Carcinogenicity of Organic Fractions of Particulate Pollutants Collected in New York City and Administered Subcutaneously to Infant Mice

S. Asahina, J. Andrea, A. Carmel, E. Arnold, Y. Bishop, S. Joshi, D. Coffin, and S. S. Epstein

SUMMARY

Groups of randomly bred infant Swiss mice were given s.c. injections of suspensions of (a) an organic extract of particulate atmospheric pollutants collected in New York City; (b) derived acidic, basic, neutral, aliphatic, aromatic, and insoluble fractions; and (c) three oxyneutral subfractions, in total doses of 10, 20, and 40 mg. Each test group consisted of a minimum of 44 mice; concurrent controls were comprised of 81 untreated and 86 solvent-treated mice.

There were wide variations in the overall carcinogenicity and incidence of tumors in various organs in different test groups. High incidences of hepatomas were found in males given injections of the basic fraction and to a lesser extent in males and females given injections of the organic extract. The incidence of lymphomas was high, particularly in females given injections of basic, aliphatic, and aromatic fractions and oxyneutral subfractions. Additionally, high incidences of solitary and multiple pulmonary adenomas were found in mice given injections of basic, neutral, and aromatic fractions and oxyneutral subfractions. Coexistent multiple tumors were also found in mice given injections of basic and aromatic fractions and oxyneutral subfractions. Injection site tumors were rare.

INTRODUCTION

The carcinogenicity in mice of organic extracts of atmospheric pollutants has been demonstrated in various studies (3, 4, 9, 11–14, 17, 18). Skin painting or s.c. injection with such extracts has generally yielded local tumors (papillomas, carcinomas, or sarcomas) sometimes accompanied by multiple pulmonary adenomas. A notable exception was the high incidence of remote tumors (hepatomas and lymphomas, besides pulmonary adenomas) together with the virtual absence of local tumors, following s.c. administration of relatively low doses of organic extracts in infant mice (9). Marked variations in the carcinogenicity of organic extracts of particulate atmospheric pollutants from various urban sites have been reported following administration to neonatal (9) and adult (12) rodents.

Except BaP, a very little is known about the wide range and diversity of chemical carcinogens in polluted air (6); further, the role of these various classes of chemicals in determining the carcinogenicity of atmospheric pollutants is largely unknown. Evidence for the multiplicity of atmospheric carcinogens includes (a) tumor production by BaP-free atmospheric pollutants such as aliphatic aerosols of a synthetic smog (13, 14) and by aliphatic and oxyneutral fractions of crude organic extracts of particulate atmospheric pollutants (12); (b) the lack of parallelism between the carcinogenicity of organic extracts and their BaP concentrations (9, 12); and (c) the diverse pattern and multiplicity of tumors developing following injection of pollutant extracts in infant mice (Ref. 9; K. Fujii and S. S. Epstein, unpublished data).

The objective of the present study was to determine the relative carcinogenicity of various fractions and subfractions of an organic extract of atmospheric particulate pollutants collected in New York City. These studies are preliminary to attempts to define new classes of chemical carcinogens in individual organic fractions and subfractions of particulate atmospheric pollutants.

MATERIALS AND METHODS

Samples of approximately 10 kg of airborne particulate matter were collected on 20 Cambridge air conditioner filters over a period of 6 months, from August 11, 1965, to February 23, 1966; each filter contained 96 sq ft of filtration surface with 85% efficiency. The filters were mounted in a blower-filter assembly in an air-conditioning plenum on the 5th floor of the Pan Am office building in New York City. The air-conditioning plenum faced east, and the average collection was 80 g of crude organics per individual filter. The estimated total air flow filtered on all filters during this time was $10^8$ cu m. The prevailing wind was from the southwest approximately 70% of the time and from the east 30% of the time.

The particulate samples were extracted exhaustively with...
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benzene at 75°. The benzene was removed completely by evaporation under vacuum, yielding 1079 g of a crude extract of benzene-soluble organics. The extracts were fractionated and subfractionated by the Pressure Chemical Company, Pittsburgh, Pa., according to previously described techniques (27, 28) with minor modifications (Chart 1).

In pilot studies, the Cambridge and standard glass fiber filters showed good general agreement for gross measurement of airborne particulate matter when compared with the standard National Air Sampling Network high-volume method (27). Nevertheless, concentration of polycyclic organics in the Cambridge filter extracts, expressed as μg/g extract, were lower due to the larger amount of organic material extracted (T. W. Stanley, personal communication).

Uniform suspensions of the organic extract; acidic, basic, neutral, aliphatic, aromatic, and insoluble fractions; and pentane-9%, -12%, and -36% ether oxyneutral subfractions were prepared in redistilled tricaprylin at concentrations of 25, 50, and 100 mg/ml and stored in ampuls at room temperature. With the use of previously described techniques (7, 9), each of 3 concentrations of each suspension was injected s.c. into the napes of the necks of randomly bred Swiss mice (Ha/ICR) in volumes of 0.1, 0.1, and 0.2 ml on Days 1, 7, and 14 of life, respectively, yielding total doses of 10, 20, and 40 mg (Table 1). Controls received either solvent alone or were untreated. Eight litters were initially randomly assigned to each control group and 4 to 6 to each treatment group. Following weaning and sexing at 28 days, all animals were housed in hanging stainless steel cages with wire grid floors in groups of 5 or fewer of each sex and were given Purina laboratory chow and tap water ad libitum. Mice were inspected daily, and litter weights were taken weekly for the

Chart 1. Scheme of fractionation of the organic extract of particulate atmospheric pollutants.
Table 1
Tumors induced in Swiss mice following neonatal s.c. infections of suspensions of a benzene-soluble organic extract of particulate atmospheric pollutants and fractions and subfractions thereof

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Total dosage (mg)</th>
<th>Treated</th>
<th>Weaned</th>
<th>Sacrificed</th>
<th>Hepatomas</th>
<th>Solidary</th>
<th>Multiple</th>
<th>Lymphomas</th>
<th>Miscellaneouse</th>
<th>% weaned mice with any tumors</th>
<th>% weaned mice with multiple tumors</th>
<th>% weaned male mice with uropathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninjected</td>
<td></td>
<td>81 (8)</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>0</td>
<td>4.3</td>
<td>0</td>
</tr>
<tr>
<td>Tricaprylin</td>
<td></td>
<td>86 (8)</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>0</td>
<td>4.3</td>
<td>0</td>
</tr>
<tr>
<td>Organic extract</td>
<td></td>
<td>70 (6)</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>28.2</td>
<td>12.5</td>
<td>7.7</td>
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<tr>
<td>Acid fraction</td>
<td></td>
<td>50 (5)</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>6.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Basic fraction</td>
<td></td>
<td>51 (5)</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>46.4</td>
<td>43.5</td>
<td>21.4</td>
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<tr>
<td>Neutral fraction</td>
<td></td>
<td>63 (5)</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>19.1</td>
<td>20.8</td>
<td>2.9</td>
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<tr>
<td>Aliphatic fraction</td>
<td></td>
<td>54 (5)</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>11.9</td>
<td>31.8</td>
<td>3.0</td>
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<tr>
<td>Aromatic fraction</td>
<td></td>
<td>66 (5)</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>22.4</td>
<td>40.7</td>
<td>5.2</td>
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<tr>
<td>Oxynuclear pentane-9% ether</td>
<td></td>
<td>66 (5)</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>32.7</td>
<td>25.4</td>
<td>5.4</td>
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<tr>
<td>Oxynuclear pentane-12% ether</td>
<td></td>
<td>65 (5)</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>32.7</td>
<td>25.4</td>
<td>5.4</td>
</tr>
<tr>
<td>Oxynuclear pentane-36% ether</td>
<td></td>
<td>64 (5)</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>22.4</td>
<td>40.7</td>
<td>5.2</td>
</tr>
<tr>
<td>Insoluble fraction</td>
<td></td>
<td>65 (5)</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>M F</td>
<td>15.5</td>
<td>17.2</td>
<td>1.7</td>
</tr>
</tbody>
</table>

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*a Numbers in parentheses, number of litters.  
*b p < 0.05. Significantly greater than tricaprylin controls.  
*c p < 0.01.  
*d One osteogenic sarcoma.  
*e One gastric adenocarcinoma.  
*f Three squamous cell papillomas of forestomach.  
*g One renal adenoma, one renal carcinoma with solitary hepatoma, pulmonary adenoma and adrenal medullary tumor.  
*h One mammary tumor.  
*i Two with a coexistent pulmonary carcinoma.  
*j One dermoid cyst, one mammary tumor.  
*k One with a coexistent pulmonary carcinoma.  
*l One papilloma of glandular stomach.  
*m One histiofibrosarcoma of upper limb.  
*n One granulocytic neoplasm.
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1st month of life. Individual animal weights were taken approximately every 28 days thereafter until the experiment was terminated. All survivors were sacrificed between 49 and 51 weeks. With occasional exceptions due to autolysis, cannibalism, or accidental loss, all mice were autopsied and tissues from any lesion or tumor, and usually also from the liver, lung, heart, kidney, spleen, adrenals, thymus, lymph nodes, and sternal marrow, were fixed in Telyesniczky's fluid, sectioned at 5 μm, and stained with hematoxylin and eosin; occasional tissues were also stained with silver reticulin.

RESULTS

Mortality before weaning was high in neonates receiving high doses of the benzene-soluble extract and in others receiving the higher doses of the acidic and basic fractions. Such mortality, which precluded determination of carcinogenicity in these groups, was largely restricted to the first few days of life, in contrast to other treated and control groups in which it was evenly distributed during the prewean period. Preweaning mortality was not sex related in any treatment or control group, as comparable numbers of males and females were identified at weaning. Following weaning, substantial sex differences in survival generally developed due to a nonspecific syndrome of obstructive uropathy in male mice. The incidence of uropathy was highest in untreated controls, possibly reflecting the greater health and spermatogenic activity of these males but was otherwise unrelated to treatment (8). Tumor incidences in treated groups were generally compared with tricaproin rather than with untreated controls.

Average weights at weaning in surviving mice in most test groups were comparable to or higher than those of controls. Increments in weight between weaning and sacrifice were consistently higher in males of all control and test groups. On the basis of results in test groups with more than 10 mice surviving, variability of weight increments between the 3 dose levels was low in each group, and weight increments in test groups were generally reduced in relation to those of untreated controls of the same sex.

With a basis of the number of mice at risk at weaning, the number of tumor-bearing animals and the overall percentage of tumor-bearing animals are presented in Table 1. As these numbers were not sufficiently large to give a clear dose-response relationship and as the same doses were used for each treatment group, for purposes of comparison the number of tumor-bearing animals were combined for all doses in each treatment group. The overall incidence of tumor-bearing females receiving aromatic and oxyneutral pentane-9% and pentane-12% ether fractions was markedly enhanced compared with solvent controls (p < 0.01), in males receiving basic fraction, and in females receiving basic and aliphatic fractions (p < 0.05). Although not statistically significant, the incidence of tumor-bearing females in all other treatment groups was higher than that of the solvent-treated controls, with the exception of the acid fraction, and was also higher for males in many groups. With the exception of mice given injections of the acidic fraction and females receiving the benzene-soluble extract, multiple-tumor-bearing mice were found in all treated groups, in contrast to a zero incidence in controls. The highest incidence of multiple-tumor-bearing animals was found in males given injections of the basic fraction.

The individual total incidence of hepatomas, solitary and multiple lung tumors, lymphomas, and other miscellaneous tumors, in all control and treatment groups, is listed in Table 1; the identity of all tumors was established histologically.

Hepatomas were found almost exclusively in males in the solvent controls and test groups; of 1061 test animals, hepatomas developed in 52 mice, 96% of which were males. The highest incidence of hepatomas was found in males that received injections of the basic fraction and the benzene-soluble extract (43 and 23%, respectively). These hepatomas were solitary or multiple and varied in size from the microscopic to a size exceeding the normal mouse liver. Generally, the tumors were of the classic, well-differentiated, and solid hepatocellular or trabecular type with conspicuous hyaline and refractile eosinophilic cytoplasmic inclusion bodies; occasionally, dilated sinusoids were prominent with a consequent hemangiomatous pattern. There was no relationship between the type and behavior of tumors and the fractions administered; no extrahepatic metastases were noted. The incidence of hepatic nodules, in which the basic parenchymal and interstitial hepatic architecture was preserved and cell atypia was absent, was low in all test and control groups; these nodules were not scored as hepatomas.

No multiple pulmonary adenomas were found in either control group. Contrastingly, a relatively high incidence of solitary and multiple adenomas was noted in mice treated with various test fractions. The incidence of both solitary and multiple adenomas did not appear sex related. The highest incidence of solitary pulmonary adenomas was found in males that received injections of the oxyneutral pentane-9% ether subfraction (18%), and in females given injections of neutral fraction (13%). Multiple pulmonary adenomas occurred most frequently in males and females given injections of the aromatic fraction (9 and 14%, respectively), and the oxyneutral pentane-12% ether fraction (7 and 11%, respectively). The adenomas were generally typical, consisting of closely packed and noncapsulated masses of uniform cuboidal or polygonal cells with eosinophilic cytoplasm and round or oval nuclei. Squamous metaplasia was occasionally seen in multiple adenomas, particularly in mice given injections of the aromatic fraction. Malignant transformation was found in 2 females and 1 male (Table 1); no metastases were detected.

No lymphomas were found in control males and the incidence in control females ranged from 4 to 6%. The incidence of lymphomas in the test groups was clearly sex related; of 1061 mice, 108 developed lymphomas, and 74% of these mice were females. The highest incidence of lymphomas in males was found in the group treated with aliphatic, aromatic, oxyneutral pentane-9% ether and insoluble fractions (7 to 13%). In females, the highest incidence developed in groups treated with basic, insoluble, aliphatic, aromatic, and oxyneutral fractions (12 to 30%). Reticulum cell type B neoplasms were the most common lymphoma in all groups, comprising 36 and 59% of all lymphomas in males and females, respectively.
females, respectively, and were generally detected in mice over 50 weeks old. Generalized lymphocytic and lymphoblastic lymphomas were less common, comprising 32 and 20% of all lymphomas in males and in females, respectively, and generally resulted in mortality in mice less than 40 weeks old. Localized thymic lymphomas were seen with similar frequency, but usually in older mice. Reticulum cell type A neoplasms were rare; one instance of plasma cell, granulocytic, and an unclassifiable neoplasm of hematopoietic origin was found among the various test groups. A variety of miscellaneous tumors were also found in the various test groups (Table 1).

The incidence of hepatomas was remarkably sex related; the rarity of induced hepatomas in female mice has been noted in various recent studies (7, 9, 29). The incidence of hepatomas was particularly high in males treated with the lowest dose of oxyneutral pentane-12% ether fraction.

In addition to these neoplastic changes, dysplastic changes in gastric mucosa were observed in a male and a female injected given injections of the highest dose of oxyneutral pentane-12% ether fraction and in a male treated with the lowest dose of oxyneutral pentane-36% ether fraction.

DISCUSSION

These studies have revealed striking differences in the acute toxicity and overall carcinogenicity and organ specificity of various organic fractions of particulate atmospheric pollutants. These data clearly indicate the presence of many more carcinogens than BaP alone in atmospheric pollutants.

Apart from hepatomas, the total tumor incidence in mice given injections of the parent benzene-soluble extract was relatively low; additionally, the variety of tumor types noted in these animals was relatively restricted. This may reflect the relatively low content of carcinogenic constituents in the parent extract in contrast to those of its concentrated fractions and subfractions.

The incidence of hepatomas was remarkably sex related; the rarity of induced hepatomas in female mice has been noted in various recent studies (7, 9, 29). The incidence of hepatomas was particularly high in males treated with even the lowest doses of the basic fraction. Excluding the possibility of the presence of antineoplastic effects in the benzene-soluble extract, it appears that hepatocarcinogens in the benzene-soluble extract are mainly concentrated by fractionation into the basic fraction. The basic fraction is known to contain dialkylated benz(c)acridines (20–22). The high carcinogenic activity of 7,9-dimethylbenz(c)acridine and 7,10-dimethylbenz(c)acridine (10, 15) and their high photodynamic activity (10), together with the highly photodynamic activity of the basic fraction (S. S. Epstein, unpublished data), suggest that these may be major carcinogenic candidates in this fraction. Sawicki et al. (21, 22) also reported that various dibenzacridines and a relatively large amount of quinoline compounds were also contained in this fraction; available data, however, indicate that these compounds were generally noncarcinogenic (1, 2).

The neonatal mouse is clearly highly sensitive to carcinogens inducing pulmonary adenomas and yields data comparable to those described in adult strain A mice used in short-term carcinogenicity studies (1, 23, 24). The adenomas induced in the present studies were generally benign, did not kill the animals, and occasionally fused into conglomerated masses which were found at terminal sacrifice. For these reasons, adenomas were not counted but were merely scored as single or multiple. The incidence of pulmonary adenomas was not sex related and was highest in mice into which aromatic, basic, and neutral fractions had been injected. Generally, there was no clear relationship between dose of fraction and tumor incidence; this may reflect the presence of anticarcinogens (16, 26) in these relatively undefined fractions.

The incidence of lymphomas was markedly sex related and the relatively short latency for the induction of undifferentiated, in contrast with differentiated lymphomas, is consistent with similar results following injection of organic extracts of particulate atmospheric pollutants from various cities in the United States (7) and following administration of known chemical carcinogens (5, 19). The high total incidence of tumors in females given injections of the aliphatic fraction was due to the high incidence of lymphomas. This fraction possibly contained various long-chain hydrocarbons, which have not been identified as significant carcinogens. Nevertheless, n-dodecane is a known cocarcinogen (25). Cocarcinogenic activity might also be anticipated due to the presence of other aliphatic hydrocarbons. It is also interesting that gastric tumors were found in 4 mice that received injections of this aliphatic fraction.

The extreme rarity of injection site tumors and the relatively high incidence of a variety of remote tumors in this study is consistent with previous findings following injection of neonatal mice with organic extracts of pollutants from 6 other cities in the United States (9). This is in contrast with a low remote tumor yield in adult mice (12). This discrepancy could be due to differences in the bioassay systems; differences in the metabolic pathway of newborn and adult mice; and quantitative and qualitative differences in the particulates, extracts, and fractions tested. On the basis of comparisons at the 20- to 25-mg dose level and the numbers of animals at risk, the incidence of hepatomas in male mice treated with the New York City extract was similar to that previously reported for Chicago, New Orleans, Philadelphia, and Washington; higher than for Los Angeles; and lower than for Cincinnati. The relatively low incidence of lymphomas induced by the New York City organic extract is in accord with a similar incidence induced by extracts from 4 cities, other than Cincinnati and Philadelphia. Nevertheless, the low incidence of multiple pulmonary adenomas in extract-treated mice in these experiments is in marked contrast with the higher incidence previously reported for all 6 cities studied. Sampling considerations apart, it is likely that these differences in part reflect the relatively low efficiency of Cambridge filters in trapping small-size particulate pollutants and the low BaP concentration of New York City extract [87 μg/g of benzene-soluble extract (T. W. Stanley, personal communication)]. This is in contrast with the higher efficiency of National Air Sampling Network high-volume samplers and the higher BaP concentrations (320 to 670 μg/g of organic extracts) in 5 of 6 cities studied (9). More importantly, extraction of particulates by benzene at 75°
appears to result in relatively low BaP concentrations (T. W. Stanley, personal communication). Standard Soxhlet extraction of particulates collected on Cambridge filters in the Western Electric Building in New York City yielded BaP values comparable with those found in New York City samples collected on standard high-volume samplers in the winter of 1967. These samples, extracted by standard methods, yielded BaP concentrations of 300 and 340 µg/g.

The data reported here confirm the sensitivity of neonatal mice in the carcinogenic bioassay of organic extracts and derived fractions of particulate atmospheric pollutants. The induction of various combinations of remote tumors (hepatomas, lymphomas, and pulmonary adenomas) by the benzene-soluble extract, its fractions, and its subfractions clearly indicates the existence of a variety of different chemical carcinogens in organic extracts of air pollutants, with the exception of BaP which is largely concentrated in aromatic fractions. These carcinogens are as yet largely undefined. The high carcinogenicity of the basic fraction may be due to its content of dialkylated benzo(acycridines, such as 7,9- or 7,10-dimethylbenz(acycridine.

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REFERENCES


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