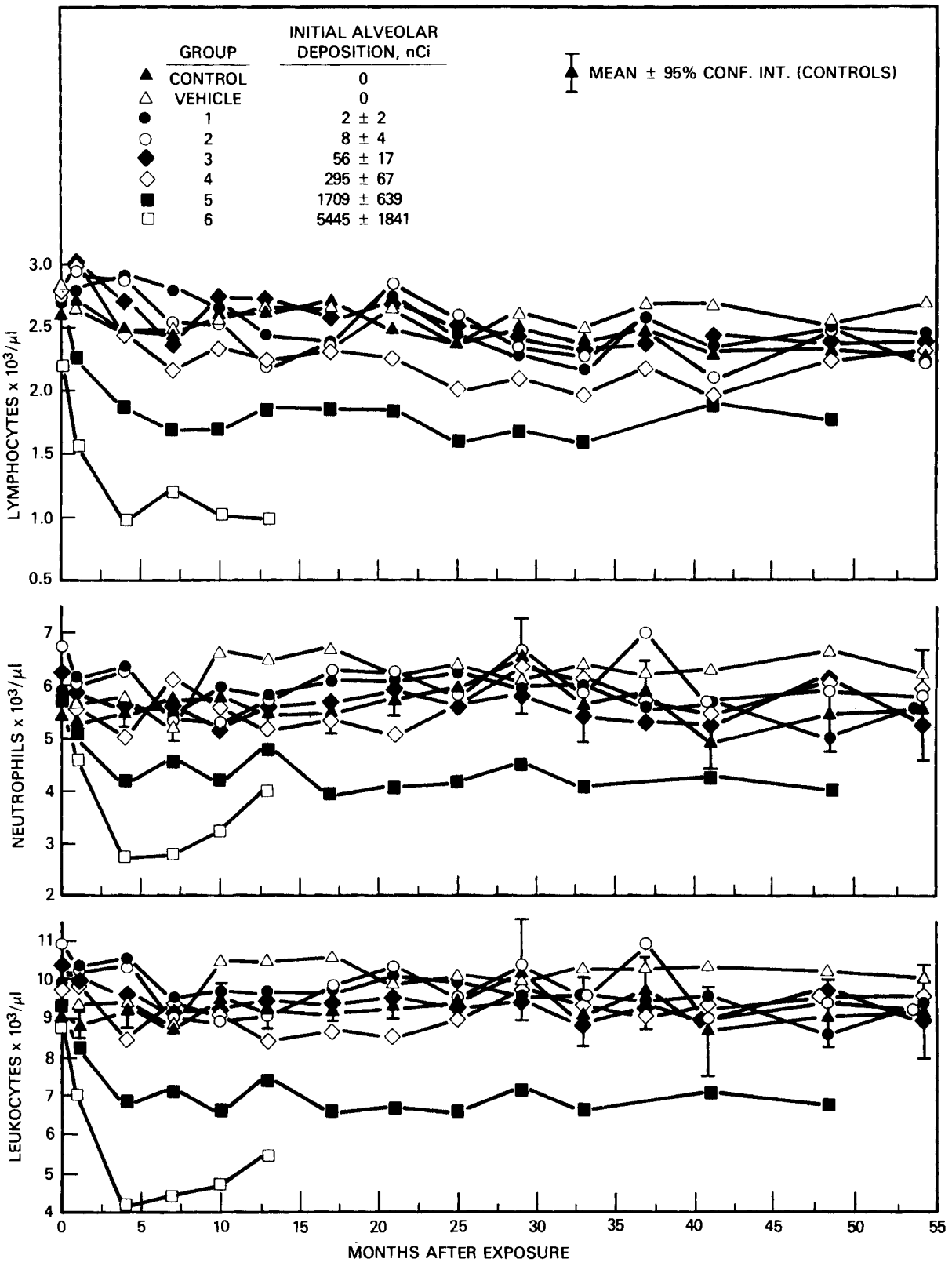

FIGURE 1. Mean Leukocyte, Neutrophil and Lymphocyte Values in Dogs After Inhalation of $^{239}Pu(NO_3)_4$.

(mean initial alveolar deposition, ~1700 nCi), and Group 6 (~5500 nCi). Numbers of leukocytes in Group 4 dogs (~300 nCi) tended to be consistently (but not significantly) lower than those from control dogs. The reduction in white cells in Groups 5 and 6 is due to an effect on most leukocyte types (neutrophils, lymphocytes, monocytes and eosinophils). Lymphocyte reductions differed significantly from values for controls only in Groups 5 and 6. This is in contrast to the effects of both $^{239}PuO_2$ and $^{238}PuO_2$, which significantly depressed lymphocyte concentrations by 21 mo after exposure to initial lung burdens of ~80 nCi or more. The lymphocytopenia at lower dose levels of plutonium oxides may be related to the more-extensive translocation of plutonium oxide to the tracheobronchial lymph nodes.

All five dogs at the highest dose level, and two of 20 dogs at the medium-high dose level, died from radiation pneumonitis 14 to 51 mo after exposure. Histopathologic examination of these dogs' lungs revealed interstitial fibrosis, alveolar epithelial hyperplasia, increased numbers of alveolar macrophages, occasional small emphysematous cavities and, at times, very small nodules of squamous metaplasia at the termini of respiratory bronchioles.

Small, multiple, bronchioloalveolar carcinomas occurred in two dogs with radiation pneumonitis and in two additional dogs euthanized because of osteosarcomas. Typically, these arose in subpleural areas in proximity to areas of interstitial fibrosis or small cavities communicating with bronchioles. They were composed of irregular proliferations of cuboidal epithelial cells, forming aggregates of epithelial cells extending into adjacent alveoli. No metastases or invasions of nonpulmonary parenchyma were observed.

Osteosarcomas were present in seven dogs euthanized 42 to 59 mo after exposure: six dogs from the Group 5 dose level and one dog at the Group 4 dose level. The osteosarcomas occurred singly, in humerus, pelvis, sacrum, cranium, cervical vertebra, thoracic vertebra, and lumbar vertebra. These dogs also had radiation pneumonitis, as described previously, as well as radiation osteosis. The osteosis was generally characterized by peritrabecular fibrosis, composed of relatively hypocellular collagen fibers, and was observed partially surrounding trabeculae in vertebrae, femora, and ribs.

Autoradiographs of liver sections from dogs euthanized 3 to 5 yr after inhalation exposure to the higher dose levels of $^{239}Pu(NO_3)_4$ were compared with liver sections from dogs exposed to levels of $^{239}PuO_2$ that yielded similar concentrations of plutonium in the liver at similar intervals after exposure. The autoradiographs showed that the nitrate-exposed dogs had >99% of plutonium activity in diffusely distributed single tracks (only rarely in alpha stars), whereas the oxide-exposed dogs had >99% of the plutonium activity concentrated in alpha stars (only rarely in single tracks). The difference in microdistribution and character of the alpha activity probably influenced the biological effect.

Serum enzyme assays have been performed throughout the postexposure period in an attempt to diagnose specific damage to liver and/or bone by plutonium translocated by the lung. Although periodic elevations occurred in mean values for glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, and alkaline phosphatase, there were no dose-related or dose-consistent elevations in these values. The periodic excursions in mean values were usually because of high values in one or two dogs at a particular sampling period, and occurred in all treatment groups, including controls.