LUNG CANCER AND INHALED URANIUM ORE DUST IN RATS

R.E.J. Mitchel and J.S. Jackson
AECL, Chalk River Laboratories,
Chalk River, Ontario K0J 1J0

ABSTRACT

Using a nose only inhalation system, 187 nine week old male Sprague-Dawley rats were exposed to two different concentrations of natural uranium ore dust aerosol (44% U) without significant radon content. Inhalation exposures averaged about 4.2 h/day, 5 days/week for 65 weeks at which point lung uranium burdens in the two groups averaged 0.9 and 1.9 mg/g dry weight. Animals (63) exposed to the air stream without dust served as controls. After inhalation exposure ceased, the rats were allowed to live for their natural lifetime, a maximum of about 900 days after the start of dust inhalation.

Lung uranium burdens were measured at the time of death of each animal. Lung burdens were found to decline exponentially after dust inhalation ceased, and the rate of decline was independent of the initial lung burden.

All lungs were examined at necropsy and histologically for lung tumors. Lung tumors of lung origin were observed in both exposed groups and in the control group. The frequency of primary malignant lung tumors was 0.016, 0.175 and 0.328 and primary non-malignant lung tumors 0.016, 0.135 and 0.131 in the control, low and high aerosol exposed groups respectively. Absorbed dose to the lung was calculated for each animal in the study. The average maximum doses for all the animals exposed to the low or high concentration of dust aerosol were 0.87 Gy and 1.64 Gy respectively. The average risk of malignant lung tumors from inhaled natural uranium ore dust was therefore about 0.20 tumors/animal/Gy. For animals with lung tumors, the average doses were 0.98 and 1.90 in the exposed groups. In both exposed groups, the frequency of primary malignant or non-malignant lung tumors was significantly greater than in the control group ($p < 0.02$) and the frequency of primary malignant lung tumors in the two exposed group were significantly different from each other ($p = 0.05$).

The frequency of primary lung tumors (malignant and non-malignant) was calculated as a function of dose increment for both exposed groups individually and combined. The data suggested that, in spite of the above result, lung tumor frequency does not increase with dose even though a risk that doubled with dose could have been detected. However, when malignant lung tumor frequency was calculated as a function of dose rate (as measured by the lung burden at the end of dust inhalation) a positive correlation was seen, suggesting dose rate may be a more important determinant of risk than dose. No strong lobe-to-lobe biases in tumor frequency were found. For the same absolute tumor incidence, lung tumor latency was longer in the group exposed to the low dust aerosol concentration, as compared to the group exposed to the higher concentration but on a relative basis there was no latency change.

Uranium particulates in lung were rapidly transferred to bronchial lymph nodes. Lymph node specific burdens were variable, ranging from 1 to 60 fold greater than the specific lung burden in the same animal. No lymph node tumors were observed.

We conclude that chronic inhalation of natural uranium ore dust alone in rats creates a risk of primary malignant and non-malignant lung tumor formation. The evidence suggests that risk is not directly proportional to dose and certainly does not double as dose doubles in the range below 1.5 Gy.

OBJECTIVE

To estimate, in rats, the risk of lung cancer associated with chronic inhalation of uranium ore dust.

BACKGROUND

The risk of lung cancer in uranium miners and mill workers arises from the inhalation of ore dust and radon daughters, as well as from external γ-radiation. Although there is considerable experimental evidence defining the risk of lung cancer in rats from external γ-radiation or inhalation of radon daughters, little similar evidence is available for inhaled uranium ore dust, in either the rat or man. Specifically, no evidence is available for exposures of long duration and hence the component of risk associated with this exposure is unknown.

Since uranium and its decay chain products produce high LET radiation, it might be expected that such exposures would produce a risk of lung cancer similar to that arising from exposure to other high LET emitters, particularly those present as a dust aerosol deposited in lungs. Some data on lung carcinogenesis in rats exposed to other radioactive aerosols is available. Exposure to a single inhalation of $^{239}PuO_2$ produced benign lung adenomas but no malignant carcinomas in animals given 1 Gy or less over their lifetime. Both tumor types were present at higher doses but displayed different dose effect relationships at doses of more than 1 Gy (1). A tumor frequency of 20% has been reported in F344 rats exposed to 0.8-1.0 Gy of inhaled $^{239}PuO_2$(2).

Survival in Wistar rats after a single inhalation of $^{239}PuO_2$ was reported to be reduced only in animals with lung doses >30 Gy (3). These workers also report a linear response of lung tumors with dose, at doses from 2.3 to 44 Gy for a single inhalation, although below 1 Gy the incidence was very low. Another study of inhaled $^{239}PuO_2$ reported no difference in the incidence of lung tumors whether rats were exposed to a single inhalation or repeatedly over one year, for similar total doses in the range 0.90 to 4.4 Gy (4).

In a study of lung carcinogenesis in hamsters induced by multiple intratracheal installations of ^{210}Po, the frequency of lung cancer by the resulting alpha irradiation was only slightly influenced by dose protraction over 120 days as compared to 10 days (5). At lung doses of 0.24 Gy, risk was slightly elevated but at 2.4 Gy the risk was slightly reduced.

Exposure of rats to radon daughter products produced lung cancer after exposures as low as 25 WLM (80 $mJ.h.m^{-3}$) over 4-6 months. However, the frequency was not different from controls when the same cumulative dose was protracted over 18 months (6). This result suggests that lung cells are able to repair the damage from high LET radiation, and that dose rate significantly influences risk of lung cancer formation for these types of radiation.

Analysis of a study of lung cancer in rats exposed to varying radon concentrations carried on a constant concentration of uranium ore dust aerosol suggested an "inverse dose rate effect" ie. an increase in the lifetime probability of a tumor with a decrease in exposure rate. This result was attributed not to radon, but to promotion effects of the uranium ore dust (7).

Radon was shown to induce micronuclei (MN) in lung fibroblasts taken from the lungs of rats, Syrian hamsters and Chinese hamsters exposed to 496 WLM. Elevated MN levels were detected 4 h after the end of exposure, but these declined as a function of time after exposure (8), implying that these lesions are repairable.

In an *in vivo* study of rat lung fibroblasts (9), radon exposure was 10.6 times as effective as acute whole body ^{60}Co γ-exposure in producing MN.

A study in human lymphocytes indicated that a single low dose of X-rays (2 cGy) prior to radon exposure induced an adaptive response that resulted in about half the number of chromosome deletions as compared to radon exposure alone, suggesting that the repair of high LET damage is inducible (10).

Taken together, these results suggest that exposure of lungs to high LET radiation from inhaled uranium ore dust would likely produce a risk of lung cancer. Although the literature appears somewhat contradictory, it also appears that dose rate effects may be important in determining risks, particularly at low chronic doses. Accordingly, the experiments reported here were designed to estimate the lifetime risk of chronic uranium ore dust exposures to lung (without concurrent radon exposure) at two different levels of dust aerosol, to produce two different dose ranges and dose rates.

METHODS

Apparatus

Nose Only Inhalation Chambers. Four chambers were used. Ore dust was delivered to the top inlet of the chamber, distributed via an internal nozzle system to the nose of each animal, collected in the interior of the chamber and removed via a bottom port connected to an exhaust system. Uranium ore dust was delivered to the chambers as an aerosol under positive pressure. By means of the vacuum exhaust system however, the chambers were maintained under negative pressure relative to the treatment room the aerosol delivery and removal system, including the inhalation chambers was self contained and separate from the room atmosphere.

Dust Generators And Ore Dust. Three separate generators were constructed, one for each inhalation chamber that delivered dust aerosol. The chamber with the control animals was connected directly to air containing no dust.

The dust generators operated on a fluidized bed principle and consisted of a glass tube containing a horizontal sintered glass disk. A fixed volume of dust was placed on the top of the sintered disk each Monday, refilled to that volume daily and removed each Friday. This procedure ensured a reasonably constant particle size distribution in the generator. A regulated flow of air was introduced into the bottom of each glass tube, which flowed upward through the sintered disk and ore dust creating a dust aerosol. This concentrated aerosol was mixed with a second regulated flow of clean air to create the total desired airflow, with the required dust aerosol concentration, for introduction into the top of the chamber. The total airflow introduced into each chamber, regardless of dust concentration, was 32 l/min, which corresponded to 0.5 l/min from each nozzle.

Uranium ore was obtained from the Cluff Lake Uranium Mine in Saskatchewan. The ore was ground to dust in a ball mill.

One dust generator was adjusted to deliver about 50mg/m^3 to one inhalation chamber (high level dust exposure). The two remaining generators were adjusted to deliver about 19mg/m^3 to two other chambers (low level dust exposure). A fourth chamber was connected to the air supply only, without dust, and served as the control.

Dust Monitors. The aerosol dust concentration delivered to the rats in each inhalation chambers was monitored for stability by a Real-time Aerosol Sensor (RAS) monitor. Each chamber was sampled at one nozzle, at the approximate level of the rat's nose. The chambers were sampled continuously and remotely monitored by a hardwired link to a dedicated chart recorder.

Dust aerosol was analyzed, and particle size distribution measured using an APS 33 Aerodynamic Particle Sizer.

Dust aerosol concentration was also occasionally measured at the nozzles by taking a glass fiber filter sample. These filters were analyzed for uranium by neutron activation analysis, and by gravimetric procedures for total ore dust mass.

Animals

Two hundred and ninety-one male Sprague Dawley (Charles River Laboratories, Charles River, Quebec) were divided into four groups of 63 test animals plus 12 sentinel animals. The remaining twenty-seven animals were spares.

Group	Color code	Treatment
50 mg/m^3	red	high ore dust level
19 mg/m^3	blue	low dust level
19 mg/m^3	tan	low dust level
0 mg/m^3	green	no ore dust

These 4 groups of animals were 6 weeks old, approximately 151 to 175 g in weight at the time of purchase. The remaining animals were 5 weeks old. The animals were acclimatized over a period of one week. During this time they were tattooed by group color and cage number.

Ore Dust Aerosol Exposure

Following acclimatization, the rodents were loaded and unloaded into the holding tubes for increasing lengths of time with exhaust air only. Exposure to uranium ore dust started at 9 weeks of age. Replacement animals were not ramped as described. Animals in the treatment groups were exposed approximately 4.2 h/day, 5 days/wk for 65 weeks, to the indicated ore dust aerosol concentration. The spare animals were held in reserve to replace any animals that died within four months of the start of exposure.

Some data is presented in terms of "days at risk" (DAR) defined as the number of days from the start of dust inhalation to death for any individual animal.

Aerosol exposure accumulated a maximum total of 1344.1 h over a 65 week period.

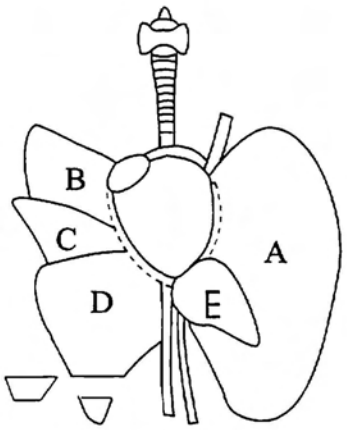
Radon Measurement

Radon levels were estimated in the air and aerosol supply for the control group and high aerosol concentration group (red- 50 mg/m^3). The charcoal radon sampler was placed in an air-tight box with two ports. One port was connected to a nozzle in one chamber port (through a filter) and the other to the pump and flow meter used to take filter samples for mass measurements. Samples were collected during the normal daily exposure and counted with a high resolution silicon detector. The clean air, prior to aerosol generation was measured at <0.5 pCi/L. After aerosol generation and subsequent removal of the dust from the air, the air contained <2 pCi/L.

Tissue Uranium Analysis

A section of lung tissue was removed from the caudal aspect of the anterior right lobe (lobe D) not including the periphery of the lobe (Figure 1). This section was weighed and freeze dried and submitted for uranium analysis using neutron activation followed by delayed neutron counting. As well, the bronchial lymph nodes were removed from selected animals at the time of death during the study and from the last 10 animals left from each of the treatment groups. The detection limit for this uranium analysis method was 0.050 µg.

Figure 1
Diagrammatic representation of rat lungs, ventral view, showing the location of tissue removed from the caudal aspect of lobe D, not including the periphery tip, for uranium analysis.



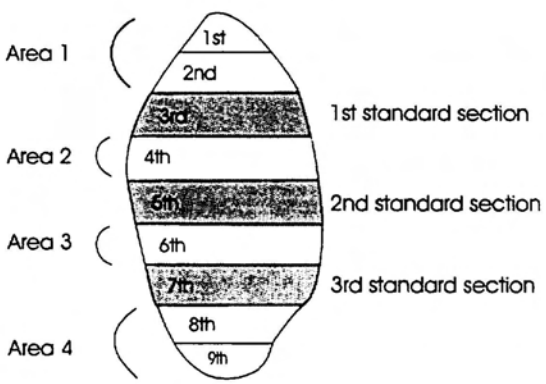
Animal Analysis

Gross Pathology. Each animal was weighed and fully examined at time of death using standard gross pathology methods. The appropriate sample for uranium analysis was taken from the caudal aspect of the anterior right lobe (lobe D) after the lung lobes were examined. This lobe was then tied off and the lungs were inflated through the trachea with gravity fed 10% buffered neutral formalin and formalin fixed. Any abnormal tissue or tumor identified during the gross pathology was removed for formalin fixation.

Histology. Each lung lobe was removed one at time, examined and then sectioned into 1-2mm sections. These sections were then examined macroscopically using a headloop (2x magnification). A systematically selected three sections per lobe (5 lobes) were placed in individual cassettes for tissue processing. Sections identified with abnormalities or possible masses in other than those systematically selected, were also submitted for tissue processing. Samples were blind coded.

The remaining lung tissue sections were divided into 4 areas beginning with the cranial portion and separated by the tissue section removed for tissue processing. These sections were placed in separate formalin vials for further reference if required. A diagrammatic representation of this process is depicted in Figure 2.

Figure 2
Diagram of the sequential sectioning of lung lobe A for gross pathology analysis and histological preparation. Sections 3, 5 and 7 were standard lung tissue sections submitted for histology processing. The remaining sections were relabelled by areas and stored separately for further reference.



Statistics

The Fisher Exact Test was used to test for significant differences in rates of tumor appearance, and the p values given are based on this test. Vertical error bars on data indicate one standard deviation. Differences in means were tested for significance using a one-way analysis of variance and p values are given.

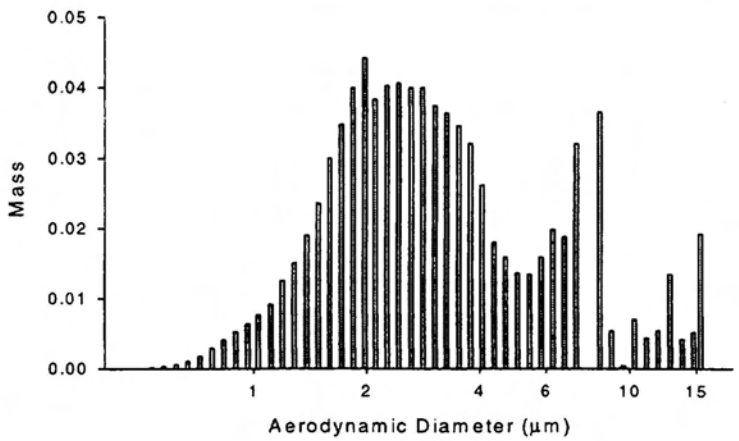
RESULTS

Ore Dust Aerosol Size

Uranium ore was ground in a ball mill to a particle size respirable by rats. Figure 3 plots the mass distribution as a function of aerodynamic diameter of the samples taken at the chamber nozzle, the point of delivery to an individual rat. About 75% of the mass was in particles < 5 μm.

Figure 3

Mass distribution after 10 minutes of operation. A new ore dust sample was placed in the generator at 0 time. The sample was taken at a chamber nozzle. Aerosol concentration, 50 mg/m^3.



Exposure Time

Rats were exposed to the aerosol ore dust five days per week for 65 weeks, holidays excluded. They were loaded into the inhalation chambers each morning and removed each afternoon. Figure 4 shows the actual dust inhalation time for each week over the entire exposure period. The average weekly exposure was about 21 hours.

Figure 4
Actual weekly inhalation time over the 65 week period of dust inhalation.

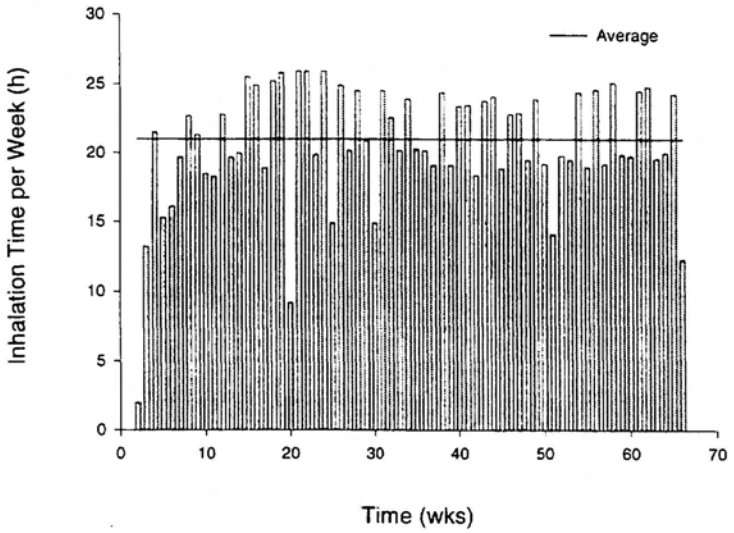
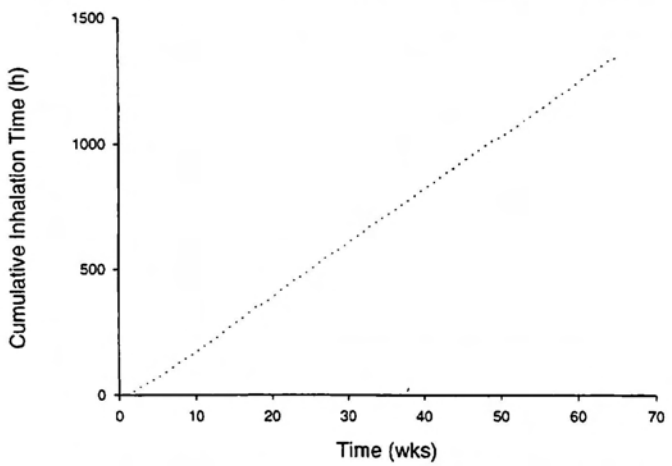


Figure 5 shows the maximum total inhalation exposure time on an accumulating basis over the entire 65 week exposure. All exposure chambers, one control, two low dose and one high dose, were operated in parallel and simultaneously. The exposure times were therefore identical for each group.

Figure 5
Accumulated inhalation exposure as a function of time.

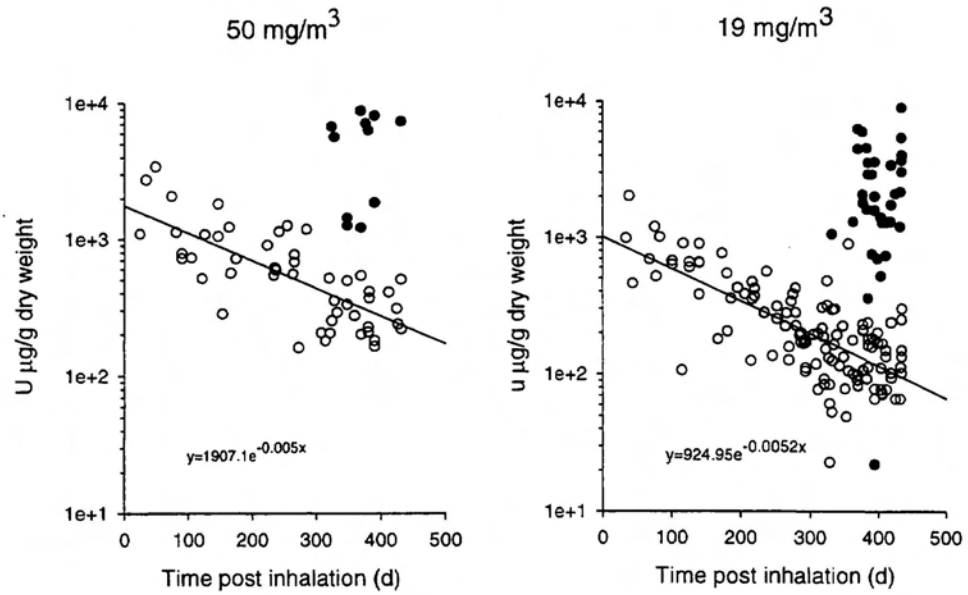


Lung Clearance

Ore dust inhalation ended for all animals 65 weeks after the beginning of inhalation by any animal. Animals were allowed to live for their natural lifespan, and after death the uranium lung burden was estimated as described above. Figure 6 shows the decline in lung uranium burden in both high and low level aerosol groups as a function of time post-inhalation for all test animals that died after the end of inhalation. Data from the two low level groups were pooled. These data were used to estimate the rate of clearance of the particulate ore dust from the lungs. Both groups (high and low level) show the same rate of decline, indicating that lung clearance rate is independent of initial burden. Extrapolation of the data sets to the point at which inhalation ended indicates the average lung burden in the

two groups at that time. This extrapolation indicates that the group exposed to the high level of aerosol dust had, on average, about twice (1.91 mg/g dry weight) the lung burden that had accumulated in the groups exposed to the lower level of aerosol dust (0.93 mg/g dry weight).

Figure 6
Lung (o) and selected bronchial lymph node (•) burdens for all the animals that died in the high and low level dust inhalation groups after the end of aerosol inhalation.



Bronchial Lymph Nodes

Bronchial lymph nodes in selected animals from both the high and low level exposure groups were separated at the time of necropsy, lyophilized and analyzed for uranium, using the same procedure as for lungs. Data from individual animals, as a function of time post exposure is given in Figure 6. Both inhalation groups show very large variations in lymph node burdens.

Lung Tumors

Table 1 shows the frequency of primary malignant lung tumors of lung origin observed in the control, low dose and high dose groups, 1/63, 22/126, and 20/61 respectively. The above table also show the frequency of malignant lung tumors of non-lung origin in each groups, as well as the six types of malignant lung tumors identified histologically, in each group of animals. Also shown is the type and group frequency of the non-malignant tumors identified. The data shows that the frequencies of the non-malignant tumors in the high and low dose groups were the same.

Table 1

Types and number of primary lung tumors as well as tumors of non lung origin observed in all the test animals.

Tumor Type	Control		Low Dose		High Dose	
	No. Tumors	Frequency of Rats with PrimaryTumors	No. Tumors	Frequency of Rats with Primary Tumors	No. Tumors	Frequency of Rats with Primary Tumors
	63 Rats Total		126 Rats Total		61 Rats Total	
Bronchioloalveolar carcinoma	0	0.000	12	0.095	9	0.148
Bronchiolar Carcinoma	0	0.000	4	0.032	6	0.098
Squamous Cell Carcinoma	0	0.000	3	0.024	4	0.066
Bronchogenic Carcinoma	0	0.000	0	0.000	1	0.016
Adenocarcinoma	0	0.000	1	0.008	0	0.000
Sarcoma	1	0.016	2	0.016	0	0.000
Total Primary Malignant	**1**	0.016	**22**	0.175	**20**	0.328
Bronchiolo alveolar adenoma	1	0.016	17	0.135	7	0.115
Bronchiolar Adenoma	0	0.000	**0**	0.000	1	0.016
Total Primary Non-Malignant	**1**	0.016	**17**	0.135	**8**	0.131
Non Lung Origin						
Osteosarcoma	1	0.016	0	0.000	0	0.000
Intravascular Carcinoma	0	0.000	0	0.000	1	0.016
Total Non- Lung	**1**	0.016	**0**	0.000	**1**	0.016

* Primary malignant and primary non-malignant tumors occur in the same animal.

Lung Dosimetry

Lung doses were calculated on the basis of the lung burden at the time of death, the rate of clearance calculated from the data in Figure 6, the total inhalation time and the time to death after the end of dust inhalation. Neutron activation analysis of the uranium ore dust indicated that it was 44% elemental uranium by weight.

Figure 7 shows the calculated absorbed dose to the lungs as a function of time for the group average in both the high and low aerosol exposure groups. The figure shows that the high exposure group accumulated dose at about twice the rate of the low exposure group. The average maximum dose received by the animals in the low exposure group was about 0.9 Gy at about 900 days after the start of inhalation, while the average maximum in the high exposure group was about twice that at the same time. The figure also shows the time of death for each animal with a primary malignant lung tumor, in both test groups. The time of tumor appearance (time of death) was very similar for both groups.

Figure 8 shows the distribution of the number of animals within increments of total lung dose, for all the animals in each exposure group and for those with lung tumors. The distribution for animals with tumors is skewed to higher doses for both test groups.

The figure also shows a plot of the frequency of animals with primary malignant and non-malignant lung tumors included in each 0.5 Gy dose increment. At higher doses in both the low and high aerosol exposure groups, this rate becomes unreliable because of the very few animals within each dose increment. However, considering this ratio only in dose increments containing 5 or more animals (0.5 to 3.0 Gy and 0 to 1.5 Gy in the high and low aerosol exposure groups respectively), three observations can be made:

1. The frequency, in either the low aerosol exposure group or the high aerosol exposure group, appears to be remarkably constant and does not increase as a function of dose.

2. The frequency in the high aerosol exposure group, in any 0.5 Gy dose increment, appears to be on average about double (0.4) that seen in the low aerosol group (0.2).

3. These results would tend to indicate that the frequency of animals with lung tumors is not directly proportional to dose.

This conclusion, that the risk of lung tumor formation was not directly proportional to dose, was tested statistically in two ways. In the first test the data for all the animals in both exposed groups (19 and 50 mg/m^3 aerosol exposure) were combined. The animals with doses between 0 and 0.74 Gy, (median dose 0.53 Gy) were identified as one group (70 animals) and the animals with doses between 0.75 and 1.49 Gy (median dose 1.04 Gy) as a second groups (80 animals). The number of animals with tumors (primary malignant and primary non-malignant) in these two groups (17 and 20 respectively) were not different (p=0.5). If tumor frequency was directly proportional to and doubled with dose as suggested from the primary malignant frequencies separately observed in the 19 and 50 mg/m^3 exposed groups (Table 1) then doubling the dose from 0-0.74 Gy to 0.75-1.49 Gy would be expected to double the tumor frequency. Since 70 animals receiving 0-0.74 Gy (median dose 0.53 Gy) developed 17 lung tumors (frequency 0.243), the group receiving 0.75-1.49 Gy (median dose 1.04 Gy) would have been expected to develop double that tumor frequency, 39 tumors in 80 animals. That expected frequency (39/80) would be significantly different from the frequency actually observed in the lower dose range group, 17/70, p=0.025 and therefore could have been detected. The expected frequency was however significantly higher than the frequency actually observed in the group receiving 0.75-1.49 Gy (p=0.024).

The statistical analysis of animals in these two dose ranges was repeated based on the frequency of primary malignant and non-malignant lung tumors observed (19/70 and 27/80 in the low and high dose ranges respectively). The two frequencies were not significantly different (p=0.3). Again hypothesizing that tumor frequency was directly proportional to dose, the high dose range would be expected to produce 44 lung tumors in 80 animals, significantly more that the 19/70 observed in the low dose range (p=0.02). That expected frequency is on the margin of significant difference from the frequency actually observed in the high dose range (27/80) p=0.06.

This analysis was again repeated considering only the frequency of primary malignant lung tumors observed, 12/70 and 15/80 in the low and high dose range groups respectively. These frequencies are not significantly different (p=0.5). Using the same argument as above, the expected frequency in the group receiving 0.75-1.49 Gy would be 28/80, and this would have been significantly different from the observed frequency in the 0-0.74 Gy dose range group (p=0.04). The expected frequency in the higher dose range group (28/80) is however significantly higher than the actual frequency observed in that group (15/80) p=0.05.

The above analysis tested the hypothesis that tumor frequency was proportional to dose using all the ore dust exposed animals combined into one data set. We also tested that hypothesis in a second way using only the animals exposed to a single ore dust aerosol concentration, 19 mg/m^3. Insufficient animal numbers in the 50 mg/m^3 exposed group precluded a similar test on that group alone. Again dividing the 19 mg/m^3 ore dust exposed group into animals receiving 0-0.74 Gy (median dose 0.53 Gy) and 0.75-1.49 Gy (median dose 1.03 Gy) showed 17/63 animals with lung tumors in the former and 15/58 in the latter. These frequencies are not significantly different (p=0.3). If the tumor frequency was proportional to dose, the 0.75-1.49 Gy dose range group would have been expected to show 33/58 animals with tumors, significantly different from the 17/61 observed in the 0-0.74 Gy dose range group (p=0.03). This expected frequency of 33/58 was again significantly higher than the 11/58 actually observed in that dose range (p=0.003). Considering the number of primary malignant and non-malignant lung tumors in the animals exposed to 19 mg/m^3 aerosol, the observed frequencies in the two dose ranges (19/61 and 16/58 in the low and high ranges respectively) are not different (p=0.5). Again hypothesizing that frequency is directly proportional to dose results in an expected frequency of 36/58 in the high dose range, significantly more than the observed frequency (19/61) in the low dose range (p=0.03) but significantly higher than that actually observed in the high dose range, 16/58 (p=0.015). Repeating this analysis for primary malignant lung tumors only in the 19 mg/m^3 dust exposed animals indicates 12/61 tumors observed in the 0-0.74 Gy dose range and 8/58 tumors observed in the 0.74-1.49 Gy dose range, not significantly different (p=0.3). If tumor frequency was directly proportional to dose, 23/58 tumors would be expected in the higher dose range, significantly different from the 12/61 observed in the low dose range (p=0.05) but significantly more than that actually observed in the high dose range (8/58) p=0.01.

The above results indicate that the hypotheses that lung tumor frequency is directly proportional to dose has failed the statistical tests.

The dose rate for each animal was not constant, but depended on the uranium lung burden that accumulated during inhalation exposure. We reanalyzed the data using the uranium lung burden at the end of inhalation as a value representative of the overall dose rate in each animal. The individual lung burdens at the end of inhalation were calculated from the measured burdens at death and the measured rate of decrease in the lung (Figure 6). Figure 9A shows the distribution of uranium lung burdens at the end of inhalation for all animals in both exposed groups. Figure 9B shows the frequency of primary malignant lung tumors within each increment of lung burden, for the two exposed groups combined. The data suggest that malignant tumor frequency increased with increased lung burden at the end of inhalation, implying that malignant lung tumor risk may increase with increasing dose rate.

While Figure 9A suggests a trend of increasing risk of malignant lung tumor formation with increasing lung burden, it does not suggest that risk doubles with a doubling of lung burden as did the data for dose (Table 5). However given the number of animals available and their distribution (Figure 9A), the magnitude of the uncertainties preclude statistical proof of this association of increasing risk with increasing dose rate (lung burden at the end of inhalation).

Figure 10A shows that for the same absolute frequency of lung tumors, the latent period was apparently longer in the group exposed to the low aerosol dust concentration. However, when plotted on a relative basis (Figure 10B) the latent period for the two groups is the same. This result suggests that the latent period for the appearance of lung tumors in the two groups is actually the same, and that only the risk (i.e. tumor number) is higher in the group exposed to the high dust concentration.

Figure 7

The calculated average absorbed dose to the lungs as a function of time for the high and low level dust inhalation groups. Those animals with primary malignant lung tumors are shown as data points at their time of death. Note that the figure does not represent the actual lung dose in these particular animals.

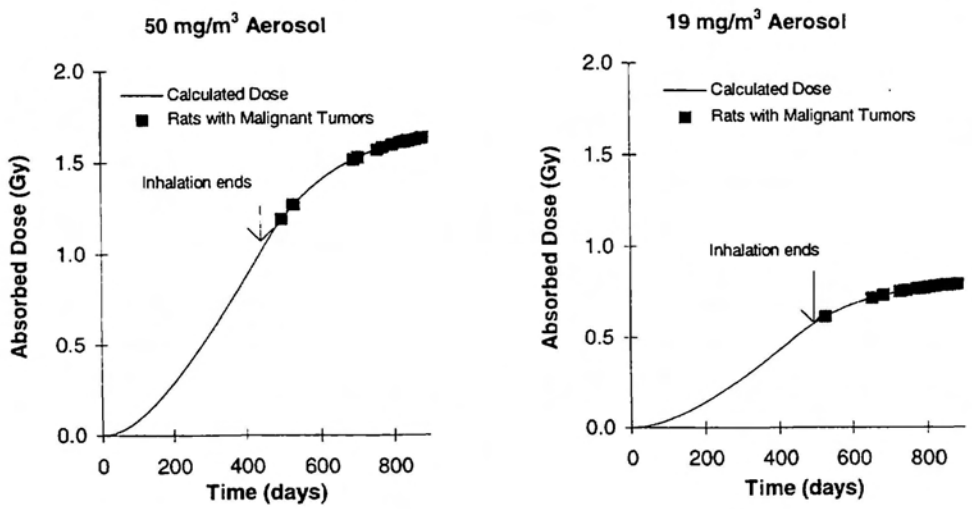


Figure 8

Lung dose distribution and frequency of animals with primary malignant and non-malignant lung tumors. Data for all animals and those with lung tumors. The two exposure groups are shown separately.

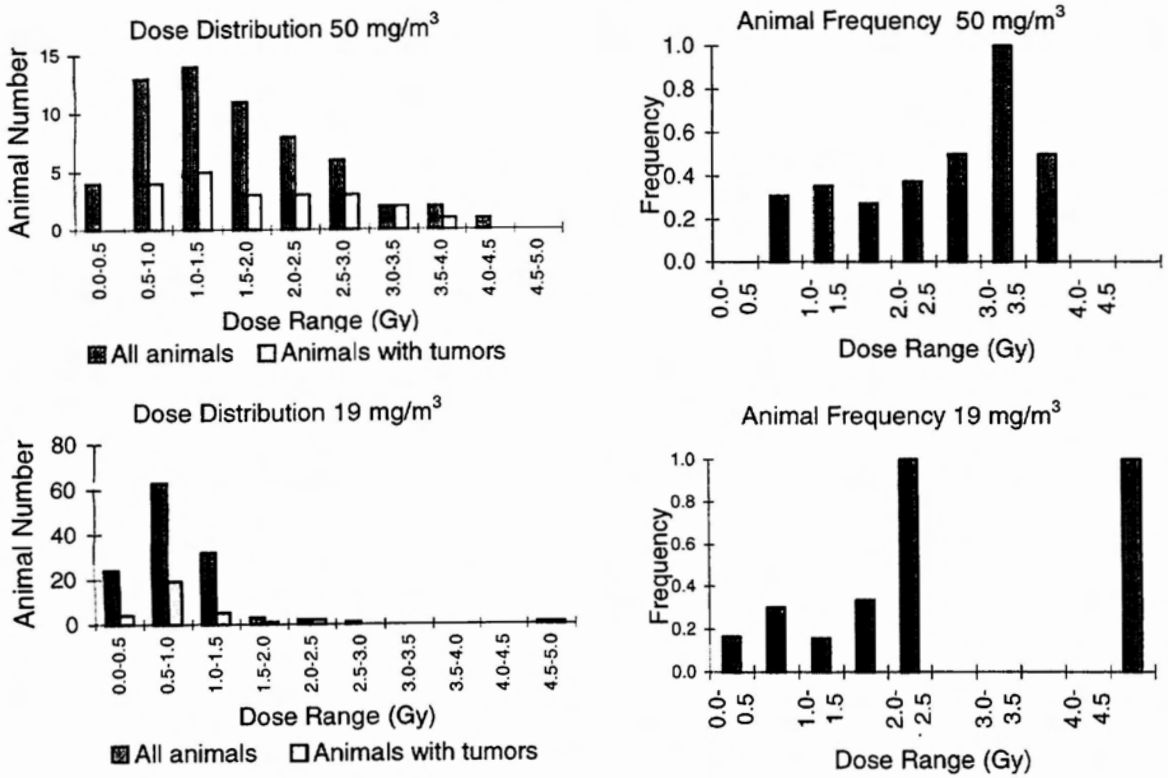


Figure 9

Distribution of uranium lung burdens at the end of inhalation for all animals in both exposure groups (Panel A). The frequency of primary malignant lung tumors for the two exposure groups (Panel B).

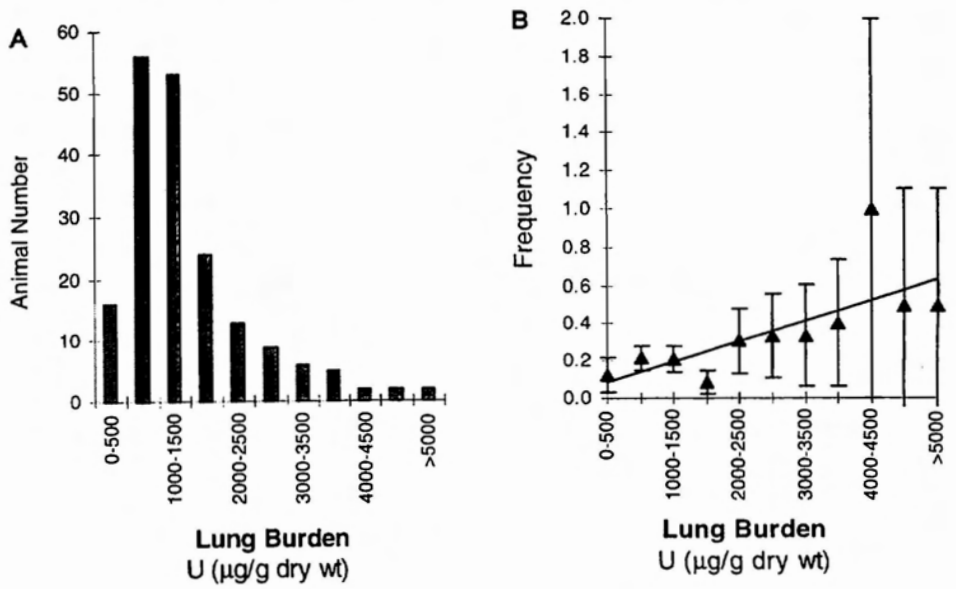
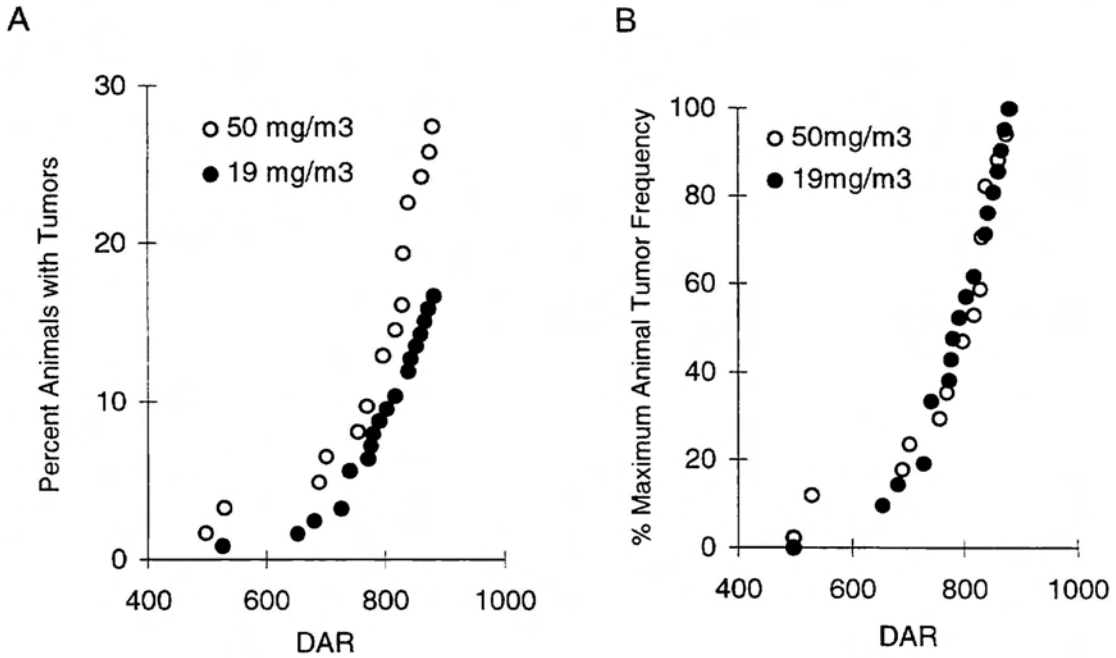


Figure 10

Lung tumor latency period. The two groups are shown separately. A. Latency period plotted as a function of the absolute tumor frequency. B. Latency period plotted as a function of the relative tumor frequency.



DISCUSSION

Lung Uranium Clearance

At the end of inhalation exposure, animals were allowed to live for the remainder of their natural lifespan, at which time the lung uranium burden was measured. This data for all animals is shown on Figure 5. The data shows that uranium clearance rates were exponential and were the same in both groups, indicating that clearance rate is independent of original lung burden. The data also indicates that at the end of dust inhalation exposure the average lung burden of the animals exposed to the high aerosol concentration was 2.06 times greater than the animals exposed to the low dust aerosol concentration. The calculated distribution of lung burdens in all animals at the end of dust inhalation is shown in Figure 9 and indicates a distribution skewed toward higher burdens. However, most animals had lung burdens in the 0.5 to 1.5 mg/g wet wt. These values of lung clearance rate, individual animal lung burden at the end of dust inhalation, individual inhalation time and time of death were used to calculate the lung dose of each animal.

Bronchial Lymph Nodes

Ore dust particles were transferred from lungs to bronchial lymph nodes. At longer times, about 400 days, after the end of dust inhalation, the lymph nodes typically displayed specific uranium burdens (Figure 6) up to 60 fold greater than the lungs in the same animal, at similar times after the end of inhalation. However, it is also evident that this ratio is extremely variable. As such, it appears that predictions of dose (and therefore risk) to lymph nodes based on lung burdens would result in large uncertainties. No bronchial lymph node tumors were detected, suggesting that the risk of tumor formation in lymph nodes is significantly less than in lungs.

Lung Tumors

The frequency of primary malignant lung tumors in the two groups of animals exposed to ore dust was significantly greater than the control group ($p < 0.002$ and $< 2 \times 10^{-5}$) and for the high concentration of ore dust group, the frequency (.328) was significantly greater (ratio 1.87, $p = 0.05$) than that seen in the animals exposed to the low concentration (0.175) (Table 1). Corrected for control tumor type and frequency (sarcoma, 0.016) these frequencies become 0.312 and 0.175 respectively for a ratio of 1.78 ($p < 0.05$). This result can be compared to the relative lung burdens in the two groups (1907 versus 925 μg/g) giving a ratio of 2.06 (Figure 6). It can also be compared to the average lung dose of all animals in the two groups, 1.64 and 0.87 Gy respectively giving a relative ratio of 1.89, or to the average lung dose of only those animals with lung tumors 1.90 and 0.98 Gy respectively for a relative ratio of 1.94. By any test, the risk of lung tumors appears to reflect the relative lung burden in the two groups and indicates that inhalation of uranium ore dust constitutes a risk for development of malignant tumors in the lung. Using the corrected frequencies of 0.312 and 0.175 with the corresponding group average doses of 1.64 and 0.87, the calculated risks of malignant lung tumor formation are 0.190 and 0.201 tumors/animal/Gy respectively. Non-malignant tumors in the lung were also significantly ($p=0.03$) elevated in the two groups breathing the ore dust. However, both groups developed a similar frequency (0.13) suggesting a less direct relationship with lung burden.

Dose, Dose Rate and Risk

The dose to the lungs of each animal was individually calculated based on the measured lung burden (μgU/g lung tissue) at the time of death. The maximum specific lung burden was fixed at the end of inhalation and then declined at the rate shown in Figure 6, and this rate was assumed to be representative of each animal. On average, the animals exposed to the high and low dust aerosol concentrations received total doses of 1.64 Gy and 0.87 Gy respectively. When only animals with tumors were examined the average doses were 1.90 and 0.98 Gy respectively, indicating that tumorgenesis was, on average, associated with the higher doses. Examination of Figure 7 indicates that tumors generally appeared late in the lifespan of the animals, while many non-tumor bearing animals died before this time (Figure 6). This indicates therefore that the higher dose in tumor bearing animals was generally related to their longer average lifespan.

While the risk of lung tumor formation appeared to be related to lung burden and therefore dose a further analysis (Figure 8) indicated this was not likely correct. When the spectrum of dose was divided into 0.5 Gy increments and the tumor frequency in each dose increment calculated, the frequency (risk) appeared to be nearly constant with dose. While the primary malignant tumor frequency in the group exposed to the high dust aerosol concentration (.328) was significantly greater ($p=0.05$) than that in the group exposed to the low dust aerosol concentration (0.175), there was no significant increase with dose up to 1.5 Gy for primary malignant tumors, all primary lung tumors or animals with lung tumors in either the 19 mg/m^3 group alone or when both the 50 and 19 mg/m^3 groups were combined. Statistical tests of the hypothesis that the risk of the above end points doubles with dose (0.2 tumors/animals/Gy, Table 5) showed that such a result could have been detected but was not, and the actual result was significantly less than that predicted by the hypotheses. These results strongly suggest that the risk of tumorgenesis from chronic lung irradiation by inhaled particulate uranium ore dust is not directly proportional to dose. It remains a possibility that the risk increases with dose, but at a rate which is not detectable with the number of animals used in this experiment. It is also possible that risk may be related to dose rate. Since dose rate was variable but depended on lung burden, the lung burden at the end of dust inhalation was calculated and used as a measure of the dose rate. Figure 9B shows that the risk of lung cancer increased as the dose rate (lung burden) increased, although given the number of animals in the study and the distribution of lung burdens the magnitude of the errors preclude statistical significance. This positive correlation with a measure of dose rate and the lack of direct correlation with dose (Figure 8) suggests that the dose rate may be more important than total dose in influencing the risk of lung cancer induced by inhaled uranium ore dust. A similar result has been reported in rats exposed to radon (6). It is conceivable that the influence of dose rate in these chronically exposed animals represents a tumor promotion effect. Since tumorgenesis is a multistep process characterized by increasing genomic instability, the rates of change in genomic instability could be influenced by the rate of high LET exposure in the cells already initiated, i.e. in those already having the first tumor initiating mutation. Figure 10B indicates a similar latent period for the appearance of malignant lung tumors in the animals exposed to the lower dust aerosol concentration as compared to those exposed to the high aerosol

concentration when latency was measured on a relative basis. When plotted on an absolute basis, Figure 10A reflects the increased risk of lung tumor formation in the animals exposed to the high dust aerosol.

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