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EXPERIMENTAL, STATISTICAL, AND BIOLOGICAL MODELS OF RADON CARCINOGENESIS

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Running Title: Models of Radon Carcinogenesis

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Abstract — Risk models developed for underground miners have not been consistently validated in studies of populations exposed to indoor radon. Imprecision in risk estimates results principally from differences between exposures in mines as compared to domestic environments and from uncertainties about the interaction between cigarette-smoking and exposure to radon decay products. Uncertainties in extrapolating miner data to domestic exposures can be reduced by means of a broad-based health effects research program that addresses the interrelated issues of exposure, respiratory tract dose, carcinogenesis (molecular/cellular and animal studies, plus developing biological and statistical models), and the relationship of radon to smoking and other copollutant exposures. This article reviews experimental animal data on radon carcinogenesis observed primarily in rats at Pacific Northwest Laboratory. Recent experimental and mechanistic carcinogenesis models of exposures to radon, uranium ore dust, and cigarette smoke are presented with statistical analyses of animal data.

INTRODUCTION

Current estimates of lung cancer risk from residential radon exposures are based on occupational risks of underground miners. Epidemiological evidence in these miners, resembling that from animal studies, clearly confirms the carcinogenicity of radon exposure.[1,2] There are, however, many differences (such as sex, age distribution, breathing patterns, and aerosol characteristics) between occupational and domestic exposures that require extrapolating miner data to infer risks of residential exposure. Such approaches include mechanistic, animal, dosimetric, statistical, and carcinogenesis modeling studies.

Basic research in radon carcinogenesis includes studies of mechanisms of effects and of DNA damage and repair as well as cellular studies. This research should ultimately confirm the carcinogenicity of low-level radon exposures and provide insights into the carcinogenic process useful for determining disease causation and suggesting treatment.

Dosimetric models link effects that have been observed at the molecular/cellular level with effects observed in animals and humans and also link miner data to exposures in homes. Such models require detailed morphometric knowledge of the respiratory tract of animals and humans as well as of physiological parameters, aerosol characteristics, deposition and clearance kinetics, and characteristics of cells at risk in radon carcinogenesis.[3-5]

Animal studies of radon-induced lung cancer are particularly valuable for understanding the carcinogenicity of human radon exposures in the home and in the workplace. Animals can be exposed to a variety of agents simulating human exposures and then sacrificed for the study of developing lesions or followed for tumor development. Both similarities and dissimilarities in animal and human exposure-response data are valuable for delineating the mechanisms of radon carcinogenesis. The questions of potential gender and age-at-exposure effects in radon carcinogenesis can be studied and the role of cigarette-smoke exposures in radon-induced lung cancers investigated. Animal tumor and non-tumorous tissues can be studied for radon-induced mutations and changes in expression of oncogenes, tumor suppressor genes, and growth factors/growth factor receptors. Further, the shape of the radon risk relationship can be determined over the full range of human exposures and the tumor data used to develop and test biological and statistical models of carcinogenesis.

This article focuses on experimental animal data on radon carcinogenesis developed primarily in adult male rats at PNL; data from similar studies at the Compagnie Générale des Matières Nucleaires (COGEMA) laboratory in France are included. Statistical risk and carcinogenesis models are also discussed.

EXPERIMENTAL MODELS AND HEALTH EFFECTS DATA

Cross[2] has reviewed experimental animal radon health effects data. This report primarily detailed the results of experiments at COGEMA and PNL but included other experimental data. The PNL experiments, which attempted to simulate the environment of the underground uranium miner, included exposures

of animals (SPF Wistar rats, Syrian Golden hamsters, and beagle dogs) to mixtures of radon, radon progeny, uranium ore dust (the carrier aerosol for the radon decay products), diesel engine exhaust, and cigarette smoke. The COGEMA experiments exposed SPF Sprague-Dawley rats to mixtures of radon, radon progeny, ambient outdoor aerosols (the carrier aerosol for the radon decay products), and cigarette smoke.

The major biological effects observed at both laboratories were respiratory tract tumors, pulmonary fibrosis, pulmonary emphysema, and life-span shortening. Fibrosis, emphysema, and life-span shortening did not occur to any appreciable extent at exposure levels less than 3.5 J h m^{-3} [<1000 WLM* (see footnote)]; however, excess respiratory tract tumors were produced in rats at exposure levels as low as 0.07 J h m^{-3} (20 WLM). Tumors were produced in animals exposed to radon decay products alone; thus, associated exposures to other irritants are not necessary for carcinoma development.

The morphology of respiratory tract lesions has been summarized by Dagle et al.[6] and Masse[7]. Principal lung tumors were pulmonary adenomas, adenocarcinomas, epidermoid carcinomas, and adenosquamous carcinomas; malignant mesotheliomas and sarcomas occasionally occurred. Malignant lung tumors were characterized by invasion and occasionally metastasized to regional lymph nodes. Most neoplasms occurred in epithelium of the distal respiratory tract. These tumors contained lamellar bodies characteristic of both alveolar type 2 epithelial cells and Clara cells. Bronchogenic tumors contained mucus granules as well as other organelles common to bronchial cells.

Extrapulmonary lesions, including tumors, were produced primarily in the nose, particularly associated with exposures to high unattached fractions of radon decay products. Significant ($p < 0.05$) excess nonrespiratory neoplasms associated with radon exposure were noted primarily in the kidneys, consistent with the dosimetry used by Pohl and Pohl-Ruhling[8], who showed that the kidney dose is about 10% of the average dose delivered to the lung whereas other tissues receive between 1% and 2%. Neoplastic lesions in non-respiratory tissues, however, were incidental findings and therefore may have been underestimated in both control and exposed animals.

The major factors found to influence the tumorigenic potential of radon exposures include radon-progeny cumulative exposure, exposure rate, unattached fraction, and associated cigarette-smoke exposures.[2] The respiratory tract cancer risk increased with an increase in radon-progeny cumulative exposure and unattached fraction and a decrease in radon-progeny exposure rate. The increase in risk with cumulative exposure is described later in the Statistical Models section. A decrease in exposure rate at a given exposure level not only increased the overall incidence of lung tumors [200% to 300% increase from 1.8 to 0.18 J h m^{-3} wk^{-1} (500 to 50 WLM wk^{-1}); Figure 1, modified from Figure 1, ref. 9] but specifically increased the incidence of epidermoid carcinomas in the PNL experiments.[10] The exposure-rate effect appeared to diminish at even lower exposure rates and at exposures less than about 1 J h m^{-3} (300 WLM). Protraction of exposures also produced a significantly higher incidence of multiple primary lung tumors (more often of a different type than the same types) and fatal primary lung tumors.

An increase in f_p (the percentage of radon decay products unattached to carrier aerosols) from 0.4% to 3% increased the tumor incidence by 14% per 10^{-3} J h m^{-3} (50% increase per WLM). The increased risk with high unattached radon decay products is particularly relevant to indoor radon exposures where the unattached levels are generally much higher than those in underground mines.

The influence of associated cigarette-smoke exposures depends in part on the temporal sequence of radon-progeny and cigarette-smoke exposures. In COGEMA rat experiments[11], synergism was observed between inhaled radon decay product and multiple daily passive cigarette-smoke exposures when smoke exposures followed completion of all exposures to the decay products. The increase in tumor incidence with added passive-smoke exposures was twofold to fourfold that from radon-only exposures at 1.8 and 14 J h m^{-3} (500 and 4000 WLM). However, when smoking preceded all radon decay product exposures, essentially no increase in tumor incidence was noted over that produced by radon decay products alone. It was not clear whether passive cigarette-smoke exposures promoted radon-induced tumors at exposures less than about 0.7 J h m^{-3} (200 WLM). The COGEMA experiments also showed no synergism when smoke exposures were less than 100 h; blood carboxyhemoglobin levels in these experiments were about 0.6%.

Early PNL experiments with beagle dogs given alternating but same-day exposures to radon progeny and mainstream cigarette smoke produced a mitigating, rather than a synergistic, effect.[12] Similar results were produced in Harwell Laboratory (UK) exposures of mice to plutonium and

cigarette smoke.[13] In the latter experiment, smoke exposures followed plutonium exposures; therefore, the radiation and smoke exposures were simultaneous (because of lung retention of plutonium) rather than sequential or alternating as in the radon experiments. Both PNL and Harwell radiation exposure levels were thought to be high enough to obscure a promotional effect of cigarette-smoke exposures.

Ongoing initiation-promotion-initiation (IPI) rat experiments at PNL also showed antagonism between radon and cigarette-smoke exposures.[14] The promotional effect of mainstream cigarette smoke was clearly evident for the preneoplastic lesion adenomatosis, but this was not true for tumors in animals sacrificed at 25, 52, and 78 weeks after beginning of exposure. Life-span IPI tumor data are currently being analyzed, but preliminary evidence also suggests antagonism. IPI radon-progeny-exposure levels were 1.1 J h m^{-3} (320 WLM); cigarette-smoke exposures were given 1 h per day for 85 days and produced 29% blood carboxyhemoglobin levels. The few cigarette-smoke-related tumors were primarily epidermoid carcinomas, in contrast to a greater prevalence of bronchioloalveolar carcinomas in the radon-only exposed groups.

The animal data in general are currently too inconclusive to be extrapolated to the complexities of the human data from combined exposures to radon and cigarette smoke. Various interpretations have been placed on human radon and cigarette-smoke data. That by Doll and Peto[15] stresses more the importance of duration of cigarette smoking on risk than the daily amount smoked. On the surface, the combined PNL IPI and COGEMA data would agree with this interpretation. At the least, therefore, redesigned IPI-type studies

including longer or multiple daily cigarette-smoke exposures would seem necessary to further analyze the influence of cigarette-smoke exposures on radon-progeny carcinogenesis.

Another experimental animal model of radon carcinogenesis included repeated intraperitoneal injections of the promoter 5,6-benzoflavone in COGEMA rats following radon inhalation exposures.[16] The histogenesis of tumors induced with this model showed unique differentiation to only squamous cell carcinomas; they appeared in less than 100 days, much faster than in mixed exposures to radon and cigarette smoke. Much as in the PNL IPI experiments, the development of pneneoplastic lesions was found to be reversible. Similar reversibility of epidermoid metaplasias has been noted in human bronchial epithelium.[17]

STATISTICAL MODELS

Statistical analyses of PNL[9] and COGEMA[18] radon lung tumor data in rats have been used to model the hazard using the Weibull function for the baseline risk; baseline age-specific risks are more uncertain in rats than they are in humans. The overall lifetime risk of lung tumors in PNL and COGEMA control rats is less than 1%. Analyses were based on the assumption that lung tumors are incidental to the death of the animal. Although a case can be made that most rat pulmonary tumors are incidental, some are fatal, and future analyses must account for this. If tumors are not fatal, less information is available on the time of occurrence of tumors, and thus less information on time-related factors.

PNL statistical analyses of radon-progeny exposures at three exposure rates show no indication of a decrease in risk per unit exposure with increasing total exposure, even to high exposure levels (see Figure 1). Similar analyses based on the assumption that lung tumors are fatal also showed no evidence of a decrease in risk with increased exposure.

The estimated linear lifetime risk coefficient, based on the combined exposure-rate data, was about 0.086 per J h m^{-3} (300 per 10^6 rats per WLM) for all primary lung tumors. Excluding adenomas, the risk coefficient is reduced to about 0.071 per J h m^{-3} (250 per 10^6 rats per WLM). Analyses based on the assumption that tumors are fatal produced risk coefficients about half as large. These values may be compared to the BEIR IV combined smokers and nonsmokers value of 0.10 per J h m^{-3} (350 per 10^6 persons per WLM) and 0.040 per J h m^{-3} (140 per 10^6 persons per WLM) for male nonsmokers.[1] Estimates from rats, therefore, when analyzed similarly to human data, are comparable to those obtained from human studies at occupational exposure-levels. Future analyses will focus on lower exposure and exposure-rate data and account for the fatality of tumors. The implications for modeling risks of indoor radon exposures [generally <0.35 J h m^{-3} (<100 WLM)] are currently under study; see refs. 9 and 18 for further detail.

General agreement of rat and human data at occupational exposure levels does not necessarily ensure agreement at environmental exposure levels. However, it is interesting to note that elevated risks of lung cancer are observed between 0.035 and 0.18 J h m^{-3} (10-50 WLM; $p \leq 0.03$) in French uranium miners,

in agreement with elevated risks in rats at these exposure levels (M. Tirmarche, Commisariat à l'Energie Atomique, personal communication).

CARCINOGENESIS MODELS

The two-mutation (recessive oncogenesis) biological model, as formulated by Moolgavkar and Knudson[19], was used to model the PNL data from exposures greater than 1 J h m^{-3} (300 WLM).[20] The two-mutation model postulates the necessity of two irreversible mutational events at the level of the cell to produce carcinoma and describes the rat lung cancer data well. Predictions of the model are that the first mutation rate and, to a somewhat lesser extent, the clonal expansion of initiated cells are strongly dependent on the rate of exposure to radon decay products. The second mutation rate was much less dependent on exposure rate. Predicted background doubling rates were 0.005 J h m^{-3} wk^{-1} (1.35 WLM wk^{-1}), 0.12 J h m^{-3} wk^{-1} (35 WLM wk^{-1}), and 1.4 J h m^{-3} wk^{-1} (400 WLM wk^{-1}) for the first mutation, clonal expansion of initiated cells, and second mutation, respectively.

Because PNL radon and decay product exposures are accompanied by ore-dust exposure, it is possible that the clonal expansion of initiated cells is caused by chronic irritation by the dust. However, excess carcinomas are produced even in the absence of associated ore-dust exposures; thus, it appears that radon decay products *per se* are complete carcinogens and are capable of initiating, promoting, and progressing normal cells to cancer.

The model also predicts a drop in hazard following cessation of radon exposures in apparent agreement with the "time-since-exposure" effect observed in the human epidemiology studies. As do the statistical modeling data, this model also shows an increase in cancer risk with protraction of radon exposures; interestingly, an optimum exposure rate is predicted for a given exposure level. Below this optimum exposure rate, the risk diminishes. These and other predictions of the model have implications for human risk assessment (see Moolgavkar et al.[20] for further details). Data from low-level radon exposures as well as data from IPI and other experiments will be used to further test the two-stage model and other biological models of radon carcinogenesis.

CONCLUSIONS

Although the broadest multilevel approach is recommended for cancer risk assessment of environmental exposures to radon, similarities in rat and human exposure-response data at occupational exposure levels suggest that the rat experimental model is particularly valuable for reducing uncertainties in low-level human exposures.

Experimental animal models are essential to addressing the complex interactions of radon and cigarette-smoke and other cofactor exposures, as well as various time-related factors in radon carcinogenesis such as an age-at-exposure or time-since-exposure effect. Tissues and data from animal experiments are also valuable for delineating the mechanisms of radon carcinogenesis and for testing and developing statistical and biological

models of radon-induced cancer. The latter type of models, in particular, are not only an essential part of a rational approach to cancer risk assessment but raise fundamental questions about the nature of the events leading to malignancy, such as the role of cell division and the interactions of neighboring and activated cells.

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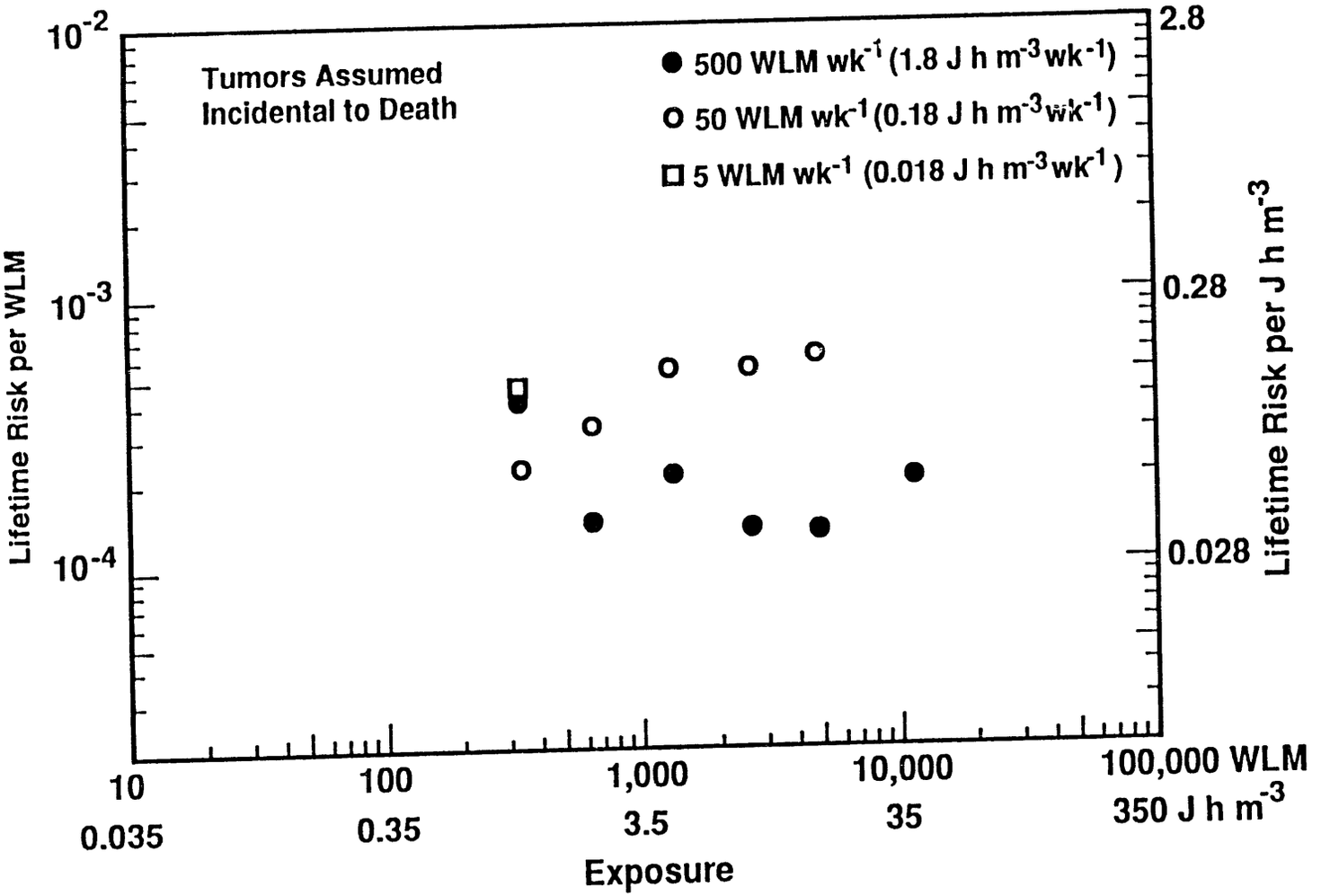
* Working level (WL) is defined as any combination of short-lived radon decay products in 1 liter of air resulting in the ultimate emission of 1.3×10^5 MeV of potential alpha energy (1 WL = 2.08×10^{-5} J m^{-3}). Working-level month: exposure equivalent to 170 h at 1-WL concentration (1 WLM = 3.5×10^{-3} J h m^{-3}).

Figure 1. Lifetime risk coefficients for radon-progeny exposure of rats (modified from Figure 1, ref. 9).


Tumors Assumed
Incidental to Death
● 500 WLM wk-1 (1.8 J h m-3 wk-1)
o 50 WLM wk-1 (0.18 J h m-3 wk-1)
□ 5 WLM wk-1 (0.018 J h m-3 wk-1)
Lifetime Risk per WLM
10-2
10-3
10-4
2.8
0.28
0.028
Lifetime Risk per J h m-3
10
100
1,000
10,000
100,000 WLM
0.035
0.35
3.5
35
350 J h m-3
Exposure

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