INTRODUCTION

An association between an excess risk of lung cancer and exposure to radon and its progeny has been demonstrated in uranium miners and other miners (1, 2). In various countries, measurements in dwellings have shown that in many homes, the radon concentrations are only one or two orders of magnitude lower than in typical underground mine situations (3). The risk related to residential exposure was estimated from the risk projections established from underground miners data in association with measurements of indoor radon concentrations. However, exposures and exposure rates were generally higher in mines compared with generally lower exposures and exposure rates in homes. The pooled analysis of data from 11 cohorts of underground miners showed that the excess relative risk (ERR) of lung cancer increased as the exposure rate decreased. This pattern called protraction enhancement effect or inverse dose-rate effect was obvious at high cumulative exposures (4) but a diminution of this effect was observed for cumulative exposures below 0.18 J h m^{-3} (50 WLM). To address concerns about extrapolating risk from miners, numerous epidemiological case-control studies were launched during the last 10 years to assess directly lung cancer risk from indoor radon, but the results are equivocal. Some recent case-control studies supported a small but significant excess risk of lung cancer from residential radon (5-8), while others reported no increased risk (9-12). The results of a meta-analysis (13) pooling eight case-control studies showed that the exposure-response trend was similar to model-based extrapolations from miners and to relative risk estimates computed directly from miners with low cumulative exposures. However, these results should be interpreted cautiously, until additional studies will be reported and the pooling of original data from different studies completed. The role of domestic radon exposure in the occurrence of lung cancer remains largely unclear.

It has been emphasised by different scientific committees that more certain understanding of the risks of indoor radon will not come from epidemiological research alone. Experimental animal studies were used in addition to epidemiological studies to investigate the effects of exposure, exposure rate and other factors in predicting risks resulting from human exposures both in the home and in the workplace. The advantage of animal data is that animal experiments are generally conducted under carefully controlled conditions and that exposure and exposure rate can be estimated more accurately. Radon animal data were obtained primarily in adult rats, and were provided mainly by the Pacific Northwest National Laboratory (PNNL, formerly PNL), in USA and our laboratory in France (14). This paper summarises the main data and the results of ongoing experiments on the influence of exposure rate on lung cancer induction.

Despite the fact that significant cumulative exposures at typical residential exposure rates, in the range of about 1.8 \times 10^{-5} J h m^{-3} per week (approximately 0.005 WLM per week) cannot be tested in a short-lived species like the rat, the rat model is valuable for reducing the uncertainties that exist in human data, particularly in regard to the exposure-rate effect. In PNL life-span animal experiments, a trend towards increasing tumour risk in relation with decreased exposure rate has been reported in Wistar rats exposed at potential alpha energy concentrations (PAEC) of 2.1 mJ m^{-3} (100 WL) and 21 mJ m^{-3} (1,000 WL), and at cumulative exposures varying from 2.3 J h m^{-3} (640 WLM) up to 18.4 J h m^{-3} (5,120 WLM) (15).

A similar trend was observed in our experiments in Sprague-Dawley rats which were exposed at cumulative exposures ranging from 3.6 J h m^{-3} (1,000 WLM) up to 21.6 J h m^{-3} (6,000 WLM) and high exposure rates varying from 0.25 mJ h m^{-3} (70 WLM per week) to 0.9 mJ m^{-3} (250 WLM per week) (6). In contrast, the results obtained at low cumulative exposure, comparable to current domestic indoor exposures showed no evidence of an inverse exposure-rate effect. Indeed, chronic radon exposure at 0.09 J h m^{-3} (25 WLM), protracted over a 18 months period at an alpha potential energy of 0.042 mJ m^{-3} (2 WL), resulted in fewer lung carcinomas in rats than a similar cumulative exposure delivered over a 4 to 6 months period at a PAEC of 2.1 mJ m^{-3} (100 WL) (16). The lung cancer incidence in rats exposed at low exposure rate (0.60%) was slightly lower than that in control animals (0.63%).
MATERIALS AND METHODS

Under the Fourth CEC Research and Development Framework Programme, a new series of experiments was carried out to investigate specifically the influence of exposure rate on lung cancer induction in rats. These studies were conducted at relatively low cumulative exposure comparable to lifetime exposures in high-radon houses or current underground mining exposures of about 0.36 J h m\(^{-3}\) (100 WLM). The animal experiments were conducted concomitantly both at CEA (France) and AEA-Technology, plc (Harwell, UK). Where possible, the experimental conditions used at the two laboratories were similar, for example both groups used rats of the same strain, sex and age. In addition, the metrology of the radon exposure atmospheres and the reporting of pathology were standardised between the two groups. The principal differences between the exposure conditions were that exposures were conducted during the working day at CEA without introduction of carrier aerosol and continuously (24 hours per day) using Carnauba wax as carrier aerosol at Harwell. The preliminary results of the experiments conducted at CEA are reported below.

**Radon exposure**

Exposures were designed to investigate the role of PAEC and protraction of exposure which have been demonstrated to be the main important parameters for lung cancer induction in experimental animals. All the CEA animal exposures were performed at the CEA-University of Limoges radon inhalation facility located in Razès (France). Radon gas emanation from uranium ore was introduced into the 10 m\(^{3}\) stainless steel chambers through a dilution system and the radon progeny were attached to the ambient aerosol (natural aerosol). The duration of exposure sessions was 6 hours. Exposures were conducted under static conditions without air renewal in the chambers. During the exposures, monitoring of the potential alpha energy concentration (PAEC), equilibrium factor F, unattached fraction f\(_p\), radon progeny concentrations and environmental conditions were performed using recognised methods agreed between AEA and CEA in previous metrology inter-comparison exercises (17). Inhalation parameters of the different exposure groups are summarised in Table 1.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>No. of rats</th>
<th>Age at start of exposure (months)</th>
<th>Cumulative exposure mJ h m(^{-3}) WLM</th>
<th>PAEC mJ m(^{-3}) WL</th>
<th>F</th>
<th>f(_p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr. 0 (RnCl) (^{(a)})</td>
<td>120</td>
<td>-</td>
<td>(\approx 0.9) (\approx 0.25)</td>
<td>(\approx 0.0004) (\approx 0.002)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gr. 1 (RnPC) (^{(b)})</td>
<td>240</td>
<td>3</td>
<td>378</td>
<td>105</td>
<td>3.91 ± 1.25</td>
<td>188 ± 60</td>
</tr>
<tr>
<td>Gr. 2 (RnFr) (^{(c)})</td>
<td>240</td>
<td>3</td>
<td>385</td>
<td>107</td>
<td>3.05 ± 0.95</td>
<td>147 ± 46</td>
</tr>
<tr>
<td>Gr. 3 (RnD3) (^{(d)})</td>
<td>240</td>
<td>3</td>
<td>361</td>
<td>100</td>
<td>1.21 ± 0.40</td>
<td>58.3 ± 19.4</td>
</tr>
<tr>
<td>Gr. 4 (RnD12) (^{(e)})</td>
<td>240</td>
<td>2.5</td>
<td>358</td>
<td>100</td>
<td>0.27 ± 0.01</td>
<td>13 ± 0.01</td>
</tr>
<tr>
<td>Gr. 5 (RnD6) (^{(f)})</td>
<td>211</td>
<td>3</td>
<td>151</td>
<td>42</td>
<td>0.37 ± 0.16</td>
<td>18.0 ± 8.0</td>
</tr>
</tbody>
</table>

\(^{(a)}\) Group 0 : untreated controls  
\(^{(b)}\) Group 1 : exposed to radon from 29-04-1996 to 28-05-1996  
\(^{(c)}\) Group 2 : exposed to radon from 11-07-1996 to 02-10-1996  
\(^{(d)}\) Group 3 : exposed to radon from 03-03-1997 to 03-06-1997  
\(^{(e)}\) Group 4 : exposed to radon from 01-12-1997 to 11-12-1998  
\(^{(f)}\) Group 5 : exposed to radon from 29-04-1996 to 14-10-1996
In these studies, experiments were performed at a cumulative exposure of 360 mJ h m\(^{-3}\) (100 WLM) and PAEC varying from 0.22 mJ m\(^{-3}\) (12-13 WL) to 3.15 mJ m\(^{-3}\) (150 WL). Group 0 (RnCt) was an unexposed control group. The cumulative radon exposure of this group was estimated to be about 0.9 mJ h m\(^{-3}\) (0.25 WLM). Group 1 (RnPC) was used as a positive control group and was exposed to radon and progeny at a cumulative exposure of about 360 mJ h m\(^{-3}\) (100 WLM) and high PAEC of 3.15 mJ m\(^{-3}\) (150 WL), which was expected to induce a lung cancer incidence of about 10%. Group 2 (RnFr) was exposed at similar cumulative exposure and similar PAEC as Group 1 [360 mJ h m\(^{-3}\) (100 WLM); 3.15 mJ m\(^{-3}\) (150 WL)], but the exposure of this group was protracted over a 3 months period, at 1 or 2 sessions per week, instead of 5 sessions per week delivered for 4 weeks in Group 1. Group 3 (RnD3) was exposed at similar cumulative radon exposure of 360 mJ h m\(^{-3}\) (100 WLM), but at a lower PAEC of about 1.2 mJ m\(^{-3}\) (50 WL). Group 4 (RnD12) was exposed at similar cumulative radon exposure of 360 mJ h m\(^{-3}\) (100 WLM), but at a lower PAEC of about 0.27 mJ m\(^{-3}\) (13 WL). Group 5 (RnD6) was initially scheduled to be exposed at the same cumulative exposure of 360 mJ h m\(^{-3}\) (100 WLM) as other groups, but at a lower PAEC of about 0.3 mJ m\(^{-3}\) (15 WL). In fact, due to works for renewal and refurbishment of the radon inhalation facility, the exposure of this group was stopped at a cumulative exposure of 151 mJ h m\(^{-3}\) (42 WLM). However, this point should be very informative, since in our experience, we did not have data on experiments conducted at such cumulative exposure and PAEC.

**Animals and histologic analysis**

Exposed rats were 12 week-old, male, specific-pathogen-free Sprague-Dawley rats (Ico: OFA SD, IFFA-CREDO, France). All the experiments were performed according to the order N° 87.848 of the French legislation and the European Directive 86/609/EEC about the Care and Use of Laboratory Animals. All the experimental procedures were in agreement with the recommendations given in Health Monitoring of Laboratory Animals of the Federation of European Laboratory Animal Science (FELASA) concerning viral, parasitological, bacterial and fungal infections. During exposure, rats were housed in wire stainless steel cages within the inhalation chambers. Litter consisted in sawdust removed daily before the beginning of exposure. Food (AO4 from UAR, France) and water were given freely. After exposure, rats were kept and regularly observed until death and euthanised when moribund. Necropsies consisted of a complete examination of all the organs and recording any abnormalities. The lungs were carefully observed and any nodules detected by a gentle palpation. Lungs, selected organs and organs with suspicious lesions were taken systematically for histopathological examination. Lungs were fixed *in situ* by intratracheal instillation of 10% neutral buffered formalin (NBF). Thoracic lymph nodes and surrounding tissues from the mediastinum, including heart, were fixed all together. If no lesion was observed, samples from liver, spleen, kidneys and the whole brain were fixed in NBF after all the organs had been systematically weighted. Any suspicious lesion from other organs was taken and fixed. Sagittal sections of the nasal and paranasal cavities were performed and any macroscopic lesion fixed. Tissues were fixed in NBF by immersion before processing and embedding in paraffin wax. Serial 5-µm thick sections were performed taking care to trim only the sufficient tissue for histopathological diagnosis in order to keep remaining tissue from the lesion available for further studies on biological markers. Routine process consisted in haematoxylin-eosin-saffron staining. In addition, selected special histochemical stainings including Alcian-blue for mucus detection in adenocarcinoma and/or immunohistochemical methods were used. Proliferative preneoplastic lesions and lung tumours were classified according to the classification published in the EULEP Color Atlas (18).

**RESULTS**

These studies are not yet fully completed. However, the majority of rats have died or were killed when moribund and then autopsied. In the first four experimental groups, group 0 (RnCt), and groups 1 (RnPC), 2 (RnFr), 3 (RnD3), and 5 (RnD6) all the rats have been autopsied. In contrast, in Group 4 (RnD12), about 32.5% of the rats, 78 of 240, are still alive. Table 2 shows the distribution of lung tumours larger than 5 mm in diameter at macroscopic examination. In our experience, lung tumours larger than 5 mm in diameter at autopsy were found to be almost exclusively malignant tumours.
Table 2. Distribution of macroscopic lung tumours with a diameter larger than 5 mm observed in rats at macroscopic examination within the different experimental groups.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Number of rats with lung tumours Ø ≥ 5 mm</th>
<th>Number of rats with single lung tumours</th>
<th>Number of rats with multiple lung tumours</th>
<th>Total number of tumours Ø ≥ 5 mm</th>
<th>Proportion (%) of tumours Ø ≥ 5 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 0 (RnCt)</td>
<td>0 / 120</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 1 (RnPC)</td>
<td>22 / 240</td>
<td>22</td>
<td>2</td>
<td>24</td>
<td>10.0</td>
</tr>
<tr>
<td>Group 2 (RnFr)</td>
<td>13 / 240</td>
<td>13</td>
<td>0</td>
<td>13</td>
<td>5.41</td>
</tr>
<tr>
<td>Group 3 (RnD3)</td>
<td>8 / 240</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>3.33</td>
</tr>
<tr>
<td>Group 4 (RnD12)</td>
<td>2 / 162</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1.24</td>
</tr>
<tr>
<td>Group 5 (RnD6)</td>
<td>5 / 211</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>2.36</td>
</tr>
</tbody>
</table>

In rats exposed to similar cumulative exposure and decreasing PAEC, the proportion of lung tumours larger than 5 mm at macroscopic examination decreases from 10% in Group 1 (RnPC) to 3.33% in Group 3 (RnD3) and 1.24% in Group 4 (RnD12). However, this last group should be regarded cautiously since all the rats from this group have not yet been autopsied. On the other hand, in group 2 (RnFr) exposed to radon at similar cumulative exposure and PAEC as group 1 (RnPC), but with exposure protracted over a 3-months period, the incidence of macroscopic lung tumours (5.41%) is marginally significantly lower than that of Group 1 (10%). In group 5 (RnD6) exposed at lower cumulative exposure of 151 mJ h m⁻³ (42 WLM) and lower PAEC of 0.37 mJ m⁻³ (18 WLM) than other groups, 5 lung tumours larger than 5 mm at macroscopic examination (2.36%) were observed.

In these experiments, the histopathological study is still in progress. All the tumours confirmed at histopathological examination as being lung carcinomas were tumours larger than 5 mm in diameter at macroscopic examination. The distribution of the histological types of lung carcinomas observed until now in the different experimental groups is listed in Table 3.

Table 3. Distribution of the histological types of lung carcinomas between the different experimental groups.

<table>
<thead>
<tr>
<th></th>
<th>Squamous cell carcinomas</th>
<th>Adenosquamous carcinomas</th>
<th>Adenocarcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (RnPC)</td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Group 2 (RnFr)</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Group 3 (RnD3)</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Group 5 (RnD6)</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Until now, in group 1 (RnPC), exposed at a 378 mJ h m⁻³ (105 WLM) cumulative exposure and high PAEC of 3.91 mJ m⁻³ (188 WL), 6 squamous cell carcinomas, 1 adenosquamous carcinoma and 7 adenocarcinomas were observed. In group 2 (RnFr), exposed at a 385 mJ h m⁻³ (107 WLM) cumulative exposure and similar PAEC of 3.06 mJ m⁻³ (147 WL) but protracted over a 3 months period, 1 squamous cell carcinoma, 1 adenosquamous carcinoma and 3 adenocarcinomas were observed. In group 3 (RnD3) exposed at 361 mJ h m⁻³ (100 WLM) but lower PAEC of 1.21 mJ m⁻³ (58 WL), 2 papillary adenocarcinomas were observed.
In group 5 (RnD6), exposed at 151 mJ h m^-3 (42 WLM) but lower PAEC of 0.37 mJ m^-3 (18 WL), 2 papillary adenocarcinomas were also observed. It should be pointed out that squamous cell carcinomas were observed only in rats exposed at high exposure rate. A full statistical analysis of the survival and tumour incidences of this study will not be possible until all animals have been analysed.

DISCUSSION
The ongoing studies on the influence of exposure rate are not yet fully completed and the histopathology study is still in progress. Full statistical analysis of all animals is required before full conclusions can be drawn. However, on the basis of autopsy macroscopic findings and of preliminary histopathological results, the results of this study could be compared with those of an historical control group of 785 rats and with those of previous experiments in rats exposed at various cumulative exposures and exposure rates.

The preliminary results of these studies indicate that at relatively low cumulative exposures of about 0.36 J h m^-3 (100 WLM), comparable to lifetime exposures in high-radon houses or current underground mining exposures, the risk of lung cancer in rats decreases with PAEC, i.e., exposure rate. They also confirm the results of previous experiments conducted at lower cumulative exposure (16), which showed that for the same cumulative exposure of 0.09 J h m^-3 (25 WLM), the relative risk (RR) of lung cancer decreases from 4.45 in rats exposed at 3.15 mJ m^-3 (150 WL), to 3.48 in rats exposed at 2.1 mJ m^-3 (100 WL) and to 0.94 in rats exposed at 0.042 mJ m^-3 (2 WL). In addition, these preliminary results indicate that the risk of lung tumour induction in rats is maximum for cumulative exposures ranging from 0.09 J h m^-3 (25 WLM) up to 360 mJ h m^-3 (100 WLM), and PAEC ranging from 1.05 mJ m^-3 (50 WL) up to 3.15 mJ m^-3 (150 WL), i.e., exposure rates ranging from 18 mJ h m^-3 per week (5 WLM per week) and 90 mJ h m^-3 per week (25 WLM per week). These data suggest that the induction of lung cancer results from a complex interplay between cumulative exposure and exposure rate, with an optimal combination of exposure rate at a given exposure level.

The significance of exposure rates in assessing the hazards of domestic radon exposure was addressed on biophysical grounds by Brenner (19), who concluded that, when cumulative exposures are sufficiently low that multiple traversals of target cells by alpha particles are rare - that is the case for typical domestic radon exposures - , all exposure-rate enhancement effects disappear. The results of recent experiments performed at Columbia University by Miller et al. using a micro-beam source (20), showed that traversal of cell nuclei by a single alpha particle induced significantly lower oncogenic transformation in the C3H10T1/2 mouse fibroblast system than does a Poisson-distributed mean of one alpha particle, suggesting that cells traversed by multiple alpha particles contribute most to the risk.

In this respect, based on dose-rate effect considerations, extrapolation of lower exposure-rate miner data to residential exposures - where no target cell is traversed by more than a single alpha particle - may overestimate risks associated with typical residential exposures and exposure rates. Our recent data in rats appear to follow this same trend and to support the hypothesis that, at low doses, the risk of lung cancer is governed by the rate at which the dose is delivered, and not by the total cumulative dose alone. Likewise, recent data from R. Mitchell et al. (21) following chronic exposures to high concentrations of natural uranium ore dust alone also indicate that malignant lung tumour risks are not directly proportional to dose, but are directly proportional to dose rate.

These data are also consistent with that of underground miners (4) showing an inverse dose-rate effect at high cumulative exposures, but a diminution of this effect at cumulative exposures lower than 0.18 J h m^-3 (50 WLM). They support both the presence of an inverse dose-rate effect at high cumulative exposure, as well as its diminution or disappearance at low cumulative exposures.
REFERENCES


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