**Brief Communication: Airborne Mutagens Bioassayed in Salmonella typhimurium**

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ABSTRACT—Particulate airborne pollutants, collected in Buffalo, New York, and Berkeley, California, were assayed for mutagenic activity in the Ames Salmonella typhimurium test system. Mutagens requiring liver enzymes for activation, as well as direct acting mutagens, were readily detected in the Buffalo sample. By contrast, only direct acting mutagens were detected in the Berkeley sample.—J Natl Cancer Inst 58: 449–451, 1977.

The presence of chemical carcinogens in the particulate matter of city air has been amply documented (1-3). However, it is less clear if these air pollutants, which are demonstrably carcinogenic to experimental animals (4-6), are present in sufficient amounts in city air to affect cancer risk in man. To assess the significance of airborne carcinogens in human cancer, it is necessary to correlate the total carcinogenic potential of air to the incidence of cancer in the people who breathe it. Chemical determinations of BP, as well as other PAH in air, have been used as indexes of the carcinogenic potential of airborne particulates (1-3), but it is uncertain what proportion of the carcinogenicity is reflected by BP levels. The recent development by Ames and associates (7) of a rapid, economical, and sensitive bacterial system for detecting mutagens and carcinogens which act as mutagens may provide an alternate method for assessing the mutagenic and carcinogenic potentials of air pollutants. In this report, we describe the application of the Ames system for the bioassay of mutagenic particulate airborne contaminants.

The principal samples of particulate air pollutants selected for testing were collected by Winkelstein and his associates (8-10) in conjunction with a series of epidemiologic studies on the relationship of air pollution to human chronic disease. Over 2,500 samples were collected at 21 locations in Buffalo, New York, and its environs. This investigation, preliminary to a more thorough follow-up of the Buffalo studies, demonstrates two types of mutagenic activities in selected filters of the Buffalo collection.

MATERIALS AND METHODS

The collection of 24-hour Buffalo air samples in 1961-63 has been detailed in (9). The glass fiber filters containing the deposited particulates were folded between cardboard, sealed with wax paper, and stored in manila envelopes prior to analysis. Under these storage conditions, organic particulate matter is generally considered to be stable (11, 12). In addition, to verify that the observed activities were not artifacts of storage, a freshly collected sample of airborne particulate matter was obtained on a synthetic fiber filter placed in an air duct of the Biochemistry Building, University of California, Berkeley. The results obtained with this more recent sample are included here. The particular Buffalo samples used for mutagenicity assays were collected on March 14–20, 1962, at station 8 in Buffalo, New York—a station located downwind from a steel mill. The amount of total suspended particulates collected at this station, ranking eighth highest among the 21 stations, averaged 126 µg/m³.

To determine the appropriate solvent for extracting the filters, benzene, acetone, chloroform, and methanol were used in Soxhlet apparatuses. Equal portions of four filters were extracted with 200 ml of each solvent for 2 hours. The extracts were then concentrated to dryness, and the residues were weighed, redissolved in dimethyl sulfoxide (10 mg residue/ml), and assayed in the Salmonella mutagen test system developed by Ames et al. (7). The total mutagenic activity of each extract equaled the specific mutagenic activity (in U revertants/100 µg) multiplied by the amount of residue obtained from the extraction. Under these conditions, chloroform and acetone extracts contained the highest total mutagenic activities, and acetone was selected for use in subsequent extractions. Acetone extracts of blank filters were not mutagenic.

Since airborne particulate matter contains PAH (1-3), an experiment was designed to estimate the percent recovery of PAH from the filters. [3H]BP (Amersham-Searle Corp., Des Plaines, Ill.) was added to 50 cm² of one filter prior to Soxhlet extraction. An aliquot of the subsequent extract was tested by thin-layer chromatography in a system that separates BP from its oxidation products (13). Four hours of extraction with 200 ml acetone provided quantitative recovery of the [3H]BP with no apparent decomposition.

Based on these preliminary studies, the following procedures were adopted for the preparation of organic extracts: a) A 100-cm² portion of the filter was cut and placed in a Soxhlet apparatus. b) The filter was extracted for 4 hours with 200 ml acetone. c) The extract was concentrated to dryness by vacuum. d) The residue was redissolved in a small volume of acetone, transferred to a preweighed vial, dried, weighed, and redissolved in dimethyl sulfoxide (10 mg residue/ml).

The mutagenic activities of the organic extracts were examined in Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100 in the presence and absence of liver S-9. The methods described by Ames et al. (7) were followed without modification. The extracts were

**ABBREVIATIONS USED**

BP = benzo[a]pyrene; PAH = polycyclic aromatic hydrocarbons; s-9 = 9,000×g supernatant; AHH = aryl hydrocarbon hydroxylase.

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mutagenic toward strains TA1537, TA98, and TA100; consequently these strains were studied for dose-response relationships. We investigated the effect of AHH induction on the expression of the mutagenic activities by substituting normal liver s-9 in place of the Aroclor-induced preparation and also by testing 7,8-benzoflavone as an inhibitor of enzyme-dependent mutagenesis (12). These latter studies were carried out in strain TA100, the strain exhibiting the highest sensitivity to the organic extracts.

RESULTS AND DISCUSSION

Particulate air pollution, collected on glass fiber filters and extracted with acetone, was mutagenic toward three S. typhimurium strains that respond to frameshift mutagens (text-fig. 1). The mutagenic activity was maximally expressed in the presence of liver s-9 from rats given Aroclor 1254 (text-fig. 2). This activity was inhibited 50% by the addition of 7,8-benzoflavone (100 µg/plate) and was reduced 90% by the substitution of control liver s-9 (text-fig. 2). These results suggest that most of the mutagenic activity of the samples can be attributed to the presence of PAH, since PAH are also activated by Aroclor 1254-inducible AHH, an enzyme specifically inhibited by benzoflavone (14). Moreover, on thin-layer chromatography (13), the major fluorescent material in the extract presented the same retardation factor value as BP (unpublished observations). Fractionation of the acetone extracts on high-pressure liquid chromatography (15) should indicate whether BP or other PAH principally account for the activation-dependent mutagenicity.

The results obtained on strains TA98 and TA1537 indicate that a second type of chemical mutagen was present in the acetone extract. In these strains, 20-25% of the total mutagenic activity was expressed in the absence of liver s-9 (text-figs. 1b, 1c), which indicated the presence of mutagens that do not require metabolic activation.

The presence of direct-acting mutagens in the extracts of selected filters from the Buffalo collection raised the possibility that these compounds may have formed by oxidation during storage. However, the presence of direct-acting mutagens in Berkeley air soot had been observed by Streitweiser and Ames in freshly collected samples (Ames B: Personal communication). Therefore, we obtained a sample of Berkeley air soot from Ames, extracted it, and tested it (text-fig. 3). In contrast to the Buffalo samples, the Berkeley sample was maximally active in the absence of liver s-9, and no BP could be detected by thin-layer chromatography. In agreement with the observations of Streitweiser and Ames (Ames B: Personal communication), liver s-9 appeared to lower the mutagenic activity (text-fig. 3).

These results indicate that the direct-acting mutagens present in the Buffalo samples are probably not artifacts of storage. It is likely that the activities of the Buffalo samples have changed somewhat during the 14-year storage period. But, since these samples were collected and stored under identical conditions, comparisons of
TEXT-FIGURE 3.—Mutagenic activity of Berkeley air pollution extract. A portion of a filter containing particulate air pollution collected in Berkeley was extracted as described in "Materials and Methods." The extract was tested for mutagenic activity with S. typhimurium strains TA100 (3a), TA98 (3b), and TA1537 (3c) by the methods of Ames et al. (7). ○ = liver s-9 included in assay; △ = liver s-9 omitted.

relative activities and correlations to epidemiologic variables should still yield meaningful data.

The chemical identities of the mutagens in air pollution extracts require further investigation, but the presence of at least two types of mutagens agrees with recent results obtained by Gordon et al. (16) in their studies of air pollution fractions. These workers found that the PAH fraction and a non-PAH fraction contained cell-transforming agents. The observation that polluted air may not reflect its total carcinogenic potential. For example, the Berkeley air sample used in this study (text-fig. 3), was mutagenic, even though BP was present only in submutagenic quantity if at all. The results of this study, and those of Gordon et al. (16), suggest that it may be useful to supplement measurements of airborne BP levels with a bioassay. The methodologic simplicity and quantitative nature of the Salmonella mutagenicity test system indicate that this bioassay may be applicable to the assessment of the toxic potential of airborne pollutants and other complex mixtures of combusted material (17).

REFERENCES