BIOLOGICAL EFFECTS OF DAILY INHALATION OF RADON AND ITS SHORT-LIVED DAUGHTERS IN EXPERIMENTAL ANIMALS*

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INTRODUCTION

Initial studies in our laboratory with hamsters exposed for their lifespan to 30 Working Levels (WL)** radon daughters produced only slight pulmonary response. Similar levels have been found in some areas of operating uranium mines. Lifespan exposures of hamsters to 600 WL radon daughters with uranium ore dust caused pulmonary lesions, including vesicular emphysema, fibrosis, metaplasia, and adenomatous lesions with anaplasia. A subsequent experiment involved lifespan exposures of hamsters to 1200 WL radon daughters, diesel engine exhaust, and uranium ore dust. These hamsters showed more extensive and severe pulmonary lesions, with accelerated development of cuboidal and squamous metaplasia of the bronchial epithelium and the appearance of squamous tumors.

Earlier studies (Morken and Scott, 1966) with mice exposed to 1750 WL radon daughters on room air dust (150 hrs/wk), showed significant reduction of lifespan. Workers in France (Perraud et al., 1972) have reported peripheral tumors in the lungs of SPF rats after 500 hours of exposures to

*This paper is based on research performed under U.S. Atomic Energy Commission Contract AT(45-1)-1830.

**A Working Level (WL) is defined as any combination of short-lived radon daughters in one liter of air that will result in the ultimate emission of 1.3×10^{-5} Mev of alpha energy from radioactive decay.

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DISCLAIMER

approximately 1 μCi per liter of radon, but interpretation of these results is extremely difficult because the levels of radon daughters were not described, and no correlation to human exposure histories based on Working Levels (radon daughter levels) was possible.

This paper presents results of a pilot study that we conducted to correlate these results obtained in three different laboratories, to provide direct interspecies comparison of the effects of exposure to identical aerosols, and to determine if there exists an increased sensitivity or resistance of one or more of these species of rodents. We prepared systems for simultaneous exposures of mice, hamsters and SPF rats in the same chambers to high levels of radon daughters, with and without concomitant exposure to uranium ore dust (carnotite). Exposure of the three species in these initial studies were made at levels of radon daughters calculated to induce tumor production or acute death.

METHODS

The animals used in this study were SPF Wistar rats, Syrian-Golden hamsters and C57 Bl/6J mice. They were all males and approximately 100 days old at the start of exposures. The animals were individually housed in two types of compartmented, stainless steel mesh cages. There were 16 compartments for mice or hamsters in one type of cage, and 8 compartments for rats in the other type. Two cages of rats, one of hamsters, and one of mice were in each of the two exposure chambers. Ten control animals of each species were individually housed in the exposure room, but not in a chamber. Food and water were available to the animals at all times. The experimental design is shown in Table 1.

The two exposure chambers were nearly cubical with volumes of 1700 liters. As seen in Figure 1, the animal cages were supported on racks that positioned the bottoms of the cages 81 centimeters below the chamber inlets for radon or radon and uranium ore dust. Unfiltered room air entered at the top of each chamber, passed through the chambers, and left via outlets centered 20 cm above the chamber bottoms. These outlet air streams passed through valved flow meters and absolute filters, and were then discharged into a high flow, low vacuum system whose outlet was diluted in the building exhaust stack prior to discharge to the atmosphere.

A separate radon generator was used for each chamber. Saturated air bubbling through acid solutions of radium chloride at a rate of 150 ml/min swept radon from the generators through traps to remove acid vapor that may have been carried out of the generators. This radon laden air was filtered just prior to introduction into the chambers. The radon entered the center top of the chambers where it mixed with incoming room air in the case of Chamber 1, and with incoming room air and uranium ore dust in Chamber 2. The uranium ore dust was introduced into Chamber 2 by means of a Wright Dust Feed Mechanism mounted on top of the chamber. Ore dust with a count median diameter of 0.24 μm was maintained at a concentration of 18 ± 4 μg per liter in the chamber during exposure periods. This level was selected to coincide with those used in our previous studies, serving to lower the unattached radon daughters to only a few percent.

Initial measurements in the empty chambers indicated that nearly twice as much radon would be required in Chamber 1 without ore dust in order to attain approximately equal Working Levels of radon daughters in the two chambers. This was felt to be due to greater losses of radon daughters to the walls of Chamber 1 than to those of Chamber 2 which contained the ore dust. As a consequence, one radon generator was loaded with approximately 1.3 Ci of radium and the other with approximately 0.7 Ci. With a total air flow of 35 liters per minute through each chamber, these levels of radium led to radon concentrations of 4.8 μCi/liter in Chamber 1 and 2.5 μCi/liter in Chamber 2.

Radon levels in the chambers were continuously monitored during exposures, and each chamber was sampled at least once during each exposure period for measurement of radon daughter concentrations, using a two channel alpha analyzer employing a solid state surface barrier detector. Concentrations of condensation nuclei were measured one or more times during each exposure period using a Gardner nuclei counter. These concentrations ranged from 2,000 to 24,000 per cc in Chamber 1 and from 57,000 to 130,000 per cc in Chamber 2.

When animals were first introduced into the chambers, measured levels of radon daughters and condensation nuclei showed little change in the chamber containing the aerosol of radon daughters with uranium ore dust (Chamber 2). However, in Chamber 1, without uranium ore dust, radon daughters were found to be reduced by a factor of at least four from levels observed in the empty chamber. Condensation nuclei were reduced from approximately 19,000 to 2,000 per cc. The radon level in the chamber remained at approximately 4.8 μCi per liter, however, so it was decided to continue the animal exposures with unequal Working Levels of radon daughters in the two chambers.

The animals were exposed in the two chambers for approximately 90 hours per week, in two continuous 45 hour periods. All animals were weighed biweekly and exposures were continued until all the animals had died or had been sacrificed when moribund. At death the nose, trachea, lungs, liver, spleen and kidneys of each animal were retained for histopathological investigation.

RESULTS

Table 2 shows the mean weights plus or minus the standard error of the means for the mice in the control and in the two exposure groups at various times after the start of exposures. As early as 55 days after the start of exposures, both exposure groups showed significant weight loss; i.e. 22% reduction from control animal weights. Table 3 shows the same data for hamsters. In this species, the reduction in weight did not occur as soon after the start of exposures but was just as dramatic as exposures progressed. As observed in the mice, the weights of the hamsters exposed to radon daughters with uranium ore dust show greater reduction from control hamster weights than do those exposed to radon daughters without ore dust. The weight data of exposed rats shown in Table 4 tell the same story. Significant reductions from control rat weights were seen in both exposure groups by 55 days after the start of exposures, with the greater weight reductions occuring in the rats exposed to radon daughters with ore dust. It is evident that all three species showed a marked weight loss in both exposure groups, with those exposed to radon daughters plus uranium ore dust showing more drastic effects in all three species.

Figure 2 shows the survival curves for each of the species exposed in Chamber 1, together with a curve showing the cummulative Working Level Hours (CWLH) of radon daughters to which they were exposed. The increase in the slope of the CWLH curve is a result of the radon daughter concentrations increasing as the number of animals in the chamber decreases. The radon level in the chamber remained at ∿ 4.8 μCi/liter. It can be seen that the first death, a mouse, occurred only 25 days after the start of exposures. The last animal to die, a hamster, had been exposed to over 8 million CWLH of radon daughters during the 178 days it survived after the start of exposures. The mice died earlier and at a faster rate than did rats and hamsters, but as the exposures continued the percent surviving showed little difference among the three species.

Figure 3 shows the survival curves for each of the species exposed to radon daughters with uranium ore dust in Chamber 2. The nearly constant slope of the CWLH curve shows that the concentrations of radon daughters in the chamber were little effected by the number of animals in the chamber. Half of the mice in this group had died by 30 days after the start of exposures, compared to nearly 120 days before 1/2 of the rats and hamsters were dead. The first rat died 52 days after the start of exposures, at which time it had been exposed to ∿ 1.6 million CWLH of radon daughters. The last hamster to die had been exposed to 7 million CWLH during the 144 days it survived after the start of exposures.

Table 5 shows the geometric mean survival times, and their 95% confidence limits, for the rats, hamsters and mice in each of the two exposure chambers. All three species show significantly shorter survival times following exposure to radon daughters with ore dust than they do following exposure to radon daughters without ore dust.

Histopathological examination of tissues from the rats, hamsters, and mice reveals varying degrees of radiation pneumonitis due to inhalation of radon daughters. The characteristic septal fibrosis, alveolar lining cells sloughed into alveolar lumena, and invasion by macrophages as well as the atypical nuclei of alveolar septal cells are illustrated in Figure 4. In animals exposed to uranium ore dust with radon daughters, one sees a pulmonary response to particulate material diagnosed as uranium ore pneumoconiosis. Macrophages laden with uranium ore dust, and the septal fibrosis characteristic of this lesion are shown in Figure 5. Bronchiolar epithelial hyperplasia was commonly seen in the lungs of animals exposed to radon daughters.

Mice and rats seem less prone to the pulmonary lesions than hamsters but are afflicted with quite severe lesions of the upper respiratory tract. Severe suppurative rhinitis and squamous metaplasia of nasal epithelium (Figure 6) was a common observation in mice and rats of this experiment. Dense exudate containing many neutrophils was seen in the nasal passages. Suppurative laryngitis and bronchitis were frequent observations in all three species, but most severely in rats (Figure 7). Suppurative tracheitis was also quite common and, in at least two mice, the tracheal epithelium had undergone a keratinizing squamous metaplasia (Figure 8). The squamous nature of the ordinarily columnar epithelial cells and the keratin layer are quite atypical of the trachea.

Hamsters also had a high incidence of rhinitis and laryngitis, but had a higher incidence of pulmonary consolidation due to septal cell hyperplasia, interstitial pneumonitis, and macrophage proliferation. An additional lesion found with approximately equal frequency in rats and hamsters was an adenomatoid metaplasia of alveolar epithelium. This lesion was found in small foci in rat lungs but involved large areas in hamster lungs. In two hamsters exposed to radon daughters without ore dust, the lesion had undergone squamous metaplasia (Figure 9). In view of previous observations in hamsters, this lesion is considered pre-malignant; i.e. a stage just previous to epidermoid carcinoma.

In all three species, the major differences between exposure to radon daughters only, and radon daughters with uranium ore seem to be the increased septal fibrosis and macrophage proliferation in the latter. The uranium ore dust particles also seem to cause a slightly higher incidence of pulmonary emphysema and septal cell hyperplasia than found in animals exposed to radon daughters only.

DISCUSSION

This pilot study of the effects of inhalation of high levels of radon daughters with and without concomitant uranium ore dust by three rodent species has shown several interesting results.

Exposures for 90 hours per week to radon daughters ranging from 2000 to 8500 WL, with and without 18 μg per liter uranium ore dust, caused marked life-shortening in all three species. Marked reduction in body weights occurred in all three species, with weight losses of 30-50% of control animal values in all species after 3 1/2 months of exposures. Mice exposed to radon daughters and ore dust were particularly susceptible in terms of mortality, although the lungs of these animals showed very little pathological change.

Classical radiation pneumonitis with alveolar septal fibrosis and occasional bronchiolar epithelial hyperplasia were the predominant deep lung lesions seen in all species. In contrast to hamsters exposed 30 hours per week to 1200 WL of radon daughters and uranium ore dust, proportionately more of the pathology was seen in the upper respiratory tracts of the hamsters in the present study. The contrast between markedly affected trachea and major bronchi vs. relatively little effects in deep lung was most evident in rats. Findings of severe suppurative laryngitis and bronchitis were frequent in rats, and may have been an important contributing factor to their death. These findings dictate further studies involving sacrifice and radioactivity analyses of tracheal and lung tissues to determine relative absorbed radiation doses at these sites for correlation with developing degenerative and proliferation changes of the respiratory tract in each species.

Our next experiment involves exposures of rats, hamsters and mice during five, 6 hour periods per week. This lowered exposure rate may allow the animals to live long enough for proliferative epithelial changes to progress beyond the stage of squamous metaplasia to possible invasive tumor formation.

REFERENCES

Morken, D. A. and J. K. Scott, 1966. The Effects on Mice of Continued Exposure to Radon and Its Decay Products on Dust. UR-669. University of Rochester, New York.

Perraud, R., J. Chameaud and J. Lafuma, 1972. Cancer Broncho-pulmonaire Experimental du Rat par Inhalation de Radon. Extraits Francais Med Chir Thor, 26, 25-41.

FIGURE CAPTIONS

Figure 1: View of multi-species exposure chambers and glovebox containing the radon generators.

Figure 2: Survival curves of rats, hamsters, and mice exposed to radon daughters without concomitant uranium ore dust, together with a curve showing the Cummulative Working Level Hours of radon daughters to which they were exposed.

Figure 3: Survival curves of rats, hamsters, and mice exposed to radon daughters with concomitant uranium ore dust, together with a curve showing the Cummulative Working Level Hours of radon daughters to which they were exposed.

Figure 4: Radiation pneumonitis in hamster lung showing characteristic septal fibrosis, alveolar lining cells sloughed into alveolar lumena, invasion by macrophages, and atypical nuclei of alveolar septal cells. (H&E, 660X).

Figure 5: Uranium ore pneumoconiosis in a hamster lung showing ore dust laden macrophages and septal fibrosis (H&E, 660X).

Figure 6: Suppurative rhinitis and squamous metaplasia of nasal epithelium seen in a mouse exposed to radon daughters and uranium ore dust (H&E, 415X)

Figure 7: Suppurative laryngitis and bronchitis in a hamster (H&E, 660X).

Figure 8: Suppurative tracheitis in a mouse showing keratinizing squamous metaplasia of tracheal epithelium (H&E, 120X; Insert X540).

Figure 9: Squamous metaplasia of alveolar epithelium from the lung of a hamster (H&E, 330X).

Table 1

EXPERIMENTAL DESIGN

GROUP	NUMBER OF ANIMALS	EXPOSURE (90 HOURS PER WEEK)
1	16 of Each Species	2000-8500 WL Radon Daughters
2	16 of Each Species	6000-7500 WL Radon Daughters with Uranium Ore Dust (18 mg/m^3)
3	10 of Each Species	Controls (Not Housed in Chamber)

Table 2

MOUSE WEIGHTS
(Mean ± S.E. in Grams)

Days Since Start Of Exposures	Radon Daughters	Daughters + Ore	Controls
0	27 ± 1	27 ± 1	28 ± 1
55	25 ± 1	25 ± 1	32 ± 1
111	23 ± 1	21	34 ± 2
132	19 ± 1	---	34 ± 2

Table 3

HAMSTER WEIGHTS
(Mean ± S.E. in Grams)

Days Since Start Of Exposure	Radon Daughters	Daughters + Ore	Controls
0	117 ± 3	120 ± 3	124 ± 3
55	121 ± 3	119 ± 4	128 ± 4
111	106 ± 4	99 ± 12	135 ± 3
132	93 ± 8	68	136 ± 3

Table 4

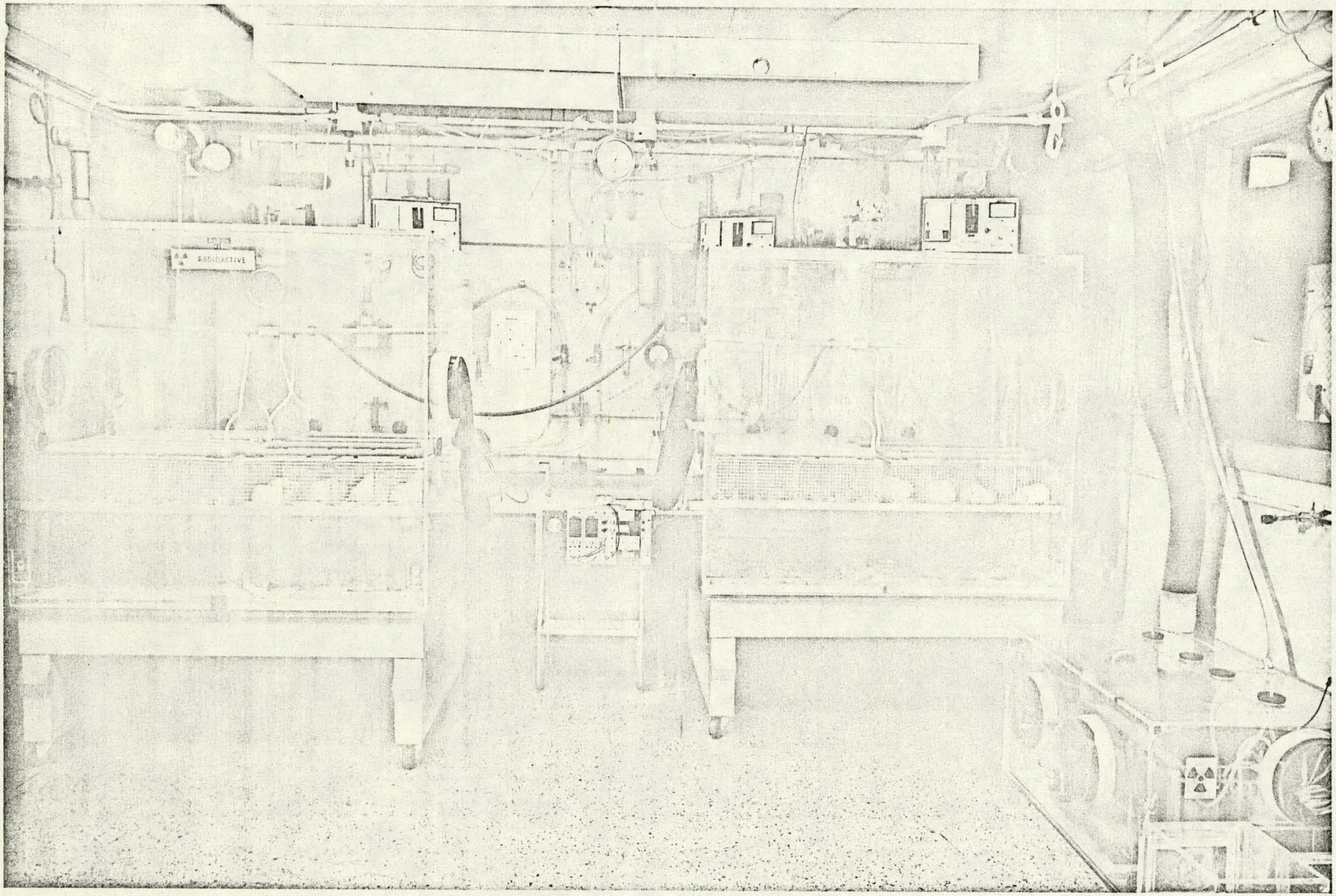
RAT WEIGHTS
(Mean ± S.E. in Grams)

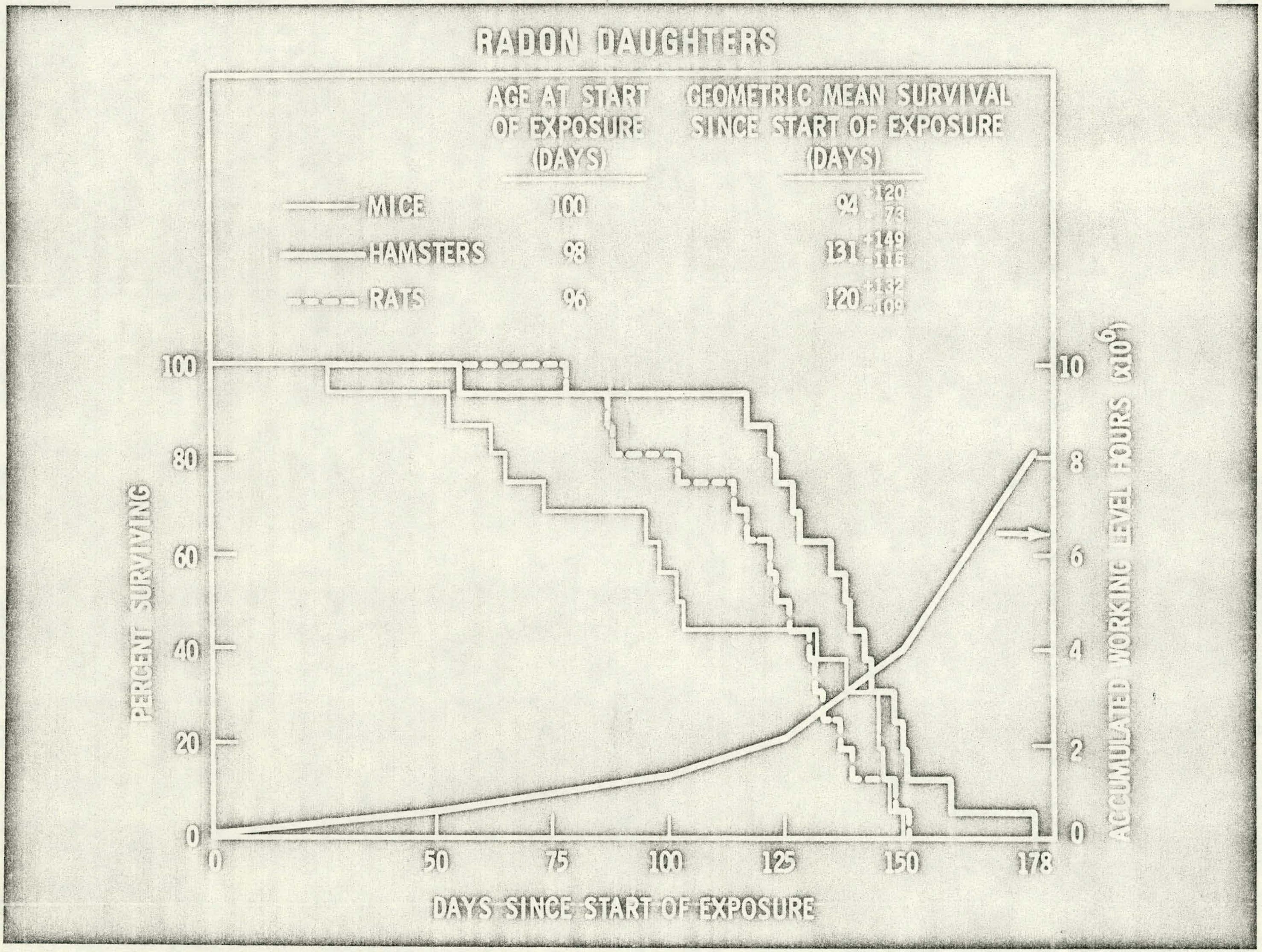
Days Since Start Of Exposures	Radon Daughters	Daughters + Ore	Controls
0	466 ± 9	442 ± 7	470 ± 10
55	479 ± 9	439 ± 10	544 ± 12
111	407 ± 17	364 ± 26	600 ± 14
132	401 ± 23	321	616 ± 15

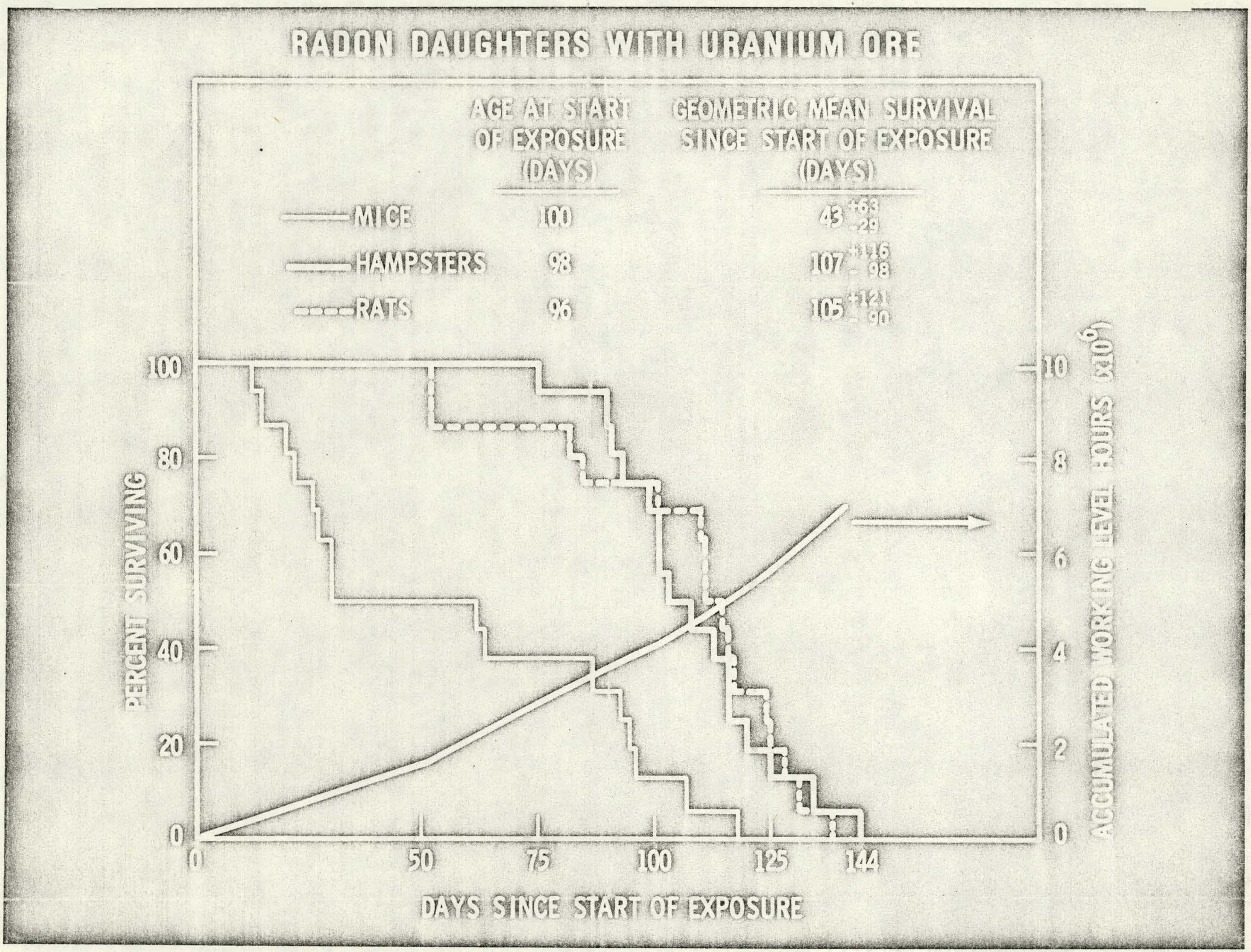
Table 5

GEOMETRIC MEAN SURVIVAL TIMES
(Days Since Start Of Exposures
With 95% Confidence Limits)

	Mice	Hamsters	Rats
Radon Daughters	94_{73}^{120}	131_{115}^{149}	120_{109}^{132}
Daughters + Ore	43_{29}^{63}	107_{98}^{116}	105_{90}^{121}


RADIOACTIVE


RADON DAUGHTERS
AGE AT START OF EXPOSURE (DAYS)
GEOMETRIC MEAN SURVIVAL SINCE START OF EXPOSURE (DAYS)
MICE 100 94 +120 -73
HAMSTERS 98 131 +149 -116
RATS 96 120 +132 -109
PERCENT SURVIVING
0
20
40
60
80
100
ACCUMULATED WORKING LEVEL HOURS (x10^6)
0
2
4
6
8
10
0
50
75
100
125
150
178
DAYS SINCE START OF EXPOSURE


RADON DAUGHTERS WITH URANIUM ORE
AGE AT START OF EXPOSURE (DAYS)
GEOMETRIC MEAN SURVIVAL SINCE START OF EXPOSURE (DAYS)
MICE 100 43 +63 -29
HAMPSTERS 98 107 +116 -98
RATS 96 105 +121 -90
PERCENT SURVIVING
ACCUMULATED WORKING LEVEL HOURS (x10^6)
DAYS SINCE START OF EXPOSURE
0
20
40
60
80
100
0
2
4
6
8
10
0
50
75
100
125
144