Investigation of Cancer Epidemiology and Study of Carcinogenic Agents in the Shanghai Rubber Industry

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ABSTRACT

Preliminary studies on crude cancer incidences among workers from 89 factories in Shanghai revealed excessive risk of cancer for workers in certain workshops of rubber tire factories. Chronic in situ animal exposures showed that compounding and Banbury mills for mastication and mixing were origins of carcinogenic contaminants. Various chronic experiments indicated the carcinogenicity of PBNA in rats and mice, especially with regard to the lungs. The high concentration of PBNA in the atmosphere of the work area seemed to be related to the excessive incidence of lung cancer among the workers. Epidemiological investigation showed that there was an excessive number of cases of lung cancer in the workshop of rubber tire factories where compounding, mixing, and milling took place.

INTRODUCTION

In the spring of 1970, a collaborative research group was organized in Shanghai to study occupational cancers. Participants in the group consisted of members of the Shanghai Institute of Experimental Biology (Academia Sinica) and other medical institutions in Shanghai.

After a preliminary retrospective epidemiological study (1961 to 1970) of crude cancer incidences in 146,292 workers from 89 different factories in Shanghai, with about 420,000 other Shanghai inhabitants over 20 years of age used as controls, it was found that workers exposed to pitch and coal tar had more cancer of the stomach, lungs, liver, and esophagus than did the controls; however, no increase in skin cancer was observed. In some rubber factories, especially rubber tire factories, cancer incidence was apparently elevated. Therefore, Rubber Tire Factory A was selected for intensive investigation.

In Situ Animal Exposures

Epidemiological studies on crude cancer incidences in different workshops of 3 rubber tire factories revealed that the first workshop, where compounding, mixing, and milling took place, was always the site with the highest relative cancer incidence among all workshops of the factory. Since the processing in this workshop involves the use of many kinds of materials and drastic physical treatment generating heat, gases, vapors, and dust, an attempt was made to locate the sites of liberation of carcinogenic agents into the atmosphere of the work area. Separate in situ animal exposure experiments were carried out at 3 suspected sites: the compounding room (Fig. 1); the Banbury mill for mastication (Fig. 2); and the Banbury mill for mixing. Young adult albino rats were kept day and night at the suspected sites unless room temperature exceeded 37⁰. Control animals were given the same stock feed and tap water as were the experimental rats but were kept in the animal house of the Institute of Experimental Biology. Each experiment lasted for 2 years; then, all animals were sacrificed and underwent detailed anatomical and histological examination. Animals dying or which had died earlier were similarly examined. As is shown in Table 1, rats kept at all 3 suspected sites for 2 years developed significantly more carcinomas, but not sarcomas, than did control rats. The induced carcinomas appeared in diverse internal organs, including the lungs (Fig. 3), stomach, cecum, pancreas, esophagus, prostate and seminal vesicle, kidneys, and the thyroid and adrenal glands. Liver and bladder carcinomas were not observed. A significant incidence of lung carcinomas and gastrocecal ulcers appeared at both Banbury mills but not in the compounding room.

Different groups of additives were involved at the 3 suspected sites. In the compounding room, all solid additives except carbon black were weighed out and mixed in various portions. In the Banbury mill for mastication, only ZnO and Accelerator M were added to the raw rubber. In the Banbury mill for mixing, all the rest of the additives (including PBNA²) were added to the master batch. Therefore, some suspected additives were separately tested by chronic animal experiments in different institutes.

After conclusion of the in situ animal exposures, reconstruction of workshop 1 was begun with improved ventilation and dust removal.

Chronic Animal Experiments with Technical Antioxidant D (Containing 98% PBNA)

These experiments were carried out in 2 separate institutes with consistent positive results. In chronic feeding experiments by gastric intubation, significantly more carcinomas and gastrocecal ulcers were induced in young adult male albino (2 years) and Wistar (1.5 years) rats than in the respective control rats. It was striking that carcinomas of the lung, prostate (Fig. 4), pancreas (Fig. 5), and kidney appeared frequently but liver and bladder cancer each occurred only in one experimental animal (3, 5). Chronic feeding of male C57BL mice induced significantly more carcinomas than it did in the controls. Both carcinomas and hemangiosarcomas of the liver were induced, as well as carcinomas of the pancreas, salivary gland, and kidney. However, lung carcinomas ranked first among the tumors induced, and the difference from their incidence in the controls was significant at p = 0.05 (5). Chronic inhalation of the aerosol of antioxidant D (180 to 230 mg/cu m) induced lung carcinomas in 27.4% of the experimental animals, the finding being statistically significant (p < 0.05) (10). Repeated gastric intubations of an oil solution of antioxidant D with weekly intubations of a small dose of CCl₄ solution induced cirrhosis of the liver in 67.5% of the male rats, and liver cancer developed in 74% of the cirrhotic

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Table 1
In situ animal exposures at suspected places in Factory A

<table>
<thead>
<tr>
<th>Place of exposure</th>
<th>No. of rats</th>
<th>No. %</th>
<th>Respiratory</th>
<th>Digestive</th>
<th>Urinary</th>
<th>Genital</th>
<th>Endocrine</th>
<th>Sarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banbury mill for mastication</td>
<td>68</td>
<td>24</td>
<td>35.3</td>
<td>13</td>
<td>8</td>
<td>11.4</td>
<td>1.5</td>
<td>9</td>
</tr>
<tr>
<td>Banbury mill for mixing</td>
<td>65</td>
<td>21</td>
<td>32.3</td>
<td>12</td>
<td>8</td>
<td>18.0</td>
<td>3.0</td>
<td>6</td>
</tr>
<tr>
<td>Compounding room</td>
<td>62</td>
<td>17</td>
<td>27.4</td>
<td>1</td>
<td>1.6</td>
<td>2</td>
<td>3.2</td>
<td>1.6</td>
</tr>
<tr>
<td>Natural control</td>
<td>62</td>
<td>2</td>
<td>3.2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1.6</td>
<td>1</td>
</tr>
</tbody>
</table>

* Difference between experimental and control groups, p < 0.01.
** Difference between experimental and control groups, p < 0.05.

Table 2
Carcinogenicity of PBNA to experimental animals

<table>
<thead>
<tr>
<th>Chemical testeda</th>
<th>Animal used</th>
<th>Method of experiment</th>
<th>Duration</th>
<th>Group</th>
<th>No. of tumor cases/ experiment</th>
<th>No. of tumor sites/ experiment</th>
<th>Predominant tumor</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technical PBNA</td>
<td>Male albino rats</td>
<td>Repeated gastric intubation of 8% oil solution of PBNA</td>
<td>Intubation, 18 mo; experiment, 24 mo</td>
<td>Experimental Oi-injected control</td>
<td>27/57</td>
<td>6/43</td>
<td>Carcinoma of lung, prostate, kidney, pancreas Sarcoma</td>
<td>5</td>
</tr>
<tr>
<td>Partially purified PBNA</td>
<td>Male Wistar rats</td>
<td>As above, in larger doses</td>
<td>Intubation, 12 mo; experiment, 18 mo</td>
<td>Experimental Oi-injected control</td>
<td>29/57</td>
<td>36/57</td>
<td>Carcinoma of lung, kidney Sarcoma</td>
<td>3</td>
</tr>
<tr>
<td>Technical PBNA</td>
<td>Male C57BL mice</td>
<td>Inhalation of PBNA aerosol, 180-230 mg/cm²</td>
<td>5/wk inhalation 1 yr; experiment, 22 mo</td>
<td>Experimental Natural control</td>
<td>23/51</td>
<td>7/47</td>
<td>Carcinoma of lung Sarcoma</td>
<td>10</td>
</tr>
<tr>
<td>Technical PBNA</td>
<td>Male C57BL mice</td>
<td>Fed in diet in increasing concentrations</td>
<td>Feeding, 18 mo; experiment, 24 mo</td>
<td>Experimental Natural control</td>
<td>48/67</td>
<td>4/19</td>
<td>Carcinoma of lung, liver Sarcoma</td>
<td>6</td>
</tr>
<tr>
<td>Technical PBNA</td>
<td>Male albino rats</td>
<td>Gastric intubation of oil solution of PBNA (4/wk); intubation of CCl₄ (1/wk)</td>
<td>Intubation, 12 mo; experiment, 18 mo</td>
<td>Experimental CCl₄ control</td>
<td>34/40</td>
<td>1/20</td>
<td>Cirrhosis of liver, carcinoma of liver Sarcoma</td>
<td>7</td>
</tr>
<tr>
<td>Technical PBNA</td>
<td>Female albino rats</td>
<td>As above</td>
<td>As above</td>
<td>Experimental CCl₄ control</td>
<td>12/15</td>
<td>0/16</td>
<td>Carcinoma of lung</td>
<td>10</td>
</tr>
<tr>
<td>Technical PBNA</td>
<td>Male ICR mice</td>
<td>Repeated hypodermic injections of DMSO solution of PBNA</td>
<td>10 mo</td>
<td>Experimental DMSO control</td>
<td>9/20</td>
<td>0/20</td>
<td>Carcinoma of lung</td>
<td>8</td>
</tr>
<tr>
<td>Pure PBNA</td>
<td>Male ICR mice</td>
<td>As above</td>
<td>As above</td>
<td>Experimental DMSO control</td>
<td>9/20</td>
<td>0/20</td>
<td>Carcinoma of lung</td>
<td>8</td>
</tr>
</tbody>
</table>

* All samples used were free of 2-naphthylamine as shown by high-pressure liquid chromatography.
** Difference between experimental and control groups, p < 0.01.
*** Difference between experimental and control groups, p < 0.05.
† DMSO, dimethyl sulfoxide.

There seemed to be a dose-response relationship in the PBNA-injected mice. Thus, the main constituent of antioxidant D, PBNA, was found to be carcinogetic to the mouse. Its induction of lung carcinomas seems to be consistent with results of other chronic experiments with antioxidant D (4). The experiments are summarized in Table 2.

Determination of PBNA in the Atmosphere of the Work Area and Studies on the Volatility of PBNA

Using thin-layer chromatography followed by UV spectrophotometry, a sensitive and convenient method for quantitative determination of PBNA in the air was designed (2). At 10 different
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sites in Workshop 1 of Factory A, the concentration of PBNA in the atmosphere of the work area was determined separately, and the average values of 20 samples at each site were compared. It was found that the average concentrations of PBNA (80 air samples) amounted to 0.404 to 0.535 mg/cu m around sites in Workshop 1 of Factory A, the concentration of PBNA in the mix roller of the Banbury mill for mixing.

At other sites of the workshop, average concentrations of PBNA varied from 0.085 to 0.385 mg/cu m, with most being less than 0.200 mg/cu m. To test the relationship between the PBNA values in Factory A.

Since the boiling point of PBNA is 395°, it volatilized even at room temperature, and its volatility increased with increasing environmental temperature. Rubber sheets containing 5% ZnO and 2% PBNA were mixed in a miniature mix roller and were then stored at different temperatures. After storage at 30° for 24 hr, 8.2% of the PBNA was lost from the mixed rubber material. At 75°, about 10% of the PBNA volatilized after 48 hr of storage, but further loss practically ceased. At 125°, a continuous volatilization of PBNA was observed, and about 50% of the PBNA contained in rubber was lost after 2.75 days of storage. Higher volatility would be expected with stirring of the mixed rubber.

Detailed Epidemiological Studies of Lung Cancer in Rubber Tire Factories

Before 1976, the use of antioxidant D had been suspended in all rubber tire factories in Shanghai. Since the long-term carcinogenic activity, if any, of antioxidant D would still be manifest for 1 or 2 more decades among the workers exposed, epidemiological studies of cancer incidences in Factory A (1971 to 1980) and Rubber Tire Factory C (a manufacturer of bicycle and other light-type tires) (1976 to 1980) were renewed in 1981 (9). Retired workers were included in the investigation. Diagnoses of all cancer cases were rechecked and confirmed. Data for many years were obtained from the sum of the total number of workers at the middle of each year under investigation. Only workers over 30 years of age were considered, with Shanghai male and female inhabitants over 30 years old used as controls for the calculation of SMR. The significance of SMRs was tested by Poisson distribution. The division of labor among workers was made according to their job at the time of cancer diagnosis or at retirement.

It was found that the incidence of lung cancer in the mixing and milling workshop at both factories were significantly elevated (p = 0.05, p < 0.05, respectively). Incidences of all types of cancer in workers in the mixing and milling workshop at Factory A were also significantly higher than those in the controls. Details of the SMRs are shown in Tables 3 and 4. These findings were particularly striking since the incidences of all types of cancer at both factories were significantly lower than those for the controls, probably due to a marked decrease in mammary and genital cancer in the female workers as a result of better prophylactic measures.

DISCUSSION

The rubber industry has received considerable attention as to possible carcinogenic risks to which its workers may be exposed (1). Our preliminary and final epidemiological studies on cancer incidences at rubber factories in Shanghai indicated that an excess of lung cancer may occur at least in certain workshops of rubber tire factories, and exposure to aerosols of antioxidant D may be one of the causes of this excessive incidence. Although our experimental results strongly suggest the carcinogenicity of PBNA, especially to the lungs, results of our in situ animal

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Table 3
SMR of all cancers and cancer of the lung, stomach, and liver in workers over 30 years old in Rubber Tire Factory A and its 4 workshops (1971–1980)
Control population, Shanghai male and female inhabitants over 30 years old, 1976–1979.

<table>
<thead>
<tr>
<th>Workshop</th>
<th>Lung cancer</th>
<th>Stomach cancer</th>
<th>Liver cancer</th>
<th>All cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of man-yr</td>
<td>No. expected</td>
<td>No. observed</td>
<td>SMR</td>
<td>No. expected</td>
</tr>
<tr>
<td>Vulcanization</td>
<td>1,754</td>
<td>1,52</td>
<td>1</td>
<td>0.66</td>
</tr>
<tr>
<td>Inner tire</td>
<td>1,106</td>
<td>0.68</td>
<td>0</td>
<td>0.79</td>
</tr>
<tr>
<td>Tire building</td>
<td>2,444</td>
<td>1.26</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mixing and milling</td>
<td>1,935</td>
<td>2.20</td>
<td>6a</td>
<td>2.74</td>
</tr>
<tr>
<td>Entire factory</td>
<td>18,852</td>
<td>17.67</td>
<td>19</td>
<td>1.68</td>
</tr>
</tbody>
</table>

Note: Difference between experimental and control groups, p = 0.05. Difference between experimental and control groups, p < 0.05.

Table 4
SMR of all cancers and cancer of the lung, stomach, and liver in workers over 30 years old in Rubber Tire Factory C and its 4 workshops (1976–1980)
Control population, Shanghai male and female inhabitants over 30 years old, 1976–1979.

<table>
<thead>
<tr>
<th>Workshop</th>
<th>Lung cancer</th>
<th>Stomach cancer</th>
<th>Liver cancer</th>
<th>All cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of man-yr</td>
<td>No. expected</td>
<td>No. observed</td>
<td>SMR</td>
<td>No. expected</td>
</tr>
<tr>
<td>Vulcanization</td>
<td>327</td>
<td>0.13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Inner tire</td>
<td>1,031</td>
<td>0.76</td>
<td>2</td>
<td>2.63</td>
</tr>
<tr>
<td>Tire building</td>
<td>989</td>
<td>0.65</td>
<td>1</td>
<td>1.54</td>
</tr>
<tr>
<td>Mixing and milling</td>
<td>629</td>
<td>0.54</td>
<td>3a</td>
<td>5.56</td>
</tr>
<tr>
<td>Entire factory</td>
<td>6,183</td>
<td>4.37</td>
<td>6</td>
<td>1.37</td>
</tr>
</tbody>
</table>

Note: Difference between experimental and control groups, p < 0.05.

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exposures indicated that PBNA was not the only material responsible for the excessive incidence of lung and other cancers. Further epidemiological investigations in Factories A and C would be valuable. It may be worthwhile to test rubber additives other than PBNA for carcinogenicity.

REFERENCES


Fig. 1. Animal exposure experiment at the Banbury mill for mastication in a rubber tire factory.
Fig. 2. Animal exposure experiment in the compounding room in a rubber tire factory.
Fig. 3. Adenocarcinoma of the lung in a rat exposed at the Banbury mill for mixing. H & E, x 180.
Fig. 4. Adenocarcinoma of the prostate in a rat after chronic p.o. administration of technical PBNA. H & E, x 180.
Fig. 5. Adenocarcinoma of pancreas, in a rat after chronic p.o. administration of technical PBNA. H & E, x 372.
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