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Mutagenicity studies of ambient airborne particles

II. Comparison of extraction methods *

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Summary

Organic materials were extracted with acetone from airborne particles by shaking, soxhletion and sonication for varying durations. 4-h, 1-h and 1/8-min extractions by shaking, soxhletion and sonication, respectively gave maximum *his*$^+$ revertants with the Ames Salmonella/microsome assay. In a comparative study of extraction methods, sonication gave the highest and soxhletion the lowest mutagenic response. It appears that sonication with acetone is the best procedure for the extraction of mutagens from airborne particles as shown by Ames assay and Ara^r assay systems in *Salmonella typhimurium*.

The development and utilization of short-term bioassays which detect cytotoxic, mutagenic and carcinogenic activities of environmental chemicals have increased dramatically in recent years. The wide acceptance of the bacterial mutagenesis bioassay using *Salmonella typhimurium* developed by Ames et al. (1975) has been a catalyst in the expanded growth of the discipline of genetic toxicology. Because of its sensitivity and simplicity this system has been extensively used for the detection of mutagenic activity of chemicals and environmental samples.

Mutagenicity study of environmental samples involves: sample collection, extraction of mutagen(s) and testing for mutagenic activity. A number of solvents and

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extraction procedures for airborne particles have been reported. For example, shaking (Whong et al., 1981a), soxhletion (Chrisp and Fisher, 1980; Epler, 1980; Hughes et al., 1980), and sonication (Pellizzari et al., 1979; Pitts et al., 1977; VanVaeck et al., 1980; Lockard et al., 1981) for varying durations of time have been used in various laboratories. Thus, the results from different studies are often not comparable.

This study was designed (a) to determine the optimum time for extraction of mutagenic materials from airborne particles by shaking, soxhletion and sonication and (b) to compare mutagenic activities of extracts from the three methods by the Ames Salmonella/microsome assay and the arabinose-resistance (Ara^r) Salmonella assay systems.

Materials and methods

Sample collection

The airborne particles were collected during July and August of 1982 by a Hi-Vol Sampler (General Metal Works, EPA Model) on 8 in. × 10 in. high-purity glass microfiber filters. Detailed sampling procedures and location of sampler have been reported in an earlier study (Whong et al., 1981a). The sampling was carried out for 48 h continuously with a flow rate of 60 ft^3/min on a building roof (10 m above the ground). The control and experimental filters were equilibrated at room temperature (22–26°C) and relative humidity (50–60%) for at least 24 h before and after collection and were weighed to the nearest 0.1 mg.

Sample extraction

In each experiment 3 experimental glass microfiber filters were used. Each filter was divided into 12 equal parts with a paper cutter. In order to determine optimum duration of extraction 2 pieces were randomly chosen from each filter and shredded to extract with 150 ml of acetone whose efficacy as a solvent was previously demonstrated (Krishna et al., 1983). Various durations of extraction were: 0.5, 1, 2, 4, 8 and 16 h for shaking; 1, 2, 4, 8, 16 and 24 h for soxhletion and 0.125, 0.5, 2, 4 and 8 min for sonication. Different samples were used in shaking, soxhletion and sonication. The shredded samples and control filters were put into separate 250-ml bottles for shaking and sonication and in the thimble for soxhletion. Shaking was done on a rotary shaker (250 rpm) and sonication with an ultrasonicator (Branson, Smithkline Company, B-32, 117 V) at room temperature. The soxhletion was done by boiling the solvent at 65°C. The extraction was stopped at appropriate intervals and each extract collected by filtering through Whatman No. 2 filter paper. The extracts were 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). Sample extracts were then assayed for mutagenic activity.

Evaluation of extraction methods

Following determination of optimum time of extraction for each method, the

three methods were compared for their efficiency of extraction of mutagens with equal amounts of sample filter. 3 filters were used for each experiment. Each filter was divided into 12 equal parts and 4 pieces from each filter were randomly selected, shredded and used for extraction with 200 ml of acetone for shaking (4 h), soxhletion (1 h) and sonication (1 min). The extracts were prepared for mutagenicity assays as described in the previous section.

Mutagenicity assay

The mutagenic activity was determined by the Ames Salmonella/microsomal assay system (Ames et al., 1975) and the Ara^r forward mutation test system (Ruiz-Vazquez et al., 1978). In the Ames assay, extracts were tested with TA98 and TA100 of *S. typhimurium* by the plate-incorporation test with and without S9 metabolic activation. S9 homogenate was prepared from the livers of male Wistar rats preinduced with Aroclor 1254 (500 mg/kg body weight). In the Ara^r assay system, SV50 cells (3×10^6) from an overnight culture and 0.1 ml of extract or

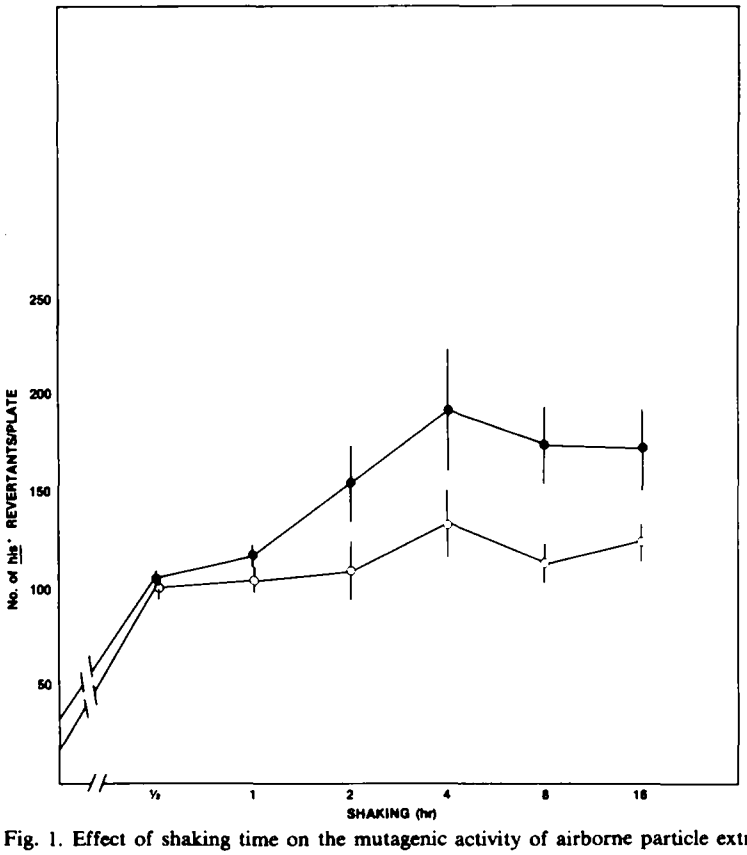

Fig. 1. Effect of shaking time on the mutagenic activity of airborne particle extracts (equivalent to 4.1 mg/plate) in *S. typhimurium* TA98, with S9 (●) and without S9 (○). Bars represent standard error of means.

DMSO were added to molten soft agar containing 0.2 ml of 20% L-arabinose and were overlayed onto a M9 bottom plate with S9 activation (Pueyo, 1978; Ruiz-Vazquez et al., 1978; Whong et al., 1981b).

For each extract a minimum of 3 plates were used for each of the 4 concentrations tested. Histidine-independent (*his*$^+$) revertants and arabinose-resistant colonies were scored after plates were incubated at 37°C for 2 and 3 days, respectively. Each experiment was repeated to check the reproducibility. Spontaneous revertants were determined in DMSO-treated plates and positive control of 2-aminoanthracene (2AA) was included in each experiment. Data were analyzed for statistical significance by analysis of variance.

Results

The data on varying times of extraction by shaking, soxhletion and sonication are presented in Figs. 1, 2 and 3. Each curve is an average of 2 independent experiments.

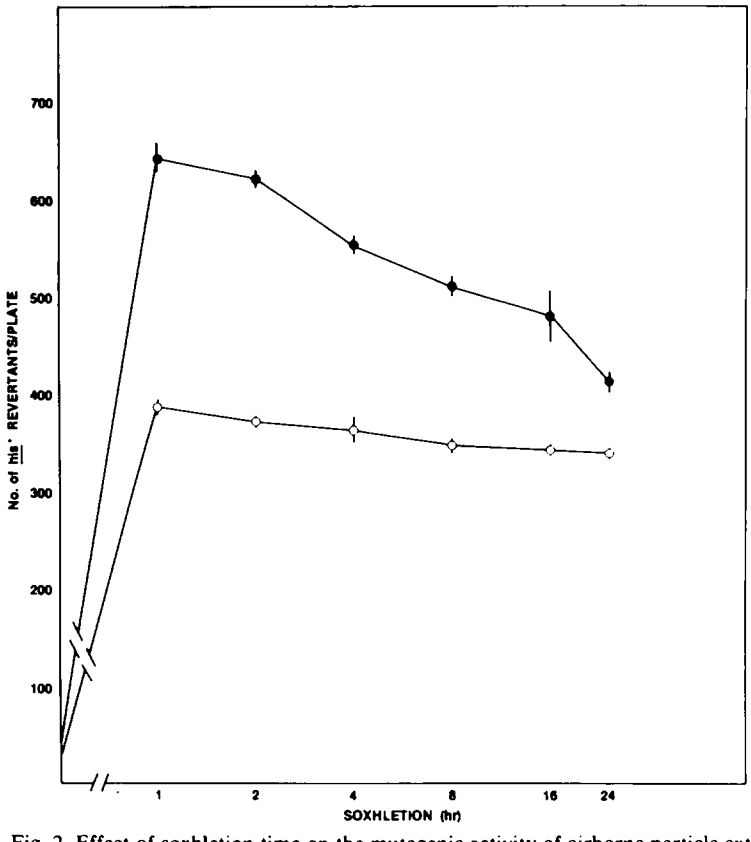

Fig. 2. Effect of soxhletion time on the mutagenic activity of airborne particle extracts (equivalent to 10.3 mg/plate) in *S. typhimurium* TA98, with S9 (●) and without S9 (○). Bars represent standard error of means.

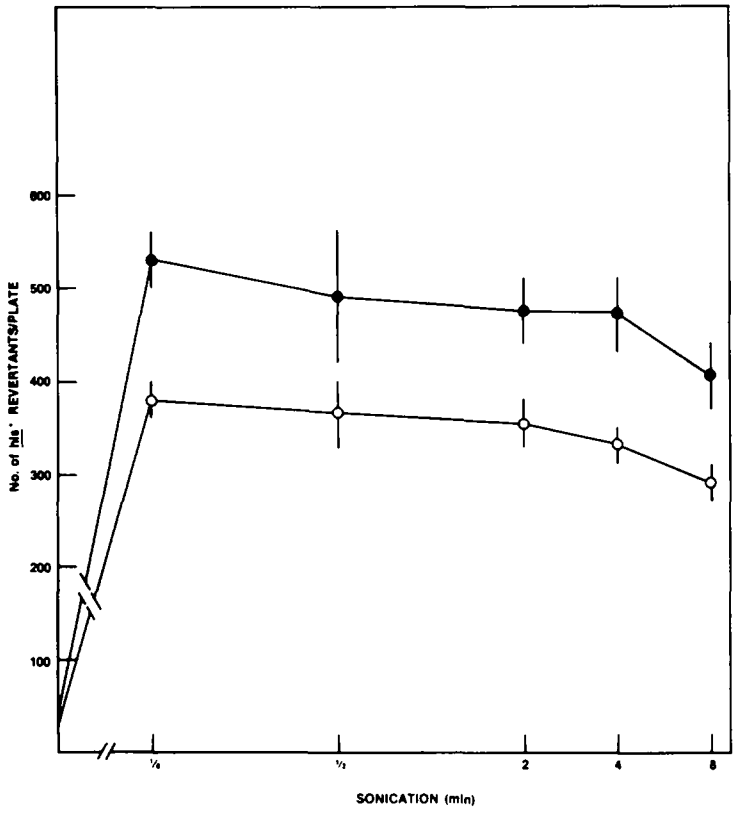

Fig. 3. Effect of sonication time on the mutagenic activity of airborne particle extracts (equivalent to 12.2 mg/plate) in *S. typhimurium* TA98, with S9 (●) and without S9 (○). Bars represent standard error of means.

The highest concentrations of extracts (based on original particle weight) used for mutagenic testing in *S. typhimurium* TA98 were: 4.1 mg/plate for shaking, 10.3 mg/plate for soxhletion and 12.2 mg/plate for sonication. The results indicate that there was a gradual increase of *his*$^+$ revertants with time of shaking up to 4 h followed by a saturation of mutagenic response (Fig. 1). The number of revertants per plate increased from 106 after 0.5 h to 193 after 4 h of shaking. Similar response was observed with and without S9 activation. TA100 responded comparably (data not shown). In soxhletion, 1 h of extraction with acetone yielded maximum *his*$^+$ revertants (Fig. 2). With further increase in time a gradual decrease in number of revertants was noticed, especially with S9 activation. With S9 activation the numbers of *his*$^+$ revertants were 645 and 416 per plate after 1 and 24 h soxhletion, respectively. Sonication for 1/8 min reached maximum mutagenic response (533 revertants per plate, Fig. 3). The number of revertants did not change materially up to 4 min of sonication. Beyond 4 min a decrease in the number of revertants was observed. Similar response was noticed with and without S9 activation. The blank

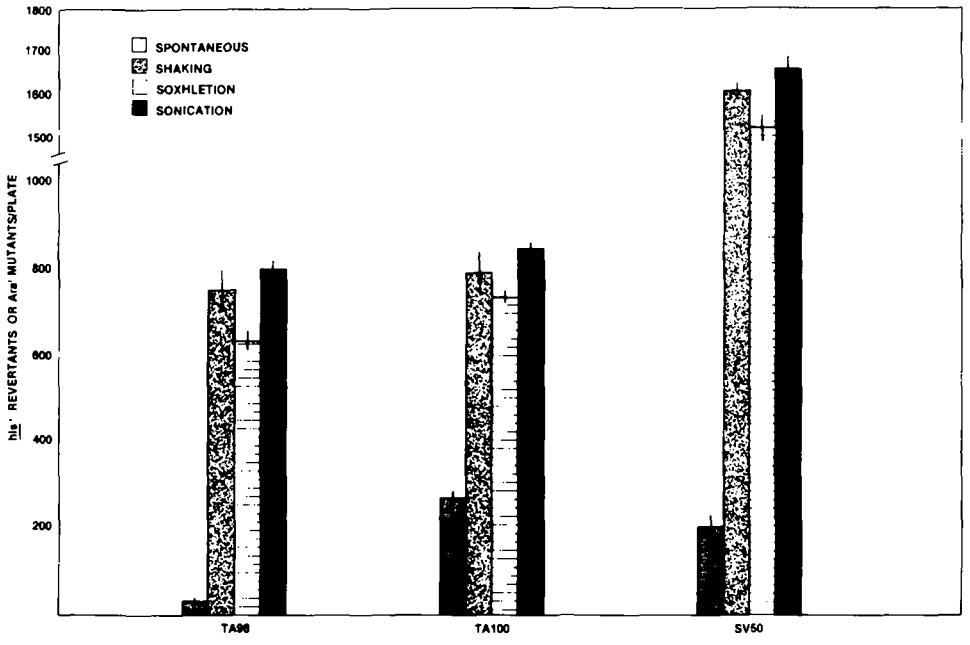

Fig. 4. Comparative mutagenic response of TA98, TA100 and SV50 of *S. typhimurium* to extracts prepared by shaking, soxhletion and sonication of airborne particles in acetone with S9 activation. The extract concentrations were: 13.8 mg particles/plate for TA98 and TA100; 10.2 mg particles/plate for SV50. Numbers represent averages of 2 Expts. The differences among extraction methods are statistically significant for TA98 and SV50 at $P < 0.05$.

control filter did not show any mutagenic activity.

The results on comparison of methods of extraction at their respective optimum time are shown in Fig. 4. In general, sonication and soxhletion extracts gave the highest and lowest mutagenic activities, respectively. Mutagenic response was comparable in TA98 and TA100 strains in the Ames assay as well as in the SV50 of the Ara^r assay system with S9 activation. A similar pattern was observed in TA98 and TA100 without S9 activation (data not shown).

Discussion

The optimum times for extraction of mutagens from airborne particles by shaking, soxhletion and sonication were 4 h, 1 h and less than 1 min, respectively. The response showing a plateau of *his*$^+$ revertants after 4 h shaking indicated that most of the extractable mutagens from airborne particles were extracted by this time. In soxhletion, maximum appearance of *his*$^+$ revertants at 1 h indicates that 1 or 2 syphoning cycles may be sufficient to extract most of the mutagens from airborne particles. Soxhletion for less than 1 h was not attempted since 1 syphoning cycle takes approximately 45 min. The gradual decrease in *his*$^+$ revertants with further

increase in duration of soxhletion indicates inactivation of mutagens from prolonged presence in boiling solvent. Temperature of boiling solvent, in combination with other physical and chemical factors perhaps affected the stability of mutagens. Fisher et al. (1979) reported complete loss of mutagenic activity of coal fly ash with heating to 350°C. Loss of mutagenic activity of fly ash was attributed to decomposition rather than volatilization of fly-ash mutagens (Chrisp and Fisher, 1980). A marked decrease of *his*$^+$ revertants with time in the presence of S9 indicates that promutagens are perhaps more heat labile than direct-acting mutagens. The relationship of *his*$^+$ revertants with time of sonication suggests that sonication in acetone for a short time was sufficient for the extraction of most of the mutagenic material from ambient air particles. This indicates that mutagens might be loosely adsorbed to the surface or located just under the surface of the airborne particles. Thus a suitable solvent without much mechanical force could be adequate for the extraction of mutagens.

Based on mutagenicity data, the optimal times for the extraction of mutagens from airborne particles by shaking, soxhletion and sonication are much shorter than those reported by other investigators (Whong et al., 1981a; Chrisp and Fisher, 1980; Epler, 1980; Hughes et al., 1980; Lockard et al., 1981).

A comparison of extraction methods by the Ames assay reveals that sonication was most efficient among the 3 methods tested. This conclusion was confirmed with the Ara^r assay system. Although the differences among various methods were not very striking, they were statistically significant for TA98 and SV50 ($P < 0.05$). These results, in general, are in agreement with those of Jungers et al. (1981), who found that acetone extracts by sonication had more mutagenic activity than soxhlet extracts. However, with fly ash, Mumford and Lewtas (1982) reported comparable extraction efficiency for soxhletion and sonication.

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