EVIDENCE OF LUNG CANCER RISK FROM ANIMAL STUDIES

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Human epidemiological data provide the most important basis for assessing risks of radon exposures. However, additional insight into the nature of exposure-response relationships is provided by animal experimentation and dosimetric determinations. Underground miners' exposure levels and those of other radon-exposed populations are uncertain, and animals can serve as surrogates in studying components of radon-exposure problems. For example, they can be exposed to well-defined levels of radon and associated pollutants, can be sacrificed for the study of developing lesions, or can be kept for their lifespans to determine late effects, such as cancer. They can also be exposed to radon levels spanning the range from environmental radon to the highest levels found in mines, thus allowing us to determine the radon exposure-response relationship.

Animal studies have now been conducted for more than 50 years to examine the levels of pollutants in underground mines that were responsible for the respiratory effects observed among miners. This work has emphasized respiratory cancer and the interaction of radon

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aerosols was shown to be an important determinant of the dose to airway epithelium. They also showed that more than 95% of the dose to airway epithelium was due to the short-lived radon daughters ^{218}Po and ^{214}Po, rather than to the parent radon gas[4]. The influence of the UR radon-daughter carrier aerosol (laboratory room air) on the pathological results of these experiments is uncertain, but it may have led to more rapid solubilization of the daughters into blood and a resulting decrease in irritation or fibrosis in comparison with that caused by ore-dust/silica aerosols.

Averaged alpha-radiation dose rates in sacrificed mice exposed for 150 hours/week (for periods ranging from 8 weeks to life) to 2000-working level (WL)* potential alpha-energy concentration (~1800 working level month [WLM]/week) were estimated to be 5, 18, 2, 60 and 280 rad/week** in whole body, kidney, liver, gastrointestinal (GI) tract/stomach and contents, and lungs/trachea/bronchi, respectively[5]. Dose to bronchial tissue may have been five to ten times that to whole lung, or as much as 2800 rad/week. In related dose experiments, averaged measured doses to whole lungs of dogs were 0.17 rad/WLM, and tracheal and bifurcation doses were 5 rad/WLM. In summary, therefore, averaged measured doses to whole lungs of animals were on the order of 0.2 rad/WLM (2 mGy WLM^{-1}) with an estimated range, in nine dogs, of 0.1 to 0.8 rad/WLM (1 to 8 mGy WLM^{-1}).

Radon Inhalation Studies at COGEMA

These studies employed adult male SPF Sprague-Dawley rats and exposures to radon alone as well as to radon along with decay products attached to ambient (outdoor) aerosols. Radon concentrations ranged from 750 to 1250 nCi/L (3×10^7 to 5×10^7 Bq m^{-3}); radon-daughter equilibrium factors ranged from about 1 to 100%. Cumulative radon-daughter exposures ranged from 20 to > 10,000 WLM; exposure rates varied from less than 10 to several hundred WLM/week, the majority between 200 and 400 WLM/week.

Co-exposure materials included stable cerium hydroxide, uranium ore dust and cigarette smoke, to determine whether the presence of dusts or cigarette smoke altered the carcinogenic effect of radon-decay prod-

* Working level is defined as any combination of the short-lived radon-decay products in 1 L of air that will result in the ultimate emission of 1.3×10^5 MeV of potential alpha-energy. Thus, WL is a unit of measure of potential alpha-energy concentration associated with short-lived radon-decay products. Working level month is an exposure equivalent to 170 hours at a 1-WL concentration.
** 1 rad = 100 erg/g; the SI unit for absorbed dose is the gray (Gy), where 1 Gy = 100 rad.

with other agents, such as ore dust, diesel-engine exhaust fumes and cigarette smoke. Many of the initial studies, however, were concerned with early effects or short-term pathological changes. These were reviewed in the final report of Subgroup I.B., Interagency Uranium Mining Radiation Review Group[1]. That report concluded that experimental work prior to the 1970s had not shown that it was possible to produce pulmonary carcinomas in animals in a systematic way from controlled exposures to radon. The exposures were primarily based on radon-gas concentrations rather than radon-daughter concentrations, which have been shown to contribute the greatest radiation dose to the lung[2]. The more recent data on radon-daughter inhalation exposures were provided by two American research centers, The University of Rochester (UR) and the Pacific Northwest Laboratory (PNL), and by the Compagnie Générale des Matières Nucleaires (COGEMA) laboratory in France. Approximately 2,000 mice, 100 rats and 80 dogs were employed in the completed UR studies, begun in the mid 1950s; 800 hamsters, 5,000 rats and 100 dogs in the ongoing PNL studies, begun in the late 1960s; and 10,000 rats in the ongoing COGEMA studies, also begun in the late 1960s.

More complete updated biological effects data resulting from chronic radon-daughter inhalation exposures of mice, hamsters, rats and beagle dogs have been ppesented by Cross[3], and the reader is referred to this reference for a more complete bibliography. Emphasis here is placed on the carcinogenic effects of radon-decay product exposure, including the influences of radon-daughter exposure rate, unattached fraction and disequilibrium, and co-exposures to other pollutants. Plausible values for the radon (radon-daughter) lifetime lung-cancer risk coefficients are also provided.

Radon Inhalation Studies at UR

These pioneering studies employed radon alone as well as radon along with radon-decay products attached to room-dust aerosols. Very few permanent pathological effects were produced in these experiments, most likely as a result of the combination of high-level exposures, with consequent lifespan-shortening, and early termination of experiments, which precluded further development of lesions. The most noteworthy accomplishment of the UR experiments was the establishment of exposure-to-dose values in whole lungs, portions of lungs and in other organs. The degree of attachment of radon daughters to carrier

sures. The risk decreases at higher WLM exposures due to lifespan shortening and appears to plateau at about 6 to 8 $\times$ 10^{-4}/WLM at 20- to 50-WLM exposures. No evidence of a threshold below 20-WLM exposures could be inferred[7].

Radon Inhalation Studies at PNL

These studies employed adult male Syrian Golden hamsters and SPF Wistar rats, as well as both adult male and female beagle dogs. Exposures were to mixed aerosols of radon, radon daughters, uranium ore dust, diesel-engine exhaust and cigarette smoke. The carrier aerosol for the radon daughters was generally uranium ore dust, except in experiments with added diesel-engine exhaust or when the decay products were attached to room-air particles. Radon concentrations ranged from 5 to 300 nCi/L (2 $\times$ 10^5 to 11 $\times$ 10^6 Bq m^{-3}); decay product equilibrium factors ranged from about 10 to 60%. Unattached ^{218}Po daughters ranged from 1.3 to 9.5% of the radon gas concentrations, and radon-daughter exposures ranged from 20 to > 10,000 WLM.

Ore-dust aerosols had mass median aerodynamic diameters (MMAD) on the order of 1.0 μm, with a geometric standard deviation (GSD) of about 2.0; the overall average AMAD of the radon-daughter aerosols was about 0.5 μm, with an overall average GSD of about 2.0. The ore dust ranged between 2 and 4% U_3O_8 and about 80% SiO_2 by weight. Cigarette-smoke exposures of dogs were by mouth- and nose-only and varied, among animals, between 10 and 20 cigarettes/day. These exposures were given alternately (but on the same day) with exposures to radon-decay products. Diesel-engine exhaust exposure of hamsters contained 50 ppm CO; 5 ppm NO_2, and < 1 ppm SO_2 and aliphatic aldehyde.

Simultaneous or same-day-exposure to radon daughters and upanium ore dust, diesel-engine exhaust or cigarette smoke increased the incidence of preneoplastic lesions but, with the exception of cigarette smoke, did not seem to affect the incidence of lung tumors in the PNL experiments[8].

Dog Studies

Lung-tumor incidence decreased when dogs received alternate (but same-day) exposures to radon-decay products and cigarette smoke. The overall incidence of tumors primary to the lung was 21% at about

ucts[6]. Associated ore-dust exposures appeared to have little influence on the tumorigenic process, although the number of animals used was too small to permit a firm conclusion. Cigarette smoke was found to be cocarcinogenic with radon daughters when exposure to smoke followed completion of exposures to the daughters. This effect was not observed, however, when smoking preceded the radon-daughter exposure. Tumor histology was not altered by cigarette smoking.

To date, the COGEMA studies have produced more than 800 lung cancers in a total of 10,000 control and exposed rats. About 15% of the animals had adenomas only. Tumor incidence depended on both exposure rate and cumulative potential alpha-energy exposures. Tumor latency period was found to increase with decrease in cumulative potential alpha-energy exposure.

Various malignant lung-tumor types were found, often in the same animal. These included epidermoid carcinomas, not always clearly differentiated, often keratinized or necrosed, and occasionally extending into the mediastinum; bronchiolar adenocarcinomas, sometimes mucus-producing, containing numerous cellular anomalies, characterized by a high number of mitoses and invasion of other lung lobes, although seldom metastatic; and bronchioloalveolar adenocarcinomas exhibiting few mitoses but frequently invading the mediastinum, diaphragm, and thoracic wall. Extrathoracic tumors were either rare, not studied, or not generally reported in the COGEMA experiments. Lifespan-shortening was generally not significant below about 5000 WLM. Lung tumors occurred between 12 and 24 months after the start of exposures (i.e., middle to late periods in the lifespan of the rat). Lung tumors never exceeded 10 g in weight, seldom metastasized and, when grafted onto other rats, were slow to kill their hosts. The experimenters therefore considered radon-daughter-induced lung tumors in rats to be mostly nonfatal or incidental to the death of the animal. This conclusion was strengthened by results of other experiments, where sacrificed rats were found to have pulmonary tumors weighing up to 25 g, yet did not appear moribund prior to sacrifice.

Besides lung carcinomas, the only two significant types of tumors noted in radon-daughter-exposed rats were cutaneous epitheliomas of the upper lip and cancers of the kidney[6]. The SPF Sprague-Dawley rat is known to be very sensitive to the latter type of tumor.

The derived range in mean lifetime lung-tumor risk coefficient, using the raw data (i.e., uncorrected for time-related factors, and lifespan differences from control animals), is about 1.5 to 7.5 $\times$ 10^{-4}/WLM exposure between approximately 20- and 5000-WLM expo-

13,000 WLM and 71 WLM/week exposures; the incidence was 37% in the group exposed to radon daughters and uranium ore dust, but only 5% in the comparably exposed group that also received cigarette-smoke exposures[9]. The overall incidence of nasal carcinoma was 8%. Lung cancers were about 70% bronchogenic carcinoma and 30% bronchioloalveolar carcinoma, using the convention that epidermoid tumors and mucus-staining adenocarcinomas are bronchogenic carcinomas, while tumors of Clara cell or Type II alveolar cell origin, as well as non-mucus-staining adenocarcinomas, are bronchioloalveolar carcinomas.

Hamster Studies

Exposures of hamsters to mixtures of radon daughters, uranium ore dust and diesel-engine exhaust produced only four squamous carcinomas in 306 radon-daughter-exposed animals (1.3% incidence)[9]. Squamous carcinoma occurred only in association with squamous metaplasia of alveolar epithelium which, in turn, occurred only in hamsters receiving exposure to radon-decay products. Because the Syrian Golden hamster was so highly refractory to carcinoma induction by inhalation exposure, no further radon-related studies were pursued with this animal.

Rat Studies

To date, about 5000 male SPF Wistar rats have served as controls or have been exposed to mixtures of radon daughters, uranium ore dust or cigarette smoke. Most of the cigarette-smoke exposed animals are still alive. The histopathology data are not complete, but general trends can be observed.

The survival times and pathology of lung tumors in PNL radon experiments are similar to those reported by COGEMA[3]. The incidence of adenoma-only tumors was also in general agreement with the French studies, although there were several groups of PNL rats in which the adenoma-only incidence exceeded 15%. However, the distinction between bronchioloalveolar carcinoma and adenoma is not always clear, suggesting that adenomas may merely be smaller tumors that would eventually become bronchioloalveolar carcinomas. This adds weight to the suggestion that all lung neoplasms should be included in a hazard analysis of the data.

Extrathoracic tumors, particularly in the nasal and laryngeal tissues, were found in the PNL experiments. This was true even in dogs with low radon-daughter unattached fractions, but it occurred more fre-

TABLE 1—*Summary of Factors Influencing the Tumorigenic Potential of Radon-Daughter Exposures*[a]

Factor	Tumorigenic Potential[b]
Radon-daughter cumulative exposure	Increases approximately linearly with exposure
Radon-daughter exposure rate	Increases with decrease in exposure rate (approximately 200 to 400% increase from about 500 to 50 WLM/week. The 500-, the 50- and the 5-WLM/week data are not significantly different at approximately 300-WLM exposures.
Radon-daughter unattached fraction	Increases with increase in unattached fraction (approximately 50% increase per WLM exposure from 2 to 10% f_a)[c]
Radon-daughter disequilibrium	Increases with increase in disequilibrium [approximately 30% increase per WLM exposure (borderline significance) from 0.4 to 0.1F][d]
Concomitant exposure to cigarette smoke	Decreases if smoking alternates on same day with radon-daughter exposure Increases if smoking follows cumulative radon-daughter exposures No effect if smoking precedes cumulative radon-daughter exposures

[a] Modified from Table III-2[11]

[b] Data pertain to raw tumor-incidence data uncorrected for time-related factors and lifespan differences from control animals.

[c] f_a is the percentage of ^{218}Po that is unattached. When expressed as percentage of radon concentrations, they are 1.3 and 5.2%, respectively.

[d] Equilibrium factor (F) is the ratio of the nonequilibrium concentration of short-lived daughters in air to the equilibrium-equivalent concentration.

quently in rats when the unattached fractions were high. Tumors of the skin and kidneys were also noted, confirming the COGEMA data, but their significance is not yet established.

An increasing trend (sometimes significant) was observed in rat lung-tumor risk per cumulative potential alpha-energy exposure with: 1) a decrease in potential alpha-energy exposure rate, 2) an increase in unattached fraction of radon daughters, and 3) an increase in radon-daughter disequilibrium. These data and other factors influencing the carcinogenic potential of radon-daughter exposures are shown in Table 1.

Lung cancers observed between exposures of approximately 300 and 5000 WLM were similar to those seen in the dog experiments discusqed above: about 70% bronchogenic carcinoma and 30% bron-

chioloalveolar carcinoma[3]. Lifetime lung-tumor risk-coefficients for rats, based on raw tumor-incidence data, range between approximately 1 and 5 × 10^{-4}/WLM for all lung tumors (benign and malignant) at cumulative exposures from 100 to 300 up to 5000 WLM. This range, of course, would be reduced somewhat if only malignant tumors are considered.

Discussion and Conclusions

Both COGEMA and PNL data indicate that tumor incidence increased with an increase in cumulative potential alpha-energy exposure (WLM) and a decrease in exposure rate (WLM/week). The PNL raw-tumor-incidence data show a tapering-off of the inverse exposure rate effect at about 300 WLM cumulative exposure and rates as low as 5 WLM/week[10]. As 5 WLM/week is a reasonable (high) representation of exposure rate in underground miners, the tentative conclusion, in the absence of more rigorous analyses or additional data, is that there is little to no inverse exposure-rate effect at the levels humans are generally exposed to.

Tumor incidence also increased with increase in radon-daughter unattached fraction and disequilibrium. The animal data suggest that the small increase with disequilibrium is of little practical importance under most environmental and occupational exposure situations. The increase with unattached fraction is also troublesome from the standpoint of increased potential for extrathoracic tumors. Because an increase in unattached fraction also increases radon-daughter plateout on surfaces, the risk increase from higher unattached daughters in a particular environment may be offset by the reduction in the radon-daughter levels from plateout. However, two different environments with identical radon-daughter levels but different unattached fractions would pose different risks.

The mean PNL and COGEMA rat lung-tumor-risk data are in good agreement with one another (Figure 1) and also with projected risk data for miners. The PNL data do not include values for high unattached fraction or high disequilibrium data on rats, but they do show the influence of exposure rate. In all cases where exposure levels are duplicated, the highest risk pertains to the lowest exposure rate, and vice versa. The risk coefficients based on raw rat data range from 1 to 5 × 10^{-4}/WLM between approximately 100- and 5000-WLM exposures. The range in projected risk for miners over the same range of exposures is 1 to 5 × 10^{-4}/WLM[3].

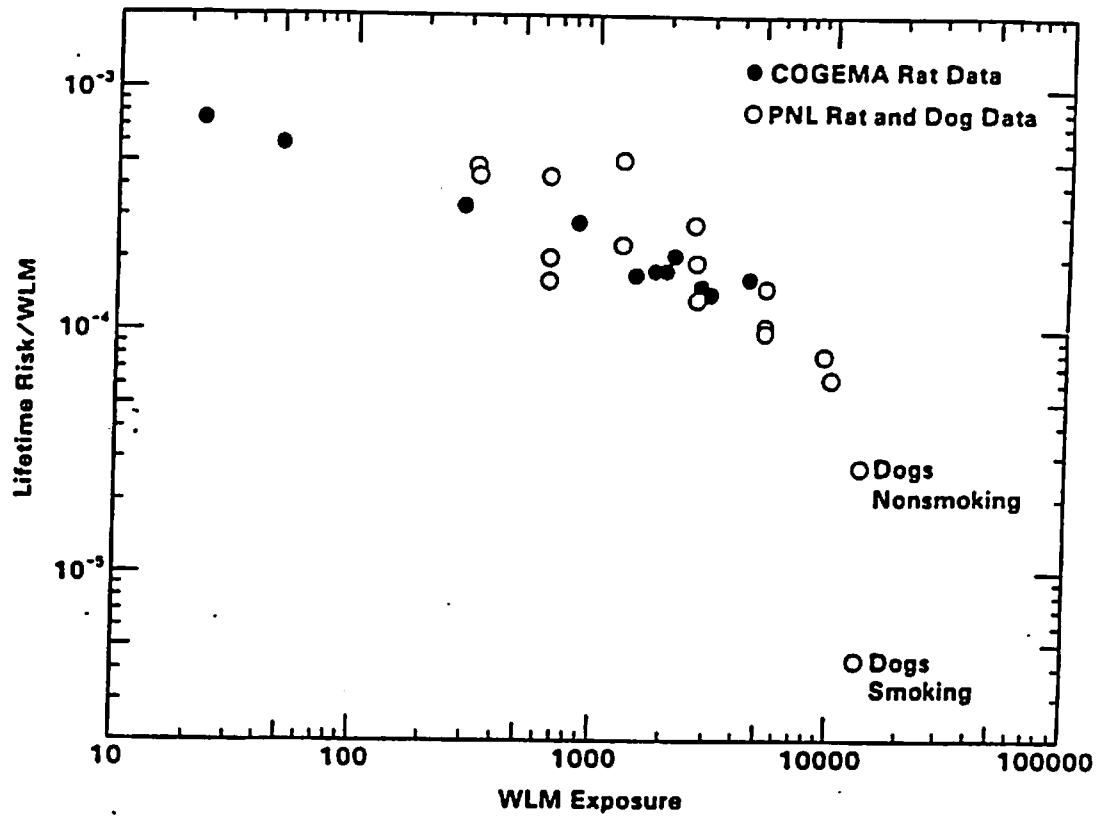

Fig. 1. Lifetime Lung-Tumor Risk Coefficients for Radon-Daughter Exposure (Reproduced from Figure III-1[11])

The mean measured dose to lung tissue from radon-daughter exposures in the UR experiments was about 0.2 rad/WLM (2 mGy WLM^{-1}), with the dose to bronchial tissue possibly five to ten times higher. Desrosiers et al.[12] calculated that hamster airway epithelium doses ranged from 0.1 to 0.3 rad/WLM (1 to 3 mGy WLM^{-1}), and that average alveolar doses ranged from 0.1 to 0.2 rad/WLM (1 to 2 mGy WLM^{-1}). Harley[13] calculated that rat airway epithelium doses for the PNL and COGEMA experiments ranged from 0.2 to 0.7 rad/WLM (2 to 7 mGy WLM^{-1}) and that average alveolar doses ranged from 0.2 to 0.4 rad/WLM (2 to 4 mGy WLM^{-1}). *In toto,* this suggests that rat lung-cancer-related doses in radon-daughter exposures range from about 0.4 to 0.8 rad/WLM (4 to 8 mGy WLM^{-1}). Therefore, the range in risk, on a dose basis, is 200 to 1250 × 10^{-6}/rad (200 to 1250 × 10^{-6} cGy^{-1}), based on raw rat data in the 100- to 5000-WLM range of exposures. The range in human risk, on a dose basis, would approximately equal that for rats.

The complex influence of cigarette-smoke exposures on radon daughter carcinogenesis is currently being elucidated in additional experiments

at PNL. In summary, the experimental data indicate that added cigarette-smoke exposures may increase or decrease, or may not affect radiation-daughter carcinogenesis, depending on the time sequence of exposures to radon daughters and cigarette smoke. The data are too tentative at this stage to extrapolate to the complexities of the human data from combined exposures to radon daughters and cigarette smoke.

The agreement of rat and human lung-cancer risk-coefficient data per unit exposure and dose may be more apparent than real. Continued hazard analyses of both human and animal data sets will eventually sort out similarities and differences. For the moment, the animal models (particularly rats) appear to be reasonable surrogates for man in determining the effects of exposures to radon and environmental pollutants.

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Discussion

J. MATUSZEK: If I were to draw an illogical conclusion from that data slide of yours with the 10 to 30 WLM data points (where you indicate a house would be), then I would say that EPA's drive to decrease concentrations doesn't gain as much as they would claim. For if you decrease an 8 picocurie per liter house to 4 picocuries per liter, your risk coefficient goes up because the rate goes down. So even though you get a lower dose, you've increased the risk coefficient and gained nothing.

F. CROSS: I think at these exposure levels, a rate effect is not very important. I tried to make that clear, maybe I didn't.

M. FRY: Would you like to speculate on why you have this reverse dose rate effect?

F. CROSS: I could certainly stumble onto an explanation. I don't know if Suresh Moolgavkar is in the audience, he proposes a two-stage model of carcinogenesis. As I understand it, if you expose to radon daughters at very high rates or levels you kill more cells than