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Mutagenicity studies of ambient airborne particles

I. Comparison of solvent systems *

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Summary

Organic materials were extracted from airborne particles by shaking with different solvent systems including acetone, benzene, cyclohexane, dichloromethane (DCM), methanol, a mixture of acetone and DCM and a combination of benzene, cyclohexane and methanol. The solvent-extracted materials were tested for mutagenic activity with the Ames Salmonella/microsomal assay system. Acetone- and cyclohexane-extracted materials gave the highest and lowest mutagenic activities, respectively. Re-extraction experiments confirmed that most of the mutagenic material from air particles cannot be extracted by cyclohexane. The sequential extraction with acetone followed by DCM gave a better mutagenic response than acetone alone or acetone in combination with DCM. Extraction with varying amounts of solvent indicated that 1 ml of acetone per mg of airborne particles reached the maximum recovery of mutagenic material.

The practicability of using mutagenic assays to identify the potential health hazard of complex mixtures has been examined by employing various coupled chemical and biological procedures. These studies involve sample collection and preparation followed by testing for chemical and/or biological activities (Epler, 1980; Chrisp and Fisher, 1980; Hughes et al., 1980; Pellizzari et al., 1979). The common mutagenicity test used is the histidine reversion assay developed by Ames

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et al. (1975). This assay is highly efficient for obtaining information on mutagenic/carcinogenic potential of uncharacterized compounds in complex mixtures.

Although there is a standard protocol for Salmonella/microsomal assay, the results on complex mixtures from different laboratories often show qualitative and quantitative variations (Mattern and Greim, 1978; Dunkel, 1979). This variation may be attributed to different sources of particles, solvents and extraction systems. For instance, solvent systems such as cyclohexane (Alfheim and Moller, 1979), benzene–hexane–isopropanol mixture (Commoner et al., 1978), cyclohexane, methylene chloride (DCM) and acetone serially (Daisey et al., 1979), methanol (Dehnen et al., 1977), cyclohexane–methanol (Pellizzari et al., 1979), methanol–benzene–methylene chloride mixture (Pitts et al., 1977; Preidecker, 1980a), acetone (Talcott and Wei, 1977), benzene (Teranishi et al., 1978; Fukino et al., 1982), methanol/cyclohexane (Tokiwa et al., 1977), have been used for the extraction of mutagenic material from ambient air particles.

The present study on the extraction of mutagens from airborne particles was designed to determine: (a) the efficacy of commonly used solvent systems, (b) the efficiency of extraction with polar and nonpolar solvents in sequence and combination, and (c) the effect of varying amounts of solvent.

Materials and methods

Sample collection

The airborne particles were collected during June and July of 1982 on the roof of a local building (10 m above the ground) by a Hi-Vol Sampler (General Metal Works EPA Model) with 8 in. × 10 in. high-purity glass microfiber filters. The sampling was done for 48 h continuously at a flow rate of approximately 60 ft^3/min. The Hi-Vol Sampler collects particles in the size range of approximately 0.1–100 μ. The control and experimental filters were equilibrated at room temperature (22–26°C) and humidity (50–60%) for at least 24 h before and after collection and weighed to the nearest 0.1 mg on a microbalance.

Sample extraction

3 glass fiber filters were used for each experiment. Each filter was divided into 12 equal parts with a paper cutter and 6 pieces (2 from each filter) were randomly chosen and shredded to extract with each solvent. Solvents used in the study were: acetone, benzene, cyclohexane, DCM, methanol (all reagent grade) and combination of benzene : cyclohexane : methanol (1 : 1 : 1, v/v). The shredded samples and controls were put into 250-ml bottles, and 150 ml of a solvent was added to each bottle. The organic materials were extracted for 16 h on a rotary shaker (250 rpm) at room temperature. The extract was filtered through Whatman No. 2 filter paper and the residue on the filter from each solvent was stored in a refrigerator for re-extraction. The extract from each solvent was concentrated separately to approximately 10 ml with a rotary evaporator (40°C) and then to dryness on a dry bath (40°C) under a stream of nitrogen gas. The dried extracts were dissolved in reagent grade dimethyl

sulfoxide (DMSO) and subjected to mutagenesis tests. The refrigerated residue of the airborne particles of the above experiment were individually subjected to re-extraction with 150 ml of acetone and extracts prepared as noted above. Acetone was used because it was found to be the most effective solvent for mutagen extraction when compared with other solvents in the study.

Sequence and combination of solvents for extraction

Acetone extraction was compared with a sequential extraction with acetone and DCM as well as a combination of the two solvents. 3 filters were used in the experiment and each filter was divided into 12 equal parts with a paper cutter. 4 pieces were randomly chosen from each filter (12 in all) and shredded for extraction with 300 ml of acetone. The remaining 24 pieces were used, 12 each, in sequential and combination extractions. In sequential extraction, the sample was extracted first with 150 ml of acetone, the residue was then re-extracted with 150 ml of DCM. The filtrates from both were mixed. In combination extraction, 150 ml of acetone: 150 ml of DCM were mixed and used for extraction. Further processing of extracts for mutagenic assay was done in the manner described earlier.

Quantifying solvent

Filters with approximately 150 mg air particles were put in 500-ml bottles and each was extracted with 50, 100, 150, 200, 300, or 400 ml of acetone on a rotary shaker (250 rpm). Extracts were prepared for mutagenicity assay as described above. The extract equivalent to 4.93 mg of air particle per plate was the highest available concentration tested.

Mutagenicity assay

Mutagenicity was determined by the Ames Salmonella/microsomal assay system (Ames et al., 1975). Solvent extracts of air particles were tested for their mutagenic activity in TA98 and TA100 of *Salmonella typhimurium* by the plate incorporation test with and without S9 metabolic activation. The S9 used was prepared from liver of Aroclor-1254-treated (500 mg/kg body weight) male Wistar/Lewis rats. Mutations were scored from histidine dependence to histidine independence. Determination of positive mutagenic response was based on criteria recommended by Ames et al. (1975).

A minimum of 2 plates were used for each of 4 concentrations tested for each solvent extract. Histidine-independent revertants were scored following incubation at 37°C for 48 h. Each experiment was repeated at least 4 times to check reproducibility. The data were analyzed for statistical significance by analysis of variance.

Results

The results from the mutagenesis assays of airborne particles with different solvents are presented in Figs. 1 and 2 for strains TA98 and TA100 of *S. typhimurium*, respectively. The concentrations indicated in all figures are based on

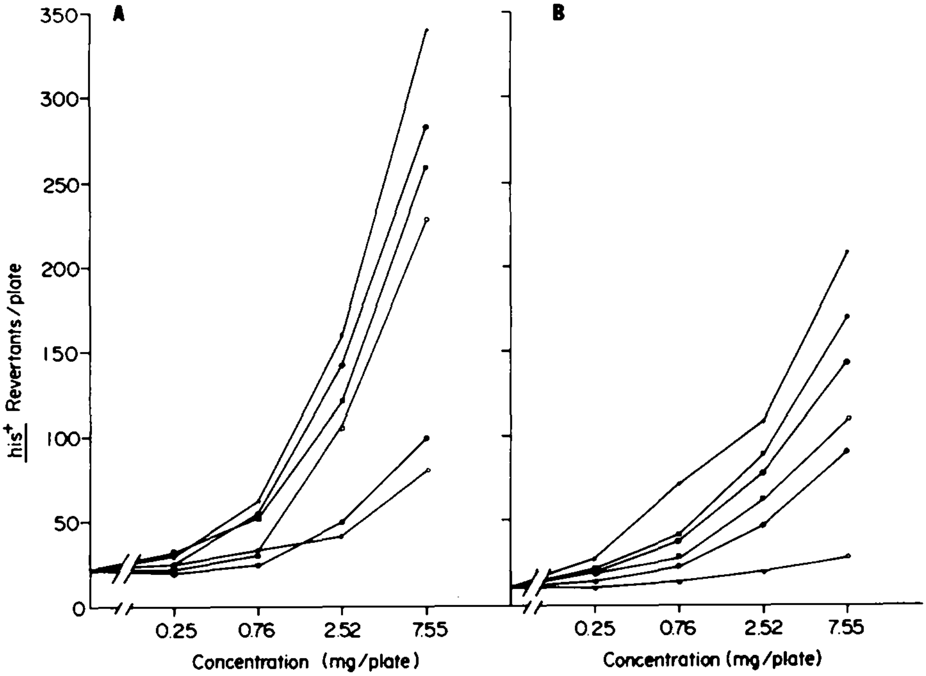

Fig. 1. Mutagenic activity of solvent extracts of air particles in *S. typhimurium* TA98: with (A) and without (B) S9 activation. ★, acetone; ■, benzene; ○, cyclohexane; ∗, DCM; □, methanol; ●, benzene–cyclohexane–methanol.

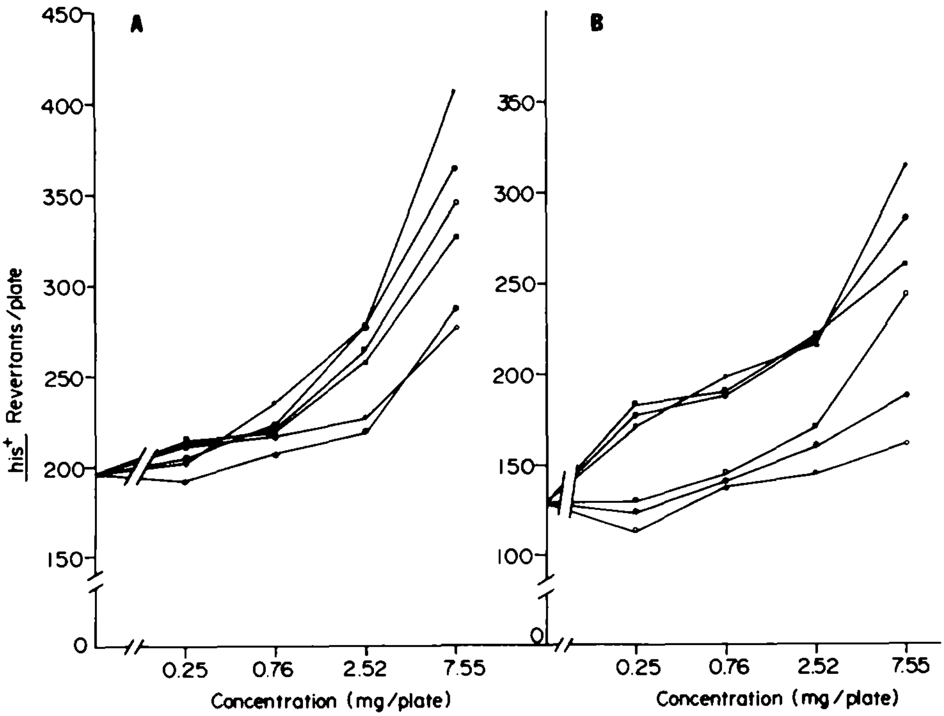

Fig. 2. Mutagenic activity of different solvent extracts of air particles in *S. typhimurium* TA100: with (A) and without (B) S9 activation. ★, acetone; ■, benzene; ○, cyclohexane; ∗, DCM; □, methanol; ●, benzene–cyclohexane–methanol.

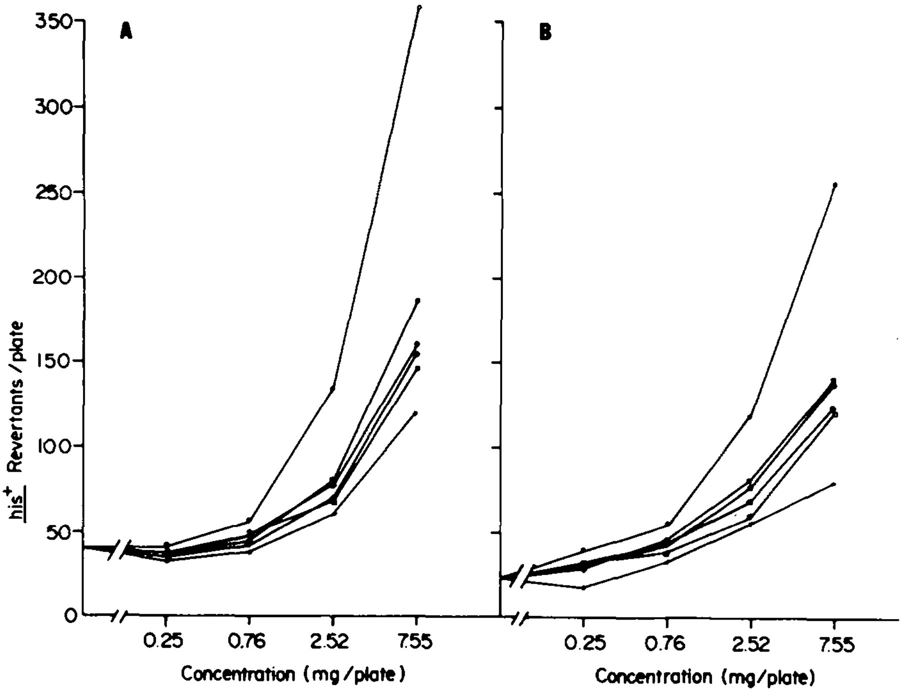

Fig. 3. Mutagenic activity of acetone extracts, from residues of air particles pre-extracted with various solvents, in *S. typhimurium* TA98: with (A) and without (B) S9 activation. ★, acetone, ■, benzene; ○, cyclohexane; •, DCM; □, methanol; ●, benzene–cyclohexane–methanol.

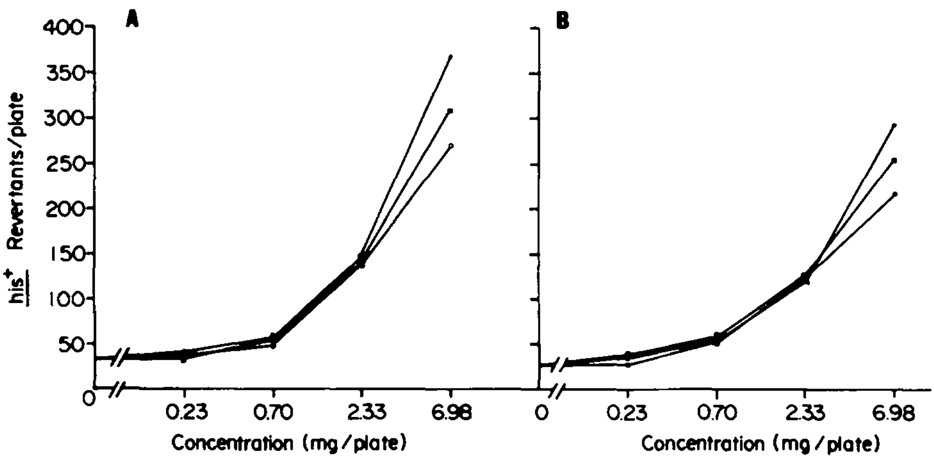

Fig. 4. The response of *S. typhimurium* TA98 to sequential and combination extraction of acetone with DCM from airborne particles: with (A) and without (B) S9 activation. ★, acetone followed by DCM (sequential); ■, acetone; ○, acetone with DCM (combination).

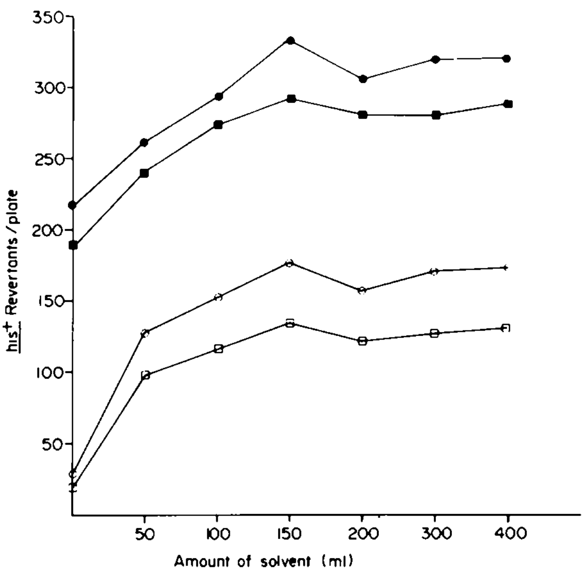

Fig. 5. The response of *S. typhimurium* to mutagens extracted with different amounts of acetone from airborne particles. TA98 with (○) and without (□) S9 activation; TA100 with (●) and without (■) S9 activation.

the original air particle weights and the curves are averages of 4 independent experiments. The acetone extract was more mutagenic than the other solvent extracts in both strains. The extracts from benzene, methanol and combination of benzene : cyclohexane : methanol were more mutagenic when compared to DCM and cyclohexane extracts. The extract from cyclohexane was least mutagenic among the solvents studied. Similar response was noticed with S9 activation in both strains. In general, S9 activation increased mutagenic activity of various solvent extracts in both testers. With the exception of cyclohexane extract, dose-dependent mutagenic response was noticed with all extracts tested. The extracts from the blank filter did not show any mutagenic activity.

The data on re-extraction of residue with acetone are shown in Fig. 3. The residue of cyclohexane when re-extracted with acetone showed the highest mutagenic activity, whereas the acetone residue showed the lowest activity. Similar results were obtained both with and without S9 activation. Furthermore, the corresponding reciprocal mutagenic response was noticed in most solvent extracts.

Data on sequence and combination of solvent extraction are summarized in Fig. 4. The sequential extraction of the airborne particles with acetone followed by DCM had the highest mutagenic activity when compared with acetone alone or acetone in combination with DCM. The extract obtained from combination of acetone and DCM had the least mutagenic activity. Similar results were found in TA100 with and without S9 activation (data not shown).

Extraction data on relative mounts of solvent to mass of airborne particles are shown in Fig. 5. The results indicated a gradual increase in mutagenic activity to 150 ml of solvent. No further increase in mutagenic response was observed with increased solvent. Comparable response was noticed with and without S9 activation. TA100 responded in a similar manner.

Discussion

Among the solvent systems studied acetone was the most effective and cyclohexane was the least effective for extraction of mutagens from airborne particles (significant at $P < 0.01$ with higher concentrations tested). Since acetone is polar and cyclohexane is nonpolar, these results suggest that air particles in Morgantown air contained mainly polar mutagens rather than nonpolar ones. The re-extraction experiment confirmed that acetone was the most effective and cyclohexane the least effective solvent for the extraction of mutagens from air particles. These results are in agreement with those of Preidecker (1980b), Jungers et al. (1981), and Talcott and Wei (1977). Preidecker found that cyclohexane was not effective for extraction of airborne mutagens. In a comparison of 4 solvent systems for the extraction of mutagens from ambient air particles, Talcott and Wei found that acetone extracted more mutagens than benzene, chloroform and methanol. Jungers et al. reported a greater mutagenic activity (per mg) of particle extracted with acetone as compared to cyclohexane, DCM and methanol. However, Goto et al. (1981), found that benzene : ethanol (4 : 1, v/v) was as good as acetone for mutagen extraction.

The mutagenic/carcinogenic activity of airborne particles has often been attributed to the presence of benzo[*a*]pyrene and other polycyclic hydrocarbons, whose concentration in urban air has been extensively measured (Pitts et al., 1977). If polycyclic hydrocarbons were responsible for the mutagenic activity found in this study, then acetone would be very effective in the extraction of mutagenic polycyclic hydrocarbons.

The response of combination extraction of acetone with DCM indicates that DCM decreases the polarity of acetone, thus reducing the efficiency of the acetone in extracting polar organic compounds (whose polarity might be close to that of acetone). However, in sequential extraction, acetone might have extracted mostly polar mutagenic organic compounds and DCM the nonpolar ones. Thus, the extract from the sequential study produced relatively more his^+ revertants than the extract from acetone alone.

The comparison of amounts of solvent indicated that a 1 to 1 ratio (ml/mg) of solvent to airborne particles reaches the maximum recovery of mutagens from airborne particles. Further increase in the amount of solvent without corresponding increase in his^+ revertants indicates that oversaturation with the solvent has no effect on recovery of airborne mutagens.

It has to be noted that studies at other locations or same location at different seasons may not yield comparable results because the amount and type of mutagens in airborne particles may vary with environmental and meteorological conditions.

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