



## Carcinogenic and Cocarcinogenic Effects of Radon and Radon Daughters in Rats

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The International Agency for Research on Cancer (1) has stated that there is sufficient evidence for the carcinogenicity of radon and its decay products both in humans and in experimental animals. An increased incidence of pulmonary neoplasms has been observed in humans in different groups of underground miners: uranium miners (2-6), iron miners (7,8) and other miners (9,10) especially those that smoke cigarettes (11-13), suggesting that cocarcinogenic mechanisms may be involved in the pathogenesis of such neoplasia.

It has also been shown that lung cancers can be induced in dogs (14) and rats (15,16) by exposure to radon and its daughters. Experimentally, a cocarcinogenic effect results in increased tumor rates after combined administration of the potential cocarcinogens (17). As combined exposure to various carcinogens is common

in some mine environments, the potential cocarcinogenic effects of environmental or industrial airborne pollutants such as tobacco smoke, mineral fibers, diesel exhausts, or minerals from metallic mine ores were studied in rats in combination with radon exposure. This review summarizes the main experimental results obtained in our laboratory over the last 20 years in rats exposed to radon and its decay products. The results of ongoing experiments using combined exposure to radon and other airborne pollutants are also reported.

### Exposure to Radon and Radon Daughters

All the experiments reported in this paper were performed at the inhalation facilities of the Laboratoire de Pathologie Pulmonaire Expérimentale (LPPE), COGE-

It has been previously established that lung cancer could be induced in rats by exposure to radon and radon daughters. Although the oat-cell carcinomas that are common in humans were not found in rats, other histological types of lung carcinomas, especially squamous cell carcinomas and primitive lung adenocarcinomas, were similar to those observed in humans. A dose-effect relationship was established for cumulative doses varying from 25 to 3000 working-level-months (WLM), which was similar for medium and high cumulative doses to that observed in uranium miners. This experimental protocol was also used to study the potential cocarcinogenic effects of other environmental or industrial airborne pollutants such as tobacco smoke, mineral fibers, diesel exhausts, or minerals from metallic mine ores that may act synergistically with radon exposure. In rats exposed to radon and tobacco smoke combined, the incidence of lung cancers was higher by a factor of 2-4 according to the cumulative radon exposure and the duration of tobacco smoke exposure. When mineral fibers were injected intrapleurally, an increased incidence of malignant thoracic tumors was observed in rats exposed to radon and fibers combined, but synergistic effects resulted in additivity. With diesel exhausts or minerals from metallic ores, a slight, nonsignificant increase in the incidence of lung carcinomas was observed compared with rats exposed to radon alone. These results demonstrated that it is possible to establish the potential cocarcinogenic action, showing either multiplicative, additive, or no effect of various environmental or industrial airborne pollutants combined with radon exposure. This radon model is valid for investigating possible interactions between two occupational exposures. *Key words:* carcinogenesis, cocarcinogenesis, diesel exhausts, dose-effect relationship, lung cancer, mineral dusts, radon, radon daughters, rats, tobacco smoke. *Environ Health Perspect* 102:64-73(1994)

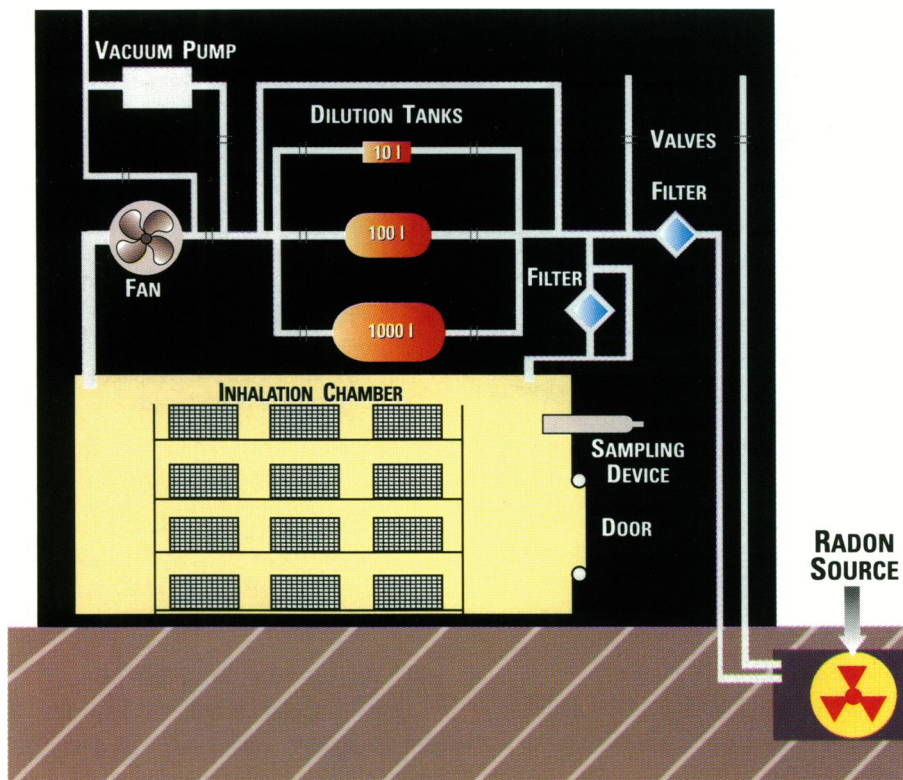


Figure 1. Simplified scheme of the radon inhalation chambers.

MA, Razès, France. These facilities consisted of two stainless-steel inhalation chambers, 10 m<sup>3</sup> each, allowing the simultaneous exposure of up to 250 rats in each chamber (15). The animals were housed in stainless-steel wire cages for whole-body exposures. A system of pipes connected each exposure chamber to three dilution tanks of 10-, 100-, and 1000-l volumic capacity, allowing the quantity of radon in the chambers to be controlled (Fig. 1).

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The radon source was two stainless-steel underground tanks containing barrels of high-grade uranium ore, full of a mixture of  $^{238}\text{U}$  and  $^{232}\text{Th}$  (uranothorianite). To perform a chosen dilution, radon gas coming from the underground source was introduced using a vacuum depression into one of the three dilution tanks. After an aging time of 10 min, corresponding to 10 periods of 55.6 sec, allowing the decay of  $^{220}\text{Rn}$  (thoron),  $^{222}\text{Rn}$  (radon) was introduced into the inhalation chamber by a closed loop system using a powerful fan. To sample air from the chambers, sampling ports fitted with airlocks were situated above the entrance to each chamber. The samplers were located 40 cm from both the ceiling and the walls to avoid sampling a region that might be affected by plate-out. Usually, the inhalation facilities were operated under static conditions with no forced air circulation. Under these experimental conditions, equilibrium between radon and radon daughters decreased according to the number of animals exposed within the chambers (18). In the majority of the experiments reported here, the decay-product equilibrium factors were about 30%. Exposures could also be performed under dynamic conditions using continuous air ventilation by the closed loop system, with a high equilibrium factor between radon gas and its daughters. Radon gas concentrations were measured using Lucas cells (19) and could vary from 1 to 400,000  $\text{KBq/m}^3$  according to the conditions of exposure. The potential alpha energy concentrations (PAEC) of radon daughters were measured on air samples from exposure chambers using the modified Thomas-Tsivoglou method (20, 21). The PAEC values obtained varied from 21  $\mu\text{mJ/m}^3$  (1 WL) to 95  $\text{J/m}^3$ . "Unattached" fractions were measured using screen/filter combination techniques and could vary from 0.11 to 0.62 according to the conditions of exposure used (22,23).

All the experiments were performed on outbred SPF Sprague-Dawley rats, OFA strain, purchased from IFFA-CREDO (France). In this strain, an excess of thyroid carcinomas was observed, but the spontaneous incidence of cancers according to age was similar to that observed in humans (19). Moreover, the incidence of spontaneous lung cancers was low. In our series, only 6 lung carcinomas among 847 control male rats (0.7%) and no lung carcinomas among 150 control female rats (0%) were observed. In all the experiments reported here, rats were allowed to live until they died or were moribund and then killed. A full necropsy was carried out on all animals. Whole lungs were fixed by intratracheal instillation of Bouin-Hollande fixing

solution, embedded in paraffin, and sectioned serially in the frontal plane. Large 20- $\mu\text{m}$  thick sections were prepared without staining and screened for lesions. When suspicious lesions were encountered, 5- $\mu\text{m}$  sections were cut, stained with Hemalun-Eosin-Saffron, and examined microscopically.

It was previously demonstrated that inhalation of  $^{222}\text{Rn}$  and its decay products at various cumulative dose induces lung cancer in rats (15,20). Although the oat-cell carcinomas that are common in humans were not found in rats, other histological types of lung carcinomas, especially squamous cell carcinomas and primitive lung adenocarcinomas, were similar to those observed in humans (21–24). Unlike human lung tumors, which are of bronchial origin, lung tumors in rats originate from terminal and respiratory bronchioles and the deep lung, or even from the bronchial tree cells. It is noteworthy that squamous cell carcinomas observed in rats after radon exposure are similar in the histology to those observed in humans (25).

The incidence of lung carcinomas in rats exposed to radon and radon daughters increased with the cumulative dose. The incidence of lung carcinomas increased for cumulative exposures up to 3000 working-level-months (WLM) and decreased thereafter (Fig. 2). For medium and high cumulative doses (i.e., corresponding to 200–3000 WLM), this pattern was similar to that observed in uranium miners, in whom effects are observed above 120–360 WLM (26). At low cumulative doses, we observed 11 lung carcinomas among 496 rats exposed at 25 WLM (2.2%) and 19 among 497 rats exposed at 50 WLM (3.8%) in our series. A significant correlation between human and experimental data has been previously emphasized (20).

The influence of the rate of radon exposure at low doses on lung carcinoma induction in rats was recently pointed out. Chronic radon exposure of 25 WLM over an 18-month period, at an alpha potential energy of 2  $\text{WL} \pm 0.36$  ( $0.042 \text{ mJ/m}^3 \pm 0.007$ ), resulted in fewer lung carcinomas in rats than a similar exposure of 25 WLM over 4–6 months at a potential alpha energy of 100 WL ( $2 \text{ mJ/m}^3$ ) (32,33). However a trend toward increasing tumor risk with decreased exposure rate has been previously reported in Wistar rats exposed at higher cumulative doses of about 640 WLM and dose rates varying from 44 to 180 WLM/week (34).

After inhalation, radon gas is solubilized and contributes to the whole-body irradiation (35). It has been assumed that doses delivered to the rest of the body are lower by at least one order of magnitude than lung doses. Although this contribu-

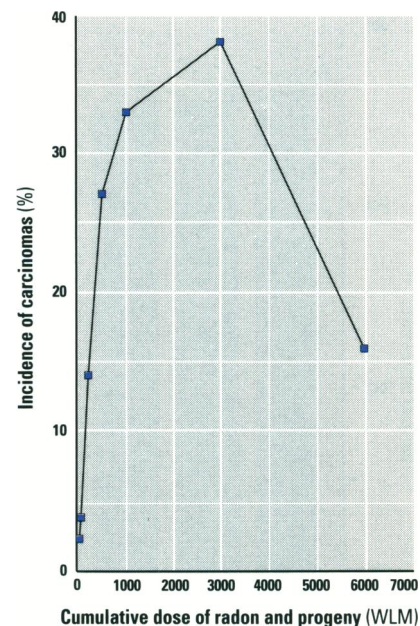


Figure 2. Dose-effect relationship.

tion to effective dose remains low, it may result in extrapulmonary tumors, especially in the kidneys, which are the most irradiated tissue in terms of average doses. In this respect, a slight, nonsignificant increased incidence of kidney carcinomas has been reported in rats after radon exposure (36). In female rats, especially the Sprague-Dawley strain, mammary tissue seems to be the major extrapulmonary target tissue of radon. A significant excess of lung carcinomas occurred after a 1600 WLM radon exposure in male rats (47% versus 0.7% in controls), and to a lesser extent in females (28% versus 0% in controls);  $p < 0.0001$ , Fisher's exact test. In contrast, highly significant excesses of mammary tissue carcinomas were observed in females (78% versus 30% in controls;  $p = 0.004$ , the Fisher's exact test), whereas no cases were observed in exposed and control male rats (20). These findings were not unexpected considering the high sensitivity to high linear energy transfer (LET) radiations of the mammary gland in the Sprague-Dawley strain (35).

As with other types of radiation, radon exposure not only increased the incidence of malignant tumors, but also shortened life spans and tumor latency periods, depending on the dose. Survival times of rats with lung carcinomas, preneoplastic lesions, and pulmonary benign tumors decreased when the cumulative exposure increased (Fig. 3), but no significant difference was observed between malignant and benign tumor-bearing rats.

In the same way, latency periods decreased when the age at exposure increased. For a cumulative exposure of 1000 WLM, the older the rats at the beginning of expo-



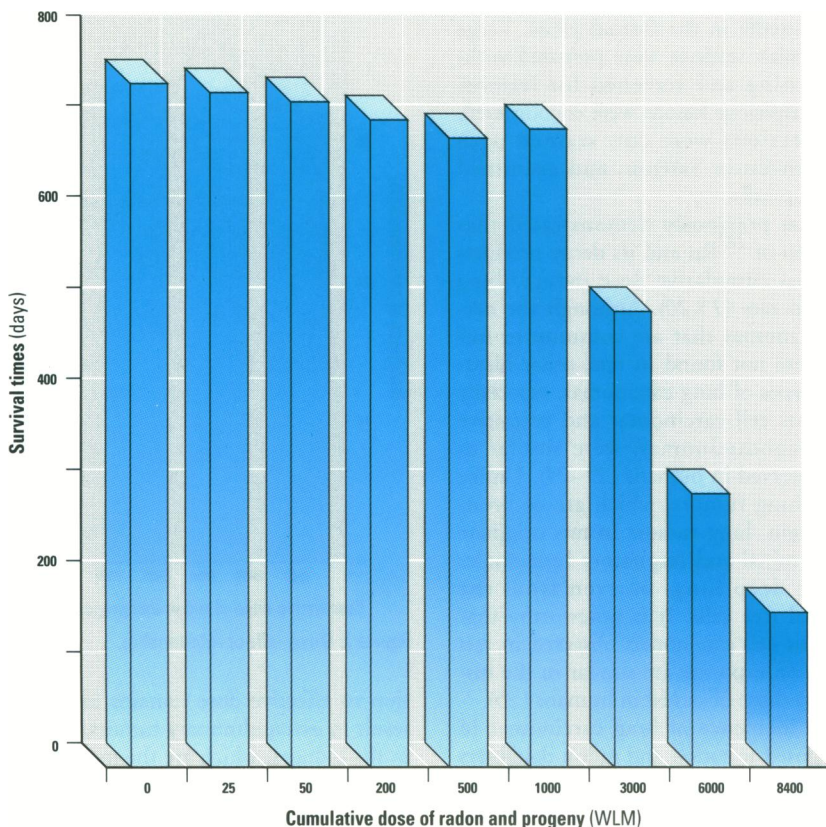


Figure 3. Mean survival times of rats after radon exposure.

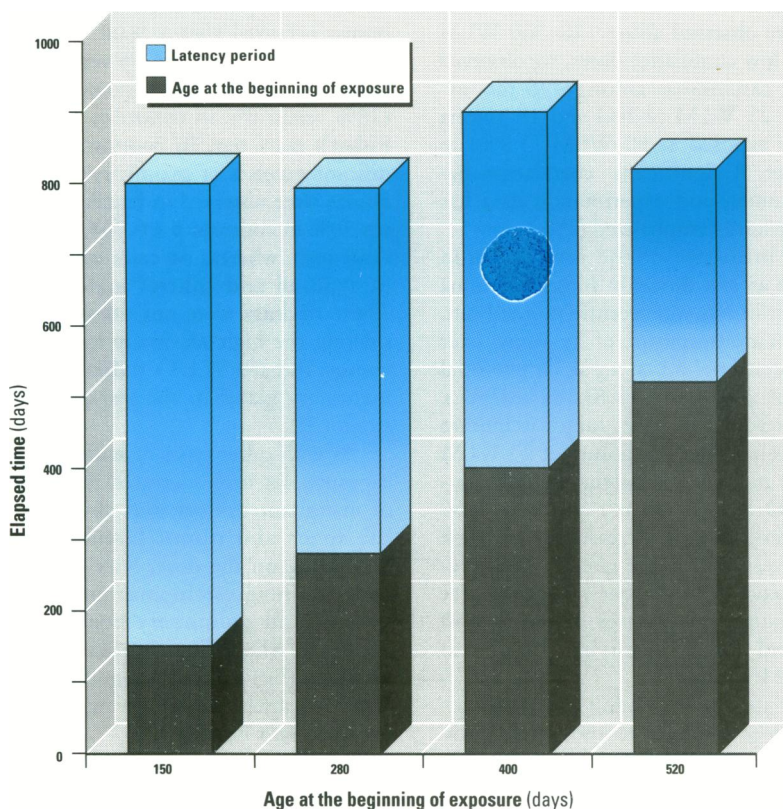


Figure 4. Latency period according to the age at the beginning of radon exposure (1600 WLM).

sure, the shorter the latency period for the occurrence of lung carcinomas (Fig. 4). On the other hand, whatever the age at the beginning of exposure, lung carcinomas occurred roughly at the same age (i.e., between 800 and 900 days after birth).

### Combined Effects of Radon and Other Airborne Pollutants

The experimental protocol of radon exposure was also used to study the potential cocarcinogenic effects of other environmental or industrial airborne pollutants, such as tobacco smoke, mineral fibers, diesel exhausts, or minerals from metallic mine ores, which may act synergistically with radon exposure, to explore the possibility of combined exposure for workers in different industries.

### Tobacco Smoke

The first experiments were carried out to investigate the effects of inhalation of radon and daughters at various cumulative doses, before or after various passive cumulative tobacco smoke exposures (37), and using cigarettes with or without filters. Exposures tobacco smoke were performed in a smoke box, 500 l in volume, allowing exposure of 50 rats simultaneously. The animals were housed in wire cages for whole-body exposures. Cigarette smoke was produced by simultaneously burning nine cigarettes (French brown tobacco). The cigarettes, with or without filters, were placed in a cigarette holder connected to the box. The cigarette smoke was introduced into the chamber by means of a slight depression produced by a vacuum pump. A ventilation system renewed the chamber atmosphere with fresh air at the end of each exposure session. In the groups exposed to radon and tobacco smoke combined, the rats were exposed to tobacco smoke 1 month after the end of radon exposure. The animals were given 10 10-min inhalation sessions daily, 5 days a week, for up to 7 months. Blood carbon monoxide levels of rats exposed to tobacco smoke were about 0.6% 1 hr after the end of daily exposure. No lung carcinomas were observed among a group of 45 rats subjected to a 350 hours of passive tobacco smoke exposure. However, alveolitis with aggregated macrophages containing tar-dense bodies and alveolar and bronchiolar metaplasias were observed in this group.

For a 1000 WLM radon exposure, the incidence of lung carcinomas was slightly lower in rats exposed to tobacco smoke before radon exposure than in rats exposed to radon alone (Table 1), but the distribution of the different histological types of tumors were similar in the two groups. In contrast, a highly significant excess of lung carcinomas ( $p = 0.0014$ , Fisher's exact

test), mainly of the squamous cell type, was observed in the group exposed to tobacco smoke after radon exposure. In this group, the incidence of lung carcinomas was higher by a factor of about 4 compared with the group exposed to radon alone.

For a 350-hr passive tobacco smoke exposure, the incidence of lung carcinomas increased with the cumulative dose of radon (Table 2). The incidence of lung carcinomas was twofold higher in the group exposed to radon at 600 WLM and tobacco smoke for 350 hr combined than in the group exposed to radon 1600 WLM only and was statistically significant ( $p=0.0003$  using the Fisher's exact test). For a cumulative dose of radon and its daughters corresponding to 1600 WLM, the incidence of lung carcinomas increased with the cumulative exposure to tobacco smoke. The synergistic effect of combined exposure to radon and tobacco smoke decreased when the cumulative exposure to tobacco smoke decreased. There were no statistically significant differences between the groups exposed to radon 1600 WLM and tobacco smoke 60 hr or 100 hr compared with rats exposed to radon 1000 WLM only, but there was a marginally significant difference between the group exposed to radon 1600 WLM and tobacco smoke 30 hr combined compared with the group exposed to radon 1600 WLM only ( $p=0.0557$ , Fisher's exact test).

The induction of lung carcinomas was less efficient in rats exposed to tobacco smoke produced by cigarettes with filters than by cigarettes without filters. As shown in Figure 5, the rates of lung carcinomas were higher, but not statistically significant, in the groups exposed to radon and tobacco smoke combined than in the group exposed to radon alone, the incidence being lower in the group exposed to filtered cigarettes than in the group exposed to unfiltered cigarettes. In the group exposed to radon and cigarettes with filters, adenocarcinomas prevailed, but the proportion of this type of tumor was quite identical to that observed in the radon-exposed group. The increased incidence of lung carcinomas in the group exposed to radon and unfiltered cigarettes was mainly related to a relative increased incidence of squamous cell carcinomas. These findings suggested a stronger synergistic effect of radon and cigarettes without filters compared to radon and cigarettes with filters and confirmed a trend toward a preferential differentiation to the squamous cell type in lung carcinomas induced in rats by combined exposure to radon and industrial or environmental airborne pollutants.

Primitive bronchopulmonary and pleural neoplasms were scored according to the following classification, derived from

**Table 1.** Incidence of the different histological types of lung carcinoma in rats exposed to tobacco smoke before or after radon exposure

Group <sup>a</sup>	No. of rats with lung carcinoma	Proportion (%) of lung carcinoma	Squamous cell carcinoma	Bronchioloalveolar carcinoma	Adenocarcinoma
Tobacco smoke (350 hr) before radon (1000 WLM)	8	16	3	1	4
Radon only (1000 WLM)	11	22	3	1	7
Tobacco smoke (350 hr) after radon (1000 WLM)	39	78	30	3	7

<sup>a</sup>Each experimental group comprised 50 rats; WLM, working-level-month.

**Table 2.** Incidence of lung carcinomas after combined exposure to radon and tobacco smoke according to the cumulative dose of radon and its progeny and to the cumulative exposure to tobacco smoke

	No. of rats	No. of lung carcinomas	Proportion (%) of lung carcinomas
Radon exposure only (40 WLM)	21	1	5
Tobacco smoke (350 hr) after radon exposure (40 WLM)	27	1	3.5
Radon exposure only (200 WLM)	63	7	11
Tobacco smoke (350 hr) after radon exposure (200 WLM)	75	16	21
Radon exposure only (1600 WLM)	208	81	39
Tobacco smoke (350 hr) after radon exposure (1600 WLM)	138	106	77
Radon exposure only (1600 WLM)	30	7	23
Tobacco smoke (100 hr) after radon exposure (1600 WLM)	35	11	31
Radon exposure only (1600 WLM)	74	22	30
Tobacco smoke (60 hr) after radon exposure (1600 WLM)	64	19	30
Radon exposure only (1600 WLM)	30	7	23
Tobacco smoke (30 hr) after radon exposure (1600 WLM)	35	1	3

WLM, working-level-month.

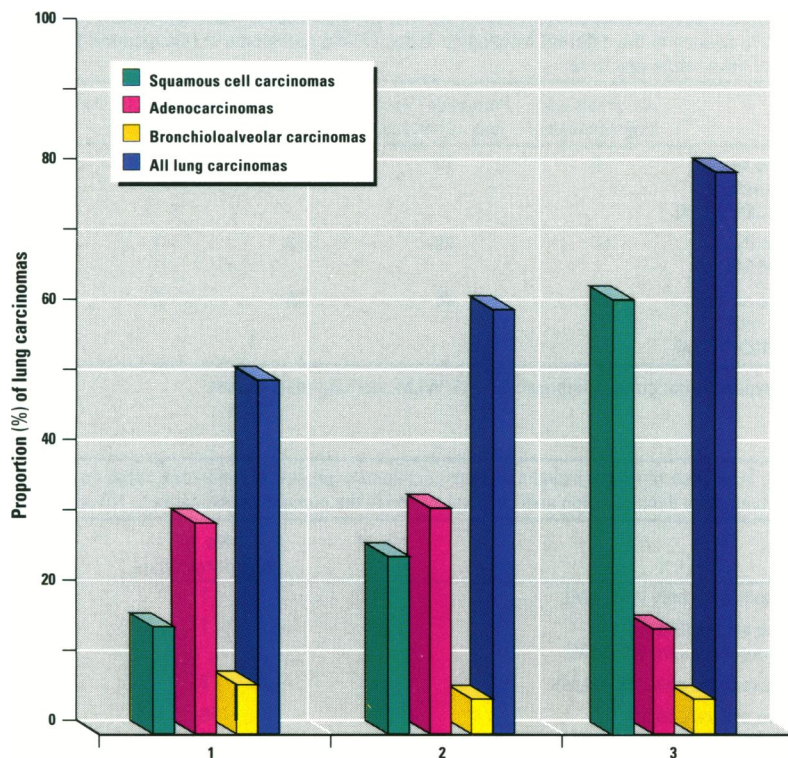
the tumor-node-metastasis (TNM) classification (38): T1, presence of a tumor <2 mm in diameter; T2, presence of a tumor 2–5 mm in diameter; T3, presence of a tumor 5–10 mm in diameter; T4, presence of a tumor >10 mm in diameter; P1, spread to the pleura; N1, lymph node involvement; M1, metastasis outside the thoracic cavity; M2, intrapulmonary metastasis or presence of several tumors in the lungs, and M3, association of M1 and M2. Primitive pleural tumors were classified according to the above system. When a pleural tumor was isolated, it was classified as P1. This procedure enabled a formula to be established and allowed simple classification and comparison of each histological specimen.

In rats exposed to radon and tobacco smoke, for the same radon exposure, the incidence of lung carcinomas was widely increased in the group exposed to radon and tobacco smoke compared with the group exposed to radon only. Tumors in the groups exposed to radon and tobacco

smoke were larger and more invasive than in the groups exposed to radon alone (Table 3). These tumors also spread more to the pleura, and intrapulmonary metastases or several tumors in the lung were observed. For the same radon exposure, the mean latency period of lung carcinomas was shorter in the group exposed to radon and then to tobacco smoke compared with the group exposed to radon alone (i.e., 682 days and 748 days, respectively, at 200 WLM radon exposure). For an identical tobacco smoke exposure of 350 hr, the mean latency period was shorter according to the cumulative dose of radon (i.e., 600 days in the 1600 WLM group and 682 days in the 200 WLM group).

All these results showed a clear cocarcinogenic effect of exposure to radon and radon daughters and tobacco smoke in rats. However, an inverse effect has been reported in beagle dogs in which cigarette smoke exposure was shown to suppress radon daughter-induced respiratory carcinomas (14,39). Such a discrepancy might





**Figure 5.** Histologic types of lung carcinomas after exposure to radon and tobacco smoke produced by filtered and unfiltered cigarettes. 1, radon 1600 WLM; 2, radon 1600 WLM + cigarettes with filters; 3, radon 1600 WLM + cigarettes without filters.

**Table 3.** Incidence of lung carcinomas in rats according to the tumor-node-metastases (TNM) system, after combined exposure to radon and tobacco smoke (350 hr)

TNM classification	Radon (40 WLM)		Radon (200 WLM)		Radon (1600 WLM)	
	Only	+ Tobacco smoke	Only	+ Tobacco smoke	Only	+ Tobacco smoke
T1	1	0	2	1	2	2
T2	0	1	0	3	4	4
T3	0	0	0	2	5	6
T4	0	0	0	2	6	22
Proportion (%) of rats with lung carcinomas	5	3.5	11	21	39	77
P1	0	0	0	6	10	20
N1	0	0	0	1	1	7
M1	0	0	0	0	0	0
M2	0	0	0	1	4	16
M3	0	0	0	0	0	1

WLM, working-level-month.

be related to the experimental exposure design. In Pacific Northwest Laboratory experiments, beagle dogs were exposed to cigarette smoke and radon daughters alternately the same day (14,39) whereas in our experiments rats were first exposed to radon daughters and then to tobacco smoke. Moreover, it has been suggested that cigarette smoke exposure might play a protective role in dogs exposed both to radon daughters and cigarette smoke by increasing mucus production and thus protecting underlying target cells from  $\alpha$  irradiation (39,40). However, it should be noted that most of the PNL dogs died prematurely from pulmonary fibrosis and that the dogs exposed to tobacco smoke had a median life span of 46 months versus 51

months for the “nonsmoking” dogs. Thus, the shorter life span might have precluded the development of many lung cancers in the dogs exposed to tobacco smoke (34,41).

### $\beta$ -Naphthoflavone

Half of radon-induced lung carcinomas in rats are of the squamous cell type (42–44). This proportion rose to 75% in rats exposed first to radon and then to tobacco smoke (Table 1). It has also been demonstrated that lung carcinomas, mostly of the squamous cell type, can be induced in laboratory animals after a single or repeated treatments by different chemical compounds (35–47). An experimental model of cocarcinogenesis has been developed in our laboratory, based on the hypothesis

that polycyclic hydrocarbons are involved in the carcinogenic activity of several compounds.

The inducibility by  $\beta$ -naphthoflavone or 5,6-benzoflavone ( $\beta$ -NF) of pulmonary microsomal enzymes from the aryl-hydrocarbon hydroxylases group, especially of cytochrome P450, has been previously demonstrated (48). The cocarcinogenic effect of  $\beta$ -NF after radon exposure has also been reported (49) in Sprague-Dawley rats exposed to radon and its daughters by inhalation (1000 WLM) over a period of 1 month followed by six intramuscular injections of  $\beta$ -NF (25 mg/kg) at fortnightly intervals. This standardized protocol induced 100% of lung squamous cell carcinomas within 100 days after the end of the treatment. Vascular alterations consisting of vasculitis, with ballooning and destruction of endothelial cells, followed by proliferative hyperplasia, were found in all the rats treated by  $\beta$ -NF. Target cells have been shown to be located at the bronchioloalveolar junction (43). The early lesions consisted of hyperplastic areas of bronchiolar-type cells in the alveolar region, which started near the respiratory bronchioles and then spread to the alveolar ducts and adjacent alveoli. Moreover, a hyperdiploid cell population appeared at an early stage during the spreading of hyperplastic lesions to the adjacent alveoli. Metaplastic squamous cells arose from bronchiolar-type cell hyperplasias, which gradually transformed to poorly differentiated squamous cell nodules, squamous papillomas, and then squamous cell carcinomas (43). On the other hand,  $\beta$ -NF has been recently shown to induce cytochrome P450 1A1 in target cells during the first stages of the cocarcinogenic process (50). The expression of this enzyme disappeared concomitantly with the development of squamous cell metaplasia. Additional studies are still in progress to determine if cytochrome P450 1A1 induction is a primeval step during squamous cell carcinoma carcinogenesis. Such a two-stage model of lung carcinoma seem to be restricted to the Sprague-Dawley strain, as it has not been possible to reproduce it in other strains of rats in which severe lung vasculitis, pleural exsudate, and fatal heart failure were frequently observed after treatment by  $\beta$ -NF (35).

### Mineral Fibers

The experimental protocol was also used to study the potential cocarcinogenic effects of radon and mineral fibers. Acid-leached chrysotile fibers were shown to exhibit less carcinogenic activity *in vivo* than untreated fibers (51,52). Because mesothelial cells are considered target cells for the carcinogenicity by mineral fibers, this experiment was designed to investigate the potential syner-

gistic action of different kinds of unleached or acid-leached asbestos fibers and other mineral dusts injected into the pleural cavity of rats that had previously inhaled radon and its daughters (53). In these experiments, 60 rats exposed to 3000 WLM radon were used as controls, and 10 groups of 10 rats were injected intrapleurally with 2 mg of mineral dust, unleached or leached asbestos fibers, glass fibers, and 2 varieties of quartz 2 weeks after the end of radon exposure at 3000 WLM. No rats were exposed to mineral fibers alone. The results of this study were compared with those of previous experiments in which rats were inoculated intrapleurally with various doses of asbestos and other mineral fibers (54).

In the 157 rats examined microscopically, 83 malignant thoracic tumors were observed: 17 out of 60 animals (28%) in the group of rats which inhaled radon alone, and 66 out of 97 animals (68%) in the group given an intrapleural injection of mineral dust. These tumors were differentiated into lung carcinomas and pleural tumors (Table 4). Lung carcinomas were differentiated into squamous cell carcinomas, bronchioloalveolar carcinomas, and adenocarcinomas. Some tumors displayed mixed patterns that combined squamous cells and adenocarcinomas and were classified as mixed pattern. Pleural tumors were differentiated into typical mesothelioma and combined pulmonary pleural tumors. These consisted of lung carcinomas, of the squamous cell, bronchioloalveolar, or adenocarcinoma type. These tumors exhibited a mesothelial pattern when they spread to the serosal surface of the pleura. This did not allow us to distinguish primitive tumors of the pleura from an extension to the pleura of a pulmonary tumor which mimics the histological pattern of a mesothelioma.

Lung carcinomas, mainly of the squamous cell and bronchioloalveolar types, occurred in all groups. No pleural tumors were observed in rats that inhaled radon only. Typical mesothelioma only occurred in the group of rats injected with asbestos fibers, whereas combined pulmonary pleural tumors were observed both in rats injected with the different mineral fibers, i.e., leached or unleached asbestos and glass fibers, and with the two varieties of quartz. The proportion of lung cancer rose from 28% in rats that inhaled radon only to 68% in those given an intrapleural injection of mineral dust after radon inhalation, demonstrating the synergistic effect of this type of insult.

The carcinogenicity of asbestos at the level of the pleura was amplified when dusts were injected intrapleurally after previous inhalation of radon and its daughters. In rats inoculated with 2 mg of asbestos after inhaling radon, the propor-

**Table 4.** Incidence of the different histological types of tumor in the different groups of rats exposed to radon and mineral dust

	Bronchopulmonary carcinoma				Pleural tumors		No. of rats with tumors
	SCC	BC	AC	MP	CPT	TM	
Radon 3000 WLM only	10	4	0	3	0	0	17/60
SFA chrysotile (54), 2 mg IP	—	—	—	—	—	5	5/12
Crocidolite (54), 2 mg IP	—	—	—	—	—	3	3/12
Radon 3000 WLM + mineral dust							
Chrysotile	1	0	1	0	2	2	6/9
Oxalic acid-leached chrysotile	8	0	1	0	0	0	9/9
Hydrochloric acid-leached chrysotile	3	2	0	0	1	1	7/9
Crocidolite	2	1	0	2	0	3	8/10
Oxalic acid-leached crocidolite	1	0	2	0	3	1	7/10
Amosite	1	1	2	0	2	2	8/10
Oxalic acid-leached amosite	1	1	1	0	0	2	5/10
JM 104 glass fibers	0	3	1	0	2	0	6/10
DQ 12 quartz	2	0	1	1	2	0	6/10
BRGM quartz	0	1	0	0	3	0	4/10

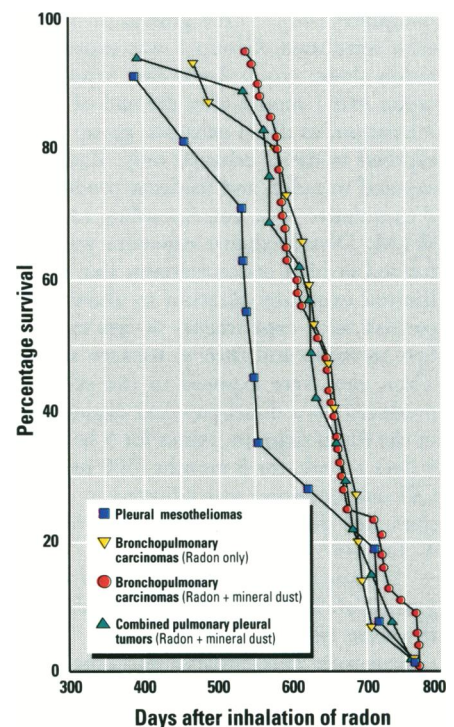
Abbreviations: SCC, squamous cell carcinoma; BC, bronchioloalveolar carcinoma; AC, adenocarcinoma; MP, mixed pattern; CPT, combined pulmonary pleural tumors; TM, typical mesothelioma; IP, intrapleural inoculation; WLM, working-level-month.

tion of pleural tumors was in the same range, about 50%, as after inoculation of 20 mg of these dusts alone (52,54). The fact that typical mesothelioma occurred only in rats intrapleurally inoculated with asbestos fibers must be related to some specific effect of fibrous dusts. Moreover, acid-leached chrysotile, previously demonstrated to be less carcinogenic than untreated chrysotile after intrapleural injection in the rat, was associated with a low incidence of mesotheliomas: none in the group of animals injected with oxalic acid-leached chrysotile and two pleural tumors (one mesothelioma and one combined pulmonary pleural tumor) in the group inoculated with hydrochloric acid-treated chrysotile. In the group inoculated with JM 104 glass fibers, no mesothelioma was found, but two combined pulmonary pleural tumors were observed.

The increased incidence of pulmonary malignancies when radon and dusts were associated compared to radon alone suggests a synergistic effect between radon and dusts, whatever the type, fibrous or not. This effect was noted even in the groups of rats inoculated with two different samples of quartz: the proportion of lung cancers and combined pulmonary pleural tumors were higher in these animals than in those that inhaled radon alone. However, no mesothelioma occurred. In the rats injected intrapleurally with mineral dust, pleural tumors, including both typical mesotheliomas and combined pulmonary pleural tumors, were mostly large (T3, T4), whereas bronchopulmonary carcinomas were fairly evenly distributed among the small (T1, T2) and most invasive (T3, T4) tumors.

As regards survival times, the limited number of rats in groups injected with

mineral dusts made comparison between these groups unreliable. Rats with mesothelioma had a shorter survival time than those with combined pulmonary pleural tumors, whose life span was in turn shorter than that of rats with bronchopulmonary carcinoma, suggesting noticeable differences in survival depending on the histological types of tumors (Fig. 6). In the groups exposed to radon and mineral fibers combined, especially in the group exposed to radon and chrysotile fibers, tumors were



**Figure 6.** Survival time of rats according to the histological type of tumor after exposure to radon 3000 WLM and mineral dust.



almost evenly distributed: about one-third (2/6) bronchopulmonary carcinomas, one-third (2/6) combined pulmonary pleural tumors, and one-third (2/6) pleural mesotheliomas. Thus, combined exposure to radon and mineral fibers resulted in an additive cocarcinogenic effect, showing grossly about one-third of lung carcinomas that could be related to radon exposure, about one-third of typical pleural mesothelioma that could be related to fiber exposure, and another third of combined pulmonary pleural tumors that could be related to the combined effect of radon and mineral dusts at the level of the pleura.

**Diesel Exhausts**

The use of diesel-powered vehicles is steadily increasing worldwide, including use in uranium mines. Among the numerous epidemiological studies on diesel-exhaust-exposed populations, only two, a case-control study (55) and a retrospective cohort study in railroad workers (56), showed significant association between diesel exhaust inhalation and lung cancer, suggesting that occupational exposure to diesel exhausts results in a small but significant excess risk of lung cancer. Experimentally, some evidence of a carcinogenic effect was previously reported in rats after exposure to diesel exhausts containing high concentrations of diesel soot particles for periods of up to 2 years (48–50).

The potential synergistic effects of diesel exhausts were investigated in rats previously exposed to radon and radon daughters (60). Three groups of 50 rats each were used. Group 1 was exposed to radon alone; group 2 was first exposed to radon and 1 month after the end of radon inhalation, to diesel exhausts; group 3 was exposed to diesel exhausts only. Rats were exposed to radon and its decay products at a cumulative dose corresponding to 1000 WLM. Diesel exhaust exposure was performed at high concentrations, but with a limited exposure duration to allow comparison with experiments in rats exposed first to radon and then to tobacco smoke. Thus, rats were exposed to the exhausts produced by a diesel-powered engine used in the Razès uranium mines for 5 hr a day, 5 days a week, for 3 months (300 hr). The CO concentration was adjusted to 20–25 ppm and the diesel particulate burden to 4–5 mg/m<sup>3</sup>.

Histopathologic analysis of the 3 groups of 50 rats revealed 28 malignant thoracic tumors in 25 animals. These tumors were differentiated into lung carcinomas, squamous cell carcinomas, bronchioloalveolar carcinomas, adenocarcinomas and pleural mesotheliomas. The incidence of each histological type of tumor in the different groups of exposed rats showed

**Table 5.** Incidence of the different histological types of malignant thoracic tumor in rats after exposure to radon and diesel exhausts

Group <sup>a</sup>	Bronchopulmonary carcinomas			PM	No. of rats with tumors
	SC	BC	AC		
Radon only (1000 WLM)	3	2	8	—	10
Radon (1000 WLM) and diesel exhausts	2	2	9	1	14
Diesel exhausts only	—	1	—	—	1

Abbreviations: SCC, squamous cell carcinoma; BC, bronchioloalveolar carcinoma; AC, adenocarcinoma; PM, pleural mesothelioma.

<sup>a</sup>Each experimental group comprised 50 rats; WLM; working-level-month.

that lung carcinomas occurred in all groups (Table 5), but only one pleural mesothelioma with a fibrosarcomatous pattern was observed in the group exposed to both radon and diesel exhausts. A slight but nonsignificant increase in the incidence of thoracic tumors was observed in rats after combined exposure to radon and diesel exhausts compared to rats exposed to radon alone. The proportion of rats with thoracic tumors rose from 20% in the group that inhaled radon only to 28% in the group exposed to radon and diesel exhausts combined, but there was only one pleural mesothelioma in the latter group. The proportion of rats with lung carcinomas was 20% in the group exposed to radon alone, but 26% in the group exposed to radon and diesel exhausts combined. However, the number of lung carcinomas was identical in both groups: 13 lung carcinomas among 10 of the rats in the group exposed to radon alone, and 13 among 13 of the rats in the group exposed to radon and diesel exhausts.

In the classification of tumors according to the TNM system, the pleural mesothelioma observed in the group exposed to radon and diesel exhausts was classified as P1. The lung carcinoma observed in the group exposed to diesel exhausts only was classified T2 and spread to the pleura. Similar degrees of tumor extension were observed among the lung carcinomas in the groups exposed to radon only and to radon and diesel exhausts. However, there were more tumors that had spread to the pleura and were classified as P1 and more intrapulmonary metastases or multiple lung tumors classified as M2 in the group

exposed to both radon and diesel exhausts than in the group exposed to radon only. Mean survival times were not noticeably different among animals with lung carcinomas (Table 6), whatever the type of exposure. Rats with no thoracic tumors had shorter survival times than those with lung carcinomas, as previously reported for rats exposed to various doses of radon and its daughters (25,34). Surprisingly, the rats with lung squamous cell carcinomas and lung adenocarcinomas that were exposed to radon only had shorter survival times than those exposed to radon and diesel exhausts. However, it is noteworthy that in rats, lung carcinomas appeared near the end of the life span (34,60). As tumors should grow to a size sufficient for detection, it is possible that the majority of short-lived animals died too soon for tumors, if any, to be detected. The one rat with a pleural mesothelioma had a shorter survival time than the rats with lung carcinomas.

These results showed that exposure to diesel exhausts only did not increase the incidence of lung cancer in rats. Combined exposure to radon and diesel exhausts induced a nonsignificant increase in the incidence of lung carcinomas compared to exposure to radon alone. They did not demonstrate that inhalation of diesel exhausts either alone or after previous radon inhalation has a clearly carcinogenic or cocarcinogenic effect.

**Minerals from Metallic Mine Ores**

It has been suggested that the carcinogenic activity of some inorganic materials might be related to their surface-reducing proper-

**Table 6.** Mean survival times (days) of rats (± SD) according to histological tumor type, after exposure to radon and diesel exhausts

Group	Bronchopulmonary carcinomas			PM	No thoracic tumors
	SC	BC	AC		
Radon only (1000 WLM)	704 ± 83	765 ± 6	692 ± 70	—	631 ± 137
Radon (1000 WLM) and diesel exhausts	768 ± 28	689 ± 44	771 ± 76	611	643 ± 168
Diesel exhausts only	—	711	—	—	698 ± 111

Abbreviations: SCC, squamous cell carcinoma; BC, bronchioloalveolar carcinoma; AC, adenocarcinoma; PM, pleural mesothelioma.

WLM; working-level-month.

ties, allowing the formation of activated species of oxygen (62). The potential carcinogenic or cocarcinogenic role of four minerals present in the ores of metallic mines (nematite, a contaminant of Quebec chrysotile; biotite, present in many granites and in the French uranium ore; iron pyrite, present in various iron and gold ores, and used here after air-aging of the powder; and iron-rich chlorite, present in iron, tungsten, and gold ores) was studied in rats, either alone or after radon exposure, in relation to potential combined exposure for workers in some of these mines.

Five groups of 30 rats each were given 4 intratracheal instillations of mineral dust, 10 mg each, suspended in phosphate-buffered saline (PBS), and 5 other groups of rats were given 4 intratracheal injections of the same mineral dusts, 1 month after the end of a 1000 WLM radon exposure. In control rats treated by PBS buffer only, two lung carcinomas were observed, a squamous cell carcinoma and an adenocarcinoma. In the groups treated with mineral dust alone, only lung carcinomas were observed: a squamous cell carcinoma in the group treated with air-aged iron pyrite and an adenocarcinoma in the group treated with chlorite (Table 7). In the group exposed to radon and PBS buffer, nine lung carcinomas were observed among five rats. In the groups treated with mineral dusts after previous radon inhalation, lung carcinomas but also one pleural mesothelioma were observed. A slight nonsignificant increase in the incidence of lung carcinomas was observed in rats exposed to both radon and minerals, especially nemalite, air-aged iron pyrite, and chlorite, compared to rats exposed to radon and PBS buffer. The occurrence of a pleural mesothelioma in the group exposed to biotite might be related to a specific carcinogenic effect of mineral dust at the level of the pleura. In the groups injected with mineral dusts after radon exposure, lung carcinomas were mostly large and more invasive and classified as T3 and T4 compared with those observed in the group treated by radon and PBS buffer (Table 8). There were also more tumors that had spread to the pleura and were classified as P1 and more intrapulmonary metastases or multiple lung tumors classified as M2 in the group exposed to both radon and mineral dusts than in the group exposed to radon and PBS buffer.

In control rats exposed to PBS buffer alone, survival times of rats with lung carcinomas were not shorter compared with those without thoracic tumors (Table 9). In the groups exposed to mineral dusts alone, survival times of rats with squamous cell carcinomas were markedly shorter

**Table 7.** Incidence of the different histological types of malignant thoracic tumor in rats after exposure to radon and minerals from metallic mine ores

	Bronchopulmonary carcinomas			PM	No. of rats with tumors
	SC	BC	AC		
Controls (PBS, IT)	1	—	1	—	2/32
Nematite (IT)	—	—	—	—	0/20
Iron pyrite (IT)	1	—	—	—	1/22
Biotite (IT)	—	—	—	—	0/30
Chlorite (IT)	—	—	1	—	1/30
Radon 1000 WLM only (historical group)	3	2	8	—	10/50
Radon (1000 WLM) and PBS (IT)	1	6	2	—	5/30
Radon (1000 WLM) and nemalite (IT)	4	2	3	—	9/30
Radon (1000 WLM) and iron pyrite (IT)	1	2	6	—	8/30
Radon (1000 WLM) and biotite (IT)	2	3	3	1	7/30
Radon (1000 WLM) and chlorite (IT)	1	2	6	—	8/30

Abbreviations: SCC, squamous cell carcinoma; BC, bronchiolalveolar carcinoma; AC, adenocarcinoma; PM, pleural mesothelioma; WLM, working-level-month; PBS, phosphate-buffered saline; IT, intratracheal.

**Table 8.** Incidence of bronchopulmonary carcinomas in rats after exposure to radon and minerals from metallic mine ores, according to the tumor-node-metastases (TNM) system<sup>a</sup>

	T1	T2	T3	T4	P1	N1	M1	M2	M3
Controls (PBS, IT)	1	1	—	—	—	—	—	—	—
Nematite (IT)	—	—	—	—	—	—	—	—	—
Iron pyrite (IT)	—	—	—	1	1	1	—	1	—
Biotite (IT)	—	—	—	—	—	—	—	—	—
Chlorite (IT)	—	—	—	1	1	1	—	1	—
Radon 1000 WLM only (historical group)	2	5	4	2	1	—	—	1	—
Radon (1000 WLM) and PBS (IT)	6	2	—	1	1	1	—	2	—
Radon (1000 WLM) and nemalite (IT)	4	3	1	1	2	1	—	1	—
Radon (1000 WLM) and iron pyrite (IT)	4	2	—	3	3	1	—	2	—
Radon (1000 WLM) and biotite (IT)	4	—	3	1	1	—	—	—	1
Radon (1000 WLM) and chlorite (IT)	5	—	1	3	2	1	—	1	—

Abbreviations: WLM, working-level-month; PBS, phosphate-buffered saline; IT, intratracheal.

<sup>a</sup>See text for definition of TNM categories.

compared with controls. In the groups of rats exposed to radon and mineral dusts, rats with no thoracic tumors had shorter survival times than those with lung carcinomas, as previously reported for rats exposed to various doses of radon and its daughters. However, survival times of rats with large and invasive squamous cell carcinomas were markedly shorter compared with controls.

**Conclusion**

These studies showed that lung carcinomas can be induced in rats by exposure to radon and its daughters. A dose-effect relationship was established for cumulative doses corresponding to exposures varying

from 25 to 3000 WLM. An excess of lung cancer was observed in rats exposed to cumulative doses similar to those to which uranium miners are exposed, but only at a high dose rate. For low doses, exposure at low dose rates resulted in fewer induced lung carcinomas than similar exposure at higher dose rates. In male rats, radon exposure induced mainly lung carcinomas and few extrapulmonary tumors, whereas in females, for the same radon exposure, only few lung carcinomas but a markedly increased incidence of mammary tissue adenocarcinomas were observed.

These results also demonstrated the potential cocarcinogenic action of various environmental or industrial airborne pollu-



**Table 9.** Mean survival times (days) of rats ( $\pm$  SD) according to histological tumor type after exposure to radon and minerals from metallic mine ores

	Bronchopulmonary carcinomas			PM	No thoracic tumor
	SC	BC	AC		
Controls (PBS, IT)	758	—	782	—	757 $\pm$ 123
Nemalite (IT)	—	—	—	—	768 $\pm$ 93
Iron pyrite (IT)	618	—	—	—	712 $\pm$ 135
Biotite (IT)	—	—	—	—	785 $\pm$ 79
Chlorite (IT)	369	—	—	—	799 $\pm$ 83
Radon 1000 WLM only (historical group)	704 $\pm$ 83	765 $\pm$ 6	692 $\pm$ 70	—	631 $\pm$ 137
Radon (1000 WLM) and PBS (IT)	807	863 $\pm$ 43	888	—	721 $\pm$ 145
Radon (1000 WLM) and nemalite (IT)	729 $\pm$ 65	865 $\pm$ 15	746 $\pm$ 38	—	753 $\pm$ 93
Radon (1000 WLM) iron pyrite (IT)	535	896 $\pm$ 11	768 $\pm$ 174	—	716 $\pm$ 140
Radon (1000 WLM) and biotite (IT)	869 $\pm$ 16	789 $\pm$ 93	864 $\pm$ 16	846	692 $\pm$ 121
Radon (100 WLM) and chlorite (IT)	456	775 $\pm$ 27	751 $\pm$ 140	—	735 $\pm$ 116

Abbreviations: SCC, squamous cell carcinoma; BC, bronchioloalveolar carcinoma; AC, adenocarcinoma; PM, pleural mesothelioma; WLM, working-level-month; PBS, phosphate-buffered saline; IT, intratracheal.

tants combined with radon exposure, showing either a multiplicative, an additive, or no effect. The strongest cocarcinogenic effect was shown by combined exposure to radon and then to tobacco smoke. Cocarcinogenic effects resulted in an increased incidence of lung carcinomas, mainly of the squamous cell type. These results also pointed out the validity of this radon model to investigate possible interactions between two occupational exposures. The interest of such an experimental model for risk assessment should be emphasized since human industrial occupational exposures are nearly always not single but multiple exposures.

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## European Organization for Research and Treatment of Cancer

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