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CARCINOGENICITY OF ^{238}Pu

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ABSTRACT

Three groups of albino, female rats were exposed to an aerosol of "soluble" ^{238}Pu derived from crushed $^{238}\text{PuO}_2$ microspheres suspended in physiological saline. Initial alveolar burdens of ^{238}Pu were 5 nCi (Group I), 18 nCi (Group II), and 230 nCi (Group III). Only 1% of the initial alveolar lung burden remained in the lung at one year, decreasing to 0.3% by 600 days after exposure. The ^{238}Pu body burden was 25% of initial alveolar burden at one year, decreasing to 12% by 1,000 days after exposure; about half of the body burden was found in the skeleton at these times. The cumulative radiation doses to the lung at two years after exposure were 9 rads (I), 32 rads (II), and 375 rads (III). Unexposed controls exhibited a median survival time of 825 days as compared to experimental survival times of 650 days (I), 675 days (II), and 550 days (III). The incidence of lung tumors in controls was 1.1% as compared to incidences in ^{238}Pu exposed rats of 6.6% (I), 23.3% (II), and 25.0% (III).

The incidence of all tumors, other than mammary tumors, was 4.3% in unexposed controls, and in ^{238}Pu exposed rats, 26.7% (I), 36.6% (II), and 46.8% (III). It was concluded that exposure of rats to small amounts of inhaled ^{238}Pu resulted in a significant incidence of tumors in the lung and other tissues.

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INTRODUCTION

Several lifespan studies have been performed with plutonium-239 deposited in the lungs of rodents and dogs, clearly demonstrating the carcinogenicity of plutonium in the lung at rather high radiation doses⁽¹⁻⁷⁾. The minimum radiation dose to lungs of animals developing lung tumors after ^{239}Pu exposure is about 50 rads delivered to rats following inhalation of soluble ^{239}Pu compounds⁽³⁾.

Lung tumors in dogs following inhalation of $^{239}\text{PuO}_2$ particles have been produced only in animals accumulating from 2000 to 20,000 rads to the lung⁽⁴⁾. Particulate plutonium deposited in the lung results in foci of intense alpha activity. The number of cells irradiated by such "hot spots" dispersed throughout the lung is considerably less than if the same activity had been more evenly distributed in the lung. Physiological translocation of particulate plutonium into subpleural areas may also greatly modify the dose distribution⁽⁸⁾. A few relatively large particles of plutonium can account for a very large share of the radiation dose delivered to the lung⁽⁹⁾.

Of the many radioisotopes tested as power sources for space, terrestrial, aquatic or medical applications, ^{238}Pu is one of the most useful for thermoelectric generation. Plutonium-238 exhibits a specific activity that is 280 times that of ^{239}Pu or a physical half-life of 86 years as compared to 24,000 years for ^{239}Pu . Although acute toxicity studies have been performed⁽¹⁰⁻¹²⁾ no study has been reported on the carcinogenic responses of animals to inhaled ^{238}Pu . This report describes the carcinogenic effects in rats of relatively "low" levels of inhaled ^{238}Pu deposited as a predominantly monomeric form.

METHODS

In June, 1966, 0.25 gram of ^{238}Pu dioxide microspheres were delivered to Battelle-Northwest from the Mound Laboratory in Ohio. The microspheres prepared in a plasma torch, contained about 80% ^{238}Pu by weight and consisted of spherical particles with diameters ranging from 10 μm to 200 μm , many angular fragments of less than 20-50 μm and numerous smaller particles attached to the surfaces of larger particles. The stock suspension of ^{238}Pu was prepared in November, 1969, and animals exposed in January, 1970. The microsphere preparation was lightly crushed in 40 ml distilled water with a porcelain mortar and pestle and then washed 10 times with 20 ml volumes of water, with one minute intervals between washings for settling out of larger fragments. Twenty ml of the upper supernatant was discarded following each washing. The lightly crushed microspheres were thoroughly crushed in 40 ml distilled water with mortar and pestle for 10 minutes. The microsphere fragments were very fragile, easily disintegrating with little pressure on the pestle. The resulting suspension was allowed to settle for several hours. The upper 20 ml containing about 400 μCi $^{238}\text{Pu}/\text{ml}$ was removed by syringe and used for our studies following appropriate dilution in physiological saline, in which it was stored for 2-3 months prior to its aerosolization.

Distinct, well defined particles with clear borders were rare when the ^{238}Pu -saline aerosol was examined with the electron microscope. The aggregates of ^{238}Pu lacked the homogeneity and electron density common to $^{239}\text{PuO}_2$ particles⁽¹³⁾ (Figure 1). The count median diameter of the aggregates was approximately 0.02 μm with a standard geometric deviation of 2.1. About 72% of the ^{238}Pu was ultrafilterable⁽¹⁴⁾. Based on this physical characterization, it is probable that the initial $^{238}\text{PuO}_2$ was solubilized in the saline in

which it was suspended⁽¹⁵⁻¹⁶⁾.

Female, 65-80 day old, Sprague Dawley, Charles River CD rats were used in this study, following a three week isolation period after being received from the supplier. Thirty-two rats received an intratracheal instillation of 0.7 μCi ^{238}Pu in 0.3 ml physiological saline. Four of these rats were placed in individual metabolism cages for 24 days and their daily excreta collected. The remaining animals were killed at intervals of one hour to 39 days after instillation.

Three groups of 35 animals each were exposed, nose only, to an aerosol of ^{238}Pu in saline. The exposure equipment and procedures have been previously described⁽¹³⁾. Exposure times varied from 10 to 30 minutes. Four animals were killed each from Groups I and II and 3 animals from Group III at 24 hours after inhalation exposure. The remaining rats were autopsied when moribund or as soon as possible after death. Thirty of 31 lifespan rats in Group I, 30 of 31 in Group II and 32 of 32 rats in Group III lived longer than 200 days after the exposure; the lifespan analyses are based on these animals. Unexposed, untreated control animals, living longer than 250 days after birth, consisted of 92 rats.

At autopsy, rats were examined grossly for tumor-like growths. The lung, heart, thymus, pulmonary lymph nodes, liver, spleen, adrenal, kidney, ovary, femur, uterus and other tissues with tumor-like growths were fixed in 10% neutral buffered formalin and paraffin sections prepared for light microscopic examination following hematoxylin and eosin staining. The amount of ^{238}Pu present in the lung, liver, total skeleton, extra-pulmonary thoracic tissues, gastro-intestinal tract, spleen, kidney, skin and that remaining in

all other tissues, along with the excreta collected from rats given ^{238}Pu by intratracheal instillation, were analyzed for ^{238}Pu contents by liquid scintillation counting following ashing and dissolving in $\text{HNO}_3\text{-HF}^{(18)}$.

Autoradiographs were prepared of lung and other selected tissue sections.

Tumor incidences were expressed as number ~~animals~~^{of} with tumors/number of rats X 100. Animals with more than one tumor of a particular type were considered statistically to have only one tumor of that type.

RESULTS

Fate of Intratracheally Instilled ^{238}Pu

Excretion - Within 24 hours, 45% of the intratracheally instilled ^{238}Pu had been excreted in the urine. After 4 days, less than 0.1% of the instilled dose was excreted daily in the urine. Only about 1.5% of the instilled dose was found in the feces the first day, decreasing to 0.1% or less per day by the seventh day after instillation (Figure 2). By 24 days, a total of 49% and 4% of the intratracheally instilled ^{238}Pu had accumulated in the urine and feces, respectively.

Tissue Distribution - Intratracheally instilled ^{238}Pu was rapidly cleared from the lung to the blood and other tissues. The lung contained about 30% of instilled ^{238}Pu at 24 hours, 20% at 4 days, 15% at 30 days and 12% at 39 days after its deposition. Translocation of ^{238}Pu to blood was greatest during the first few hours after deposition, decreasing from 0.2-0.7% of instilled dose/ml blood at 1-6 hours to less than 0.01%/ml after 30 days (Figure 3). The amount of ^{238}Pu translocated to liver rose from 0.2% of instilled dose at 1-2 hours, to 2% by 6 hours, to 3% by the tenth day and then fell to about 1% of instilled dose by the 39th day (Figure 3). A slower accumulation of

^{238}Pu into the skeleton was seen. The skeletal burden was 5% of instilled dose at 2 hours, about 10% at 24 hours, leveling off to about 20% of instilled dose thereafter (Figure 3). The body burden of ^{238}Pu decreased by 10% the first 6 hours, by 50% the first day and by 55% by the 39th day after instillation (Figure 3).

Fate of Inhaled ^{238}Pu

Determination of Initial Alveolar Deposition - From 3-4 rats in each exposure group were killed at 24 hours after inhalation of ^{238}Pu . The mean amount of ^{238}Pu measured in the lungs of autopsied animals of each group at 24 hours postexposure were 1.6 ± 1.3 for Group I, 5.9 ± 3.2 for Group II and 69.0 ± 43.6 for Group III; data is expressed in nanocuries $\pm$ 95% confidence intervals. The initial alveolar depositions of ^{238}Pu were estimated to be three times the amount present in the lung at one day following inhalation, based on the intratracheal instillation studies with the same ^{238}Pu preparation, which demonstrated an approximate 70% loss of initially deposited ^{238}Pu from the lung into the blood, tissues and excreta the first day after instillation. Thus the initial alveolar depositions were calculated as 5 nCi for Group I, 18 nCi for Group II and 230 nCi for Group III.

Microscopic Distribution of ^{238}Pu - Autoradiograms of lung sections at one day after inhalation of ^{238}Pu showed that the ^{238}Pu was associated mostly with the alveolar septae. Plutonium aggregates, as evidenced by alpha stars on the autoradiograms, were rare. Most alpha tracks in the lung were spread randomly as single tracks throughout the alveolar tissue (Figure 4). Little evidence of alveolar ^{238}Pu was present on autoradiograms of lung at one year or longer after exposure. The little remaining activity was concentrated

mostly with hemosiderin-like pigment granules located in peribronchiolar and perivascular regions of the lung.

Plutonium-238 was diffusely distributed in other soft tissues, with alpha stars, indicative of particulate plutonium, being very rarely seen. Plutonium was not concentrated in pulmonary lymph nodes. Plutonium in the femur was initially concentrated on periosteal surfaces. A year or longer after exposure, the ^{238}Pu was found distributed diffusely throughout the bone matrix, with some irregular concentration of activity being found in vascular areas of bone and in some regions of the bone marrow. Loose aggregations of ^{238}Pu associated with hemosiderin-like granules were observed at a year or longer after exposure in liver, spleen and bone marrow tissues. Autoradiographic exposures of up to two months were required to detect the ^{238}Pu activity in these soft tissues from Groups I or II a month or longer after exposure.

Tissue Distribution - Based on studies of ^{238}Pu behavior following intratracheal instillation, from 60-70% of deposited ^{238}Pu was lost from the lungs during the first day postexposure and 90% by 39 days postexposure (Figure 3). Only about 1% of the estimated initial alveolar deposition of inhaled ^{238}Pu remained in the lung at 300 days postexposure, decreasing to about 0.3% by 600 days postexposure (Figure 5).

The liver burden was about 0.7% of initially deposited activity at one year postexposure, decreasing by about half by the second year. The body burden of ^{238}Pu was about 25% of initially deposited dose at 300 days postexposure, decreasing to about 12% by 1000 days postexposure.

Detailed analyses of ^{238}Pu contents in tissues were performed on all

rats that died during the first 700 days postexposure (62/92 exposed rats). Total body burden of ^{238}Pu was determined in the remaining 30 animals which died after 700 days postexposure. Average ^{238}Pu contents in tissues on at least 19 rats per group are shown in Table I. About 1% or less of the initially deposited ^{238}Pu was present in either the lung, liver or gastrointestinal tract tissues and 0.5% or less was found in either the spleen, kidney, skin or extra-pulmonary thoracic tissues. The greatest concentration of ^{238}Pu occurred in the skeleton; 38-68% of the terminal body burden or 9-18% of the initial ^{238}Pu body burden. An average of 16-32% of initial ^{238}Pu was present in all tissues and organs of the rats during this time; this amounted to an average body burden at 200-700 days postexposure of 1.1 nCi ^{238}Pu (Group I), 5.6 nCi (Group II) and 32.7 nCi (Group III); an average of 37% (Group I), 30% (Group II) and 24% (Group III) of terminal body burden was present in all other tissues, other than lung, thoracic contents, liver, G.I. tract, spleen, kidney, skin or skeleton. The mean terminal body burdens for all 92 rats were 0.80 nCi (Group I), 4.11 nCi (Group II) and 27.8 nCi (Group III).

Dose Accumulation - The absorbed radiation doses accumulated by the lung and skeleton were calculated from data on the ^{238}Pu contents of these tissues. Dose calculations for lung and skeleton were determined from organ or tissue weights, ^{238}Pu contents and lifespans in each group. Thus radiation dose (rads) = nCi ^{238}Pu /gm X rads/nCi/gm/day X lifespan (days). The average accumulated radiation doses at 700 days after exposure to ^{238}Pu were for lung 9 rads (Group I), 32 rads (Group II) and 375 rads (Group III). The radiation dose accumulated by skeleton was determined by averaging the amount

of ^{238}Pu in skeleton, skeletal weight (assumed to be 6% of body weight at death) and lifespan. For skeleton, the accumulative mean radiation doses were 2 rads (I), 20 rads (II) and 150 rads (III). The radiation dose calculations for lung^a were determined on the basis of ^{238}Pu lung retention curves. An example of derived dose curves for lung are shown in Figure 6. About half the radiation dose delivered to the lung accumulated in the first month and 80% the first 4 months after inhalation exposure. Little further dose accumulation occurred in the lung after the first year postexposure. The dose delivered to the skeleton accumulated throughout the lifespan of the rats due to the tenacious retention of ^{238}Pu in skeletal tissue.

Toxicity of Inhaled ^{238}Pu

Mortality - The median survival time for the three groups of exposed rats were all less than for the unexposed control rats. Control animals exhibited a median survival time of 825 days, those in Group I 650 days, those in Group II 675 days and those in Group III 550 days. Exposure to the plutonium aerosol occurred at about 70 days of age. Mortality rates were similar for all groups (Figure 7). Even at the highest exposure level of ^{238}Pu (Group III), the average amount of lifespan shortening amounted to only 20% of expected lifespan, based on the survival times of unexposed control animals.

Cause of Death - The immediate cause of death or morbidity in most of the rats was difficult to ascertain. Of the ^{238}Pu exposed rats, 18% died from malignant, metastatic or infiltrative tumors involving vital organs;

$$^a \text{Dose (rads)} = \frac{\text{Initial Deposition (nCi)}}{(2.04 - 0.55e^{-0.04t} - 1.44e^{-0.006t})}$$

4% of unexposed control rats died from such tumors. Large, multiple mammary tumors were common in all groups, but did not involve vital organs. Fatty degeneration of the liver with peribiliary inflammation, liver cell necrosis, biliary hyperplasia and reactive liver cell hyperplasia were commonly seen in aging rats. Chronic nephritis with cortical inflammatory infiltrates and epithelial hyperplasia and dilated cortical tubules were very common in old rats. Adenocystic hyperplasia of the uterus and hemorrhagic degeneration of adrenal cortex were frequently observed.

Evidence of chronic murine pneumonia was seen in most animals, irrespective of treatment. About 5% of all rats exhibited bronchiectatic cavitation and 10% exhibited discrete, consolidative inflammatory lesions in one or more lobes of the lung. Peribronchiolar lymphoid hyperplasia and fibrosis were seen in most animals. Fibrosis and extensive thickening of the alveolar septae were seen only in some animals in Group III. However, patchy interstitial pneumonitis with septal infiltration of inflammatory cells was not uncommon. Animals with lung tumors did not appear to be any more severely affected by chronic murine pneumonia than those without lung tumors.

Tumor Formation: Mammary Tumors - Large numbers of mammary tumors arise spontaneously in aging rats. A higher incidence of mammary tumors occurs in female rats which live longer than 700 days. The incidence of mammary tumors in female Sprague-Dawley rats ranges from 55-85%⁽¹⁹⁻²⁰⁾.

In our study, mammary tumors constituted the greatest number of observed tumors. Mammary tumors were divided into benign fibroadenomas and malignant adenocarcinomas. Evidence of metastasis of mammary adenocarcinomas was not seen since no relationship could be found between the incidence of

carcinomas found in other tissues or organs. For example, 16.3% of the 92 ^{238}Pu exposed rats had one or more mammary adenocarcinomas, while 27.2% exhibited carcinomas in other tissues or organs. Thus on a random distribution basis, 4% of rats with extra-mammary carcinomas would be expected to also have mammary adenocarcinomas. Actually 4.3% of exposed rats exhibited both mammary adenocarcinomas and extra-mammary carcinomas, indicating no association with respect to metastatic growth between the two classes of epithelial tumors. Furthermore, no association was found between mammary adenocarcinomas and bronchiolo-alveolar adenocarcinomas of the lung.

The incidence of mammary tumors in unexposed rats was not different from that found in ^{238}Pu exposed rats (Table II). About one in every five mammary tumors was an adenocarcinoma. The incidence of mammary adenocarcinomas ranged from 10% to 20% in all groups, of fibroadenomas from 44% to 58% and of all mammary tumors, from 60% to 73%. No difference was seen in the time of first appearance of mammary tumors or in their rates of induction, irrespective of treatment.

Tumor Formation: Lung Tumors - The incidence of lung tumors in all groups exposed to ^{238}Pu was greater than in unexposed control rats (Table II). Only one lung tumor was found in control animals, a large cell undifferentiated carcinoma which exhibited a primary growth in the lung periphery and had infiltrated throughout the lung, particularly within pulmonary blood vessels (Figure 8). No undifferentiated carcinomas were found in rats exposed to ^{238}Pu . The dominant tumor type in exposed rats were bronchiolo-alveolar adenocarcinomas, which comprised about 75% of all lung tumors seen in this study. Bronchiolo-alveolar adenocarcinomas were characteristically papillary

in structure, fairly well localized in the periphery of the lung lobes and exhibited varying degrees of infiltration of tumor cells at the interface of normal lung tissue and tumor (Figures 8-9). Four of the bronchiolo-alveolar carcinomas exhibited wide spread metastatic growths in the lung. One exposed rat exhibited a large sclerosing squamous cell carcinoma, similar to an epidermoid carcinoma (Figure 8). Two tumors in the lung were of mixed cell type, containing both squamous carcinoma cells and adenocarcinoma cells. One lymphosarcoma was found in an exposed rat lung.

The incidence of lung tumors in unexposed control animals was 1.1% (1/92), in Group I, 6.6% (2/30), in Group II, 23.3% (7/30) and in Group III, 25% (8/32). The incidence of lung tumors in Groups II and III was significantly greater than in control rats at the 99.9% level of confidence. Although the percentage of rats in Group I with lung tumors was six times that in controls, statistical significance was not evident due to the low number of tumors found in Group I (Table III).

Tumor Formation: Other Tumors - Of all the 34 extra-mammary tumors found in rats exposed to ^{238}Pu , 50% were found in the lung and 50% in other areas of the body. Several endometrial carcinomas were found in the uterus of exposed rats. Other carcinomas were found originating from the ovary, renal pelvis, urinary bladder mucosa, skin, intestinal mucosa, and exocrine portion of the pancreas. Non-epithelial tumors comprised one pheochromocytoma of the adrenal gland, a leiomyosarcoma of the uterus, a reticulum cell sarcoma of an abdominal lymph node, a lymphosarcoma of the spleen and three osteogenic sarcomas (Table II). The osteosarcomas all originated from the lumbar region of the spine, all caused paralysis of the

hind legs, all found only in the highest exposure Group III and all metastasized to the lung (Figure 9). The accumulated radiation doses for animals with osteosarcoma were 51 rads, 96 rads and 152 rads. Two cases of myelogenous leukemia and one case of transitional cell carcinoma of the renal pelvis were found in unexposed rats (Table II). No leukemias were observed in rats exposed to ^{238}Pu .

Tumor Formation: All Non-Mammary Tumors - The incidence and rate of induction of all non-mammary tumors was clearly related to the amount of ^{238}Pu initially deposited in the lung (Figure 10). The incidence of non-mammary tumors in unexposed controls was 4.3%; in Group I, 26.7%; in Group II, 36.6%; and in Group III, 46.8%. The total incidence of non-mammary tumors in all groups was significantly greater than control tumor incidence at the 99.9% level (Table III). About 75% of all non-mammary tumors in all groups originated from epithelial tissues.

Of all 92 ^{238}Pu exposed rats, 28 developed 34 different tumors. Multiple tumors of different types were found in 4 rats. In one rat, a lymphosarcoma of the spleen, carcinoma of the uterus and bronchiolo-alveolar adenocarcinoma of the lung were found at autopsy. A particularly interesting animal was one that developed an osteogenic sarcoma in the lumbar vertebrae - that metastasized to the lung, a focal bronchiolo-alveolar adenocarcinoma and a transitional cell carcinoma of the urinary bladder which metastasized to the kidney and thoracic lymph nodes (Figure 9).

DISCUSSION

Crushed $^{238}\text{PuO}_2$ microspheres suspended in saline were largely in a monomeric form when delivered by inhalation to our rats. The experimental

evidence is as follows: (1) over 70% of the plutonium preparation in saline with ultrafilterable; (2) the aerosol exhibited a very small particle size of about 0.02 μm , which lacked the sharp borders and homogeneous electron density under the electron microscope, characteristic of insoluble PuO_2 particles; (3) autoradiograms of the lung one day after inhalation of ^{238}Pu showed a highly uniform distribution of mostly single tracks distributed in the alveolar tissue with very few alpha stars, indicative of particulate PuO_2 , being seen in the lung sections; (4) the lung retention of ^{238}Pu was very short with >50% of deposited activity being lost from the lung the first day after deposition of ^{238}Pu and 90% the first month; (5) the remaining ^{238}Pu was rapidly translocated from lung to blood and then mostly to skeleton.

Several conclusions regarding certain aspects of plutonium toxicology are indicated by the results of this study:

1. Leukemia was not induced by inhaled ^{238}Pu , although it was observed in two unexposed control rats. Among many studies reported only one study has shown leukemia incidence to be related to plutonium deposition in the rat⁽²¹⁾.
2. Similar to previous observations in rats and dogs, following skeletal deposition of ^{239}Pu ⁽²²⁾, osteosarcomas were observed in Pu treated animals but only the highest exposure level (Group III) and only at accumulated radiation doses of greater than 50 rads to the skeleton.
3. Exposure of female rats to ^{238}Pu had no effect on the incidence of naturally occurring, spontaneous mammary tumors. The effect of ^{238}Pu was on

the induction of tumors, rarely observed spontaneously in untreated animals.

4. About three-fourths of the ^{238}Pu induced tumors were of epithelial origin. The dose delivered to the epithelial tissues may be considered as fairly uniform and of comparable low magnitude based on the analysis of ^{238}Pu contents in tissues and examination of tissue section on autoradiograms. We shall examine this point further with respect to the lung.

5. Tumors in ^{238}Pu exposed rats are found in many other tissues, other than in the lung or bone. The incidence of tumors in any one tissue or organ, other than in lung and skeleton, is low for any particular experimental group. However, summing up the total number of such tumors in organs as the uterus, ovary, urinary bladder, G.I. tract, kidney, skin or pancreas shows a linear relationship between increasing tumor induction and increasing ^{238}Pu deposition as compared to unexposed control animals.

The physical characteristics and distribution of radiations from radionuclides deposited in tissues have an important bearing on their carcinogenic effects. A good example is the comparison in rats of the carcinogenicity of beta-emitting radionuclides with alpha-emitting nuclides deposited in the lung (Figure 11). The average energy of beta-emitters tested in these studies was from 1-2 mev. The average energy of alpha emissions from plutonium was about 5 mev or not all that much greater than for beta particles. The basic difference between the two types of radiations is in their Linear Energy Transfer (LET) with beta particles exhibiting LET's of less than 100 ev/ μm while alpha particles averaged LET's of about 100,000 ev/ μm . Beta particles penetrate millimeters in tissue while alpha particles penetrate less than 50 μm . Obviously the radiation dose around an alpha emitter is much greater than the

dose around a beta emitter. The incidence of lung tumors in rats is much greater per rad of dose delivered to the lungs for alpha emitters than for beta emitters (Figure 11). The relative biological effectiveness (R.B.E.) for alpha emitters/beta emitters is about 30 based on the data shown in Figure 11.

What about comparing alpha-emitters which are diffusely distributed in monomeric form throughout the lung with concentration of alpha emitters into polymers or oxide particulates? The initial even distribution of inhaled ^{238}Pu in the lung parenchyma resulted in a comparatively large number of tumors in the lung and in other tissues, at radiation doses that have not previously been shown to be carcinogenic. Unfortunately comparable studies with $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ particles in rats have not been published; such studies are currently being carried out at BNW over a five-fold range order of magnitude dose schedule. Studies by Meskalev (1972) and associates with inhaled ^{239}Pu citrate or ammonium $^{239}\text{Plutonyl}$ pentacarbonate have shown an apparent significant increase in the incidence of lung tumors in rats at cumulative absorbed radiation doses to the lung of about 50 rads. Studies in rats reported here show lung tumor formation at 9-32 rads following inhalation of ^{238}Pu . It is possible that a share of this ^{239}Pu was in a polymeric form resulting in greater heterogeneity of dose distribution in the lung than with our ^{238}Pu . This may account for the greater number of lung tumors or the lower doses required to induce lung tumors for ^{238}Pu than for ^{239}Pu . The greater mass of ^{239}Pu compared to ^{238}Pu required for identical radiation doses in the lung may in part result in greater polymeric association of ^{239}Pu in the aerosol and physiologically in the lung than for ^{238}Pu .

The influence of dose concentration may be more evident when dealing with relatively insoluble $^{239}\text{PuO}_2$ particles. True well-defined "hot spots" result from pulmonary deposits of such particles. The number of pulmonary cells hit by alpha particles from large Pu particles will be less than the number of cells hit from alpha emissions of smaller particles of the same total mass. Pulmonary cells adjacent to PuO_2 particles will be hit many times by alpha emissions with resultant "overkill" and "wasted amounts of radiation" from a biological-effect point of view. The probability of multiple hits on any one cell following deposition of the same amount of Pu in the monomeric form, would be much less.

To further complicate the picture, PuO_2 particles will be rather tenaciously retained in the lung while the Pu monomer may be rapidly translocated from the lung to bone and other soft tissues or excreted in the urine. PuO_2 particles in the lung will be gradually concentrated towards the periphery of the lung resulting in high radiation doses delivered to subpleural regions of the lung⁽⁸⁾. The result is often severe fibrosis in those areas further decreasing the number of epithelial "target" cells which may be hit by alpha emissions.

Finally, the nature of the particle size distribution in the aerosol deposited in the deep lung will greatly complicate the dose distribution pattern. Autoradiographic determination of $^{239}\text{PuO}_2$ particle size distribution in alveolar macrophages washed from the lungs of exposed rats within 30 days postexposure, showed that over 80% of the ^{239}Pu activity was concentrated in large particles, which represented only 2% of the deposited particles⁽⁹⁾. The dose-distribution in beagle dogs developing lung tumors from inhaled $^{239}\text{PuO}_2$ particles⁽⁴⁾ is probably similar in the degree of dose contribution

by a relatively few larger sized PuO_2 particles. Thus the disproportionate concentration of plutonium in relatively few particles of larger size, the physiological concentration of PuO_2 particles in subpleural areas of the lung and the concentration of Pu in dioxide particles or in polymers of Pu all result in "wasted" dose deposition in the lung. This conclusion is true only if one assumes that hitting of larger numbers of target cells (those which develop into tumors) will increase the probability for inducing a tumor. Studies with ^{238}Pu monomer suggest that spreading the plutonium activity evenly throughout the lung may be more carcinogenic than depositing the activity as insoluble particulates in the lung.

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FIGURE LEGEND

Figure 1. Electron micrograph of aerosolized ^{238}Pu in physiological saline. The carbon coated grids were washed with distilled water to remove sodium chloride crystals prior to taking the micrograph. X155,000

Figure 2. Excretion of intratracheally instilled ^{238}Pu in urine and feces. Each data point is a mean from four rats.

Figure 3. Tissue distribution of intratracheally instilled ^{238}Pu . Curves were drawn by eye.

Figure 4. Autoradiogram of lung (Group III) taken at one day after inhalation of ^{238}Pu . Exposure time of autoradiogram was three days. Views from two areas of the lung are shown. X220

Figure 5. Retention of pulmonary deposited ^{238}Pu in the lung. Data for the first 40 days after deposition is following intratracheal instillation; data for 200-800 days after deposition is following inhalation.

Figure 6. Cumulative radiation dose to the lung after inhalation of ^{238}Pu . Method of calculation is described in the text.

Figure 7. Cumulative mortality of control and ^{238}Pu exposed rats.

Figure 8. Pulmonary carcinomas found in the lung of rats.

A. Bronchiolo-alveolar adenocarcinoma, 790 days after inhalation of ^{238}Pu . Tumor was metastatic throughout the lung. X34

B. Epidermoid carcinoma, 657 days after inhalation of ^{238}Pu . X525

C. Bronchiolo-alveolar adenocarcinoma, 757 days after inhalation of ^{238}Pu . X670

- D. Large cell undifferentiated carcinoma in an unexposed control animal. Tumor was metastatic throughout the lung with primary tumor in subpleural region. Section shows tumor infiltrating within pulmonary vein. X220

Figure 9. Multiple tumors found in one rat, 731 days after inhalation of ^{238}Pu .

- A. Bronchiolo-alveolar adenocarcinoma in lung. X34
B. Metastatic osteogenic sarcoma in lung from primary tumor in spine. X34
C. Transitional cell carcinoma of urinary bladder. X525
D. Metastatic transitional cell carcinoma in kidney. X525
E. Metastatic transitional cell carcinoma in pulmonary lymph node. X34
F. Metastatic transitional cell carcinoma in lung. X34

Figure 10. Cumulative incidence of non-mammary tumors in rats following inhalation of ^{238}Pu .

Figure 11. Comparison of literature values for induction of lung carcinomas in rats following pulmonary deposition of beta-emitting radionuclides or alpha emitting radionuclides. Data on beta emitters is following intratracheal instillation of ^{144}Ce (24-25), ^{198}Au or ^{59}Fe (26) and ^{35}S (17). Data on alpha emitters is following inhalation of ^{239}Pu (3). The curves were drawn by eye.

TABLES

Table I. Distribution of inhaled ^{238}Pu in tissues of rats. Each value is a mean (>19 samples) of all autopsied animals taken at 200-700 days postexposure, $\pm$ 95% confidence intervals.

Table II. Tumor type, location and incidence as a function of ^{238}Pu exposure.

Table III. Incidence of tumors in the lung and all other tissues of the rat, other than mammary tumors, as a function of ^{238}Pu exposure. The level of significant difference of exposed rats from controls was determined by chi-square test.

Table I. Distribution of inhaled ^{238}Pu in tissues of rats. Each value is a mean (>19 samples) of all autopsied animals taken at 200-700 days postexposure, $\pm$ 95% confidence intervals.

Tissue or Organ	Group I			Group II			Group III		
	nCi	% Terminal Body Burden	% Initial Body Burden	nCi	% Terminal Body Burden	% Initial Body Burden	nCi	% Terminal Body Burden	% Initial Body Burden
Lung	0.05 ± 0.04	4.56	1.01	0.15 ± 0.14	2.71	0.86	1.13 ± 0.65	3.47	0.55
Thoracic Contents	0.01 ± 0.01	0.94	0.21	0.02 ± 0.01	0.36	0.11	0.05 ± 0.02	0.15	0.02
Liver	0.05 ± 0.03	5.16	1.14	0.17 ± 0.10	3.12	0.99	0.55 ± 0.24	1.67	0.26
Gastro Intestinal Tissues	0.07 ± 0.04	6.60	1.46	0.10 ± 0.04	1.78	0.56	0.16 ± 0.05	0.49	0.08
Spleen	0.02 ± 0.02	1.89	0.42	0.08 ± 0.07	1.43	0.45	0.16 ± 0.07	0.49	0.08
Kidney	0.03 ± 0.02	2.83	0.63	0.05 ± 0.04	0.89	0.28	0.11 ± 0.06	0.33	0.05
Skin	0.03 ± 0.01	2.83	0.63	0.10 ± 0.05	1.78	0.56	0.59 ± 0.54	1.80	0.29
Skeleton	0.41 ± 0.15	32.8	8.45	3.2 ± 0.8	57.6	18.3	22.2 ± 4.4	67.9	10.7
Total In All Tissues And Organs	1.1 ± 0.2	100	22.1	5.6 ± 0.9	100	31.7	32.7 ± 7.6	100	15.8

Table II. Tumor type, location and incidence as a function of ^{238}Pu exposure.

Tumor	Control	Incidence of Tumors (%)		
		Group I	Group II	Group III
Mammary Fibroadenoma	57.6	53.3	50.0	43.7
Mammary Adenocarcinoma	14.1	20.0	10.0	18.8
Pulmonary Tumors				
Bronchiole-alveolar Adenocarcinoma	----	6.6	13.3	21.9
Epidermoid Carcinoma	----	----	----	3.1
Mixed Carcinomas	----	----	6.6	----
Undifferentiated Carcinoma	1.1	----	----	----
Lymphosarcoma	----	----	3.3	----
Uterine Carcinomas	----	6.6	3.3	3.1
Uterine Leiomyosarcoma	----	----	3.3	----
Ovarian Carcinoma	----	----	----	3.1
Bladder Transitional Cell Carcinoma	----	----	----	3.1
Adrenal Pheochromocytoma	----	3.3	----	----
Renal Transitional Cell Carcinoma	1.1	3.3	----	----
Cutaneous Squamous Cell Carcinoma	----	----	3.3	----
Intestinal Adenocarcinoma	----	----	3.3	----
Pancreatic Carcinoma	----	3.3	----	----
Abdominal Reticulum Cell Sarcoma	----	3.3	----	----
Splenic Lymphosarcoma	----	----	----	3.1
Osteogenic Sarcoma	----	----	----	9.4
Myelogenous Leukemia	2.2	----	----	----

Table III. Incidence of tumors in the lung and all other tissues of the rat, other than mammary tumors, as a function of ^{238}Pu exposure. The level of significant difference of exposed rats from controls was determined by chi-square test.

<u>Group</u>	<u>Number of Animals</u>	<u>Incidence of Tumors</u>	
		<u>Lung</u>	<u>All Tumors*</u>
Control	92	1.1	4.3
I	30	6.6 (N.S.)**	26.7 ($p < 0.0001$)
II	30	23.3 ($p < 0.0001$)	36.6 ($p < 0.00001$)
III	32	25.0 ($p < 0.0001$)	46.8 ($p < 0.00001$)

*All non-mammary tumors

**Not significantly different from control