A SIMPLE METHOD FOR THE EXTRACTION OF MUTAGENS FROM AIRBORNE PARTICLES*

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Abstract. Organic materials were extracted with acetone from filters of airborne particles by soaking, shaking, soxhletion, and sonication. These extracts were tested with and without S9 for mutagenicity using Ames assay and arabinose-resistant assay of *Salmonella typhimurium*. Among the extraction methods, soaking extract had the highest mutagenic activity followed by sonication, shaking, and soxhletion in both the assays. With the samples studied, it was concluded that soaking with acetone for ½ hr is the simplest and an efficient procedure for the extraction of mutagens from airborne particles.

1. Introduction

During the past decade, much effort has been devoted to the development of sensitive and convenient experimental methods for identifying chemicals that induce mutations. Genetic toxicology tests are now being used to screen large numbers of compounds and complex mixtures in order to identify those that may pose a genetic or carcinogenic hazard to humans (Hughes *et al.*, 1980; Hollstein *et al.*, 1979). Considerations of time and expense necessitate that such screening be done with microorganisms or through other short-term tests (de Serres and Shelby, 1979; Hoffmann, 1982).

Methods of collection, extraction, chemical characterization and bioassay have been shown to be important in determining the mutagenicity of airborne particles (Hughes *et al.*, 1980; Chrisp and Fisher, 1980; Epler, 1980). Effort has been made by several laboratories to compare the efficiency of different solvent systems and methodologies for extraction of mutagens from airborne particles. Sonication and/or soxhletion of air particles in acetone have been found to be effective procedures for the extraction of mutagens (Talcott and Wei, 1977; Jungers *et al.*, 1981; Krishna *et al.*, 1983). In this study the efficacy of soaking airborne particles in acetone for extraction of mutagenic materials was evaluated.

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2. Materials and Methods

2.1. 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. The sampling was done for 48 hr continuously at a flow rate of approximately 60 ft^3/min. The Hi-Vol sampler collected respirable particles. The control and experimental filters were equilibrated at room temperature (22–26 °C) and humidity (50–60%) for at least 24 hr before and after collection and weighed to the nearest 0.1 mg on a microbalance.

2.2. Sample extraction

To determine the optimum time of soaking, each of the three sample filters was cut into 12 equal pieces. Equal number of pieces from each filter, selected at random, were shredded into 250 ml bottles and soaked in 150 ml acetone (reagent grade) for varying times. The control filter was treated in a similar manner. At appropriate time intervals, each bottle was gently shaken for about one min and the extract filtered through Whatman No. 2 filter paper. The residue was rinsed with 25 ml of acetone. The filtrates were evaporated to approximately 10 ml on a rotary evaporator (40 °C) and then to dryness on a dry bath (40 °C) with a stream of nitrogen gas. The dried extracts were dissolved in reagent grade dimethyl sulfoxide (DMSO) for mutagenesis tests.

To compare different extraction methods, three sample filters were divided into four equal parts and each was used for soaking (½ hr), shaking (4 hr), soxhletion (1 hr), and sonication (1 min). The times selected for shaking, soxhletion, and sonication were the optimal times previously determined (Krishna *et al.*, 1983). The extracts were prepared for mutagenesis tests as described previously.

TABLE I

Mutagenic activity of airborne particle extract following shorter soaking times[a]

Time (min)	His$^+$ Revertants ± SD	
	With S9	Without S9
Negative control[b]	24.0 ± 3.0	16.7 ± 2.0
Positive control[c]	863.3 ± 33.1	783.8 ± 57.4
5	998.3 ± 16.1	566.8 ± 13.4
15	976.7 ± 22.6	594.2 ± 14.9
30	988.2 ± 21.7	578.2 ± 10.9
60	722.3 ± 30.8	500.7 ± 12.9

[a] *S. typhimurium* TA98 was used as a tester. Concentration of extract at each time point was equivalent to 5.4 mg air particles/plate.
[b] 0.1 ml DMSO/plate.
[c] 0.25 μg 2-AA (with S9) and 0.1 μg TNF (without S9)/plate.

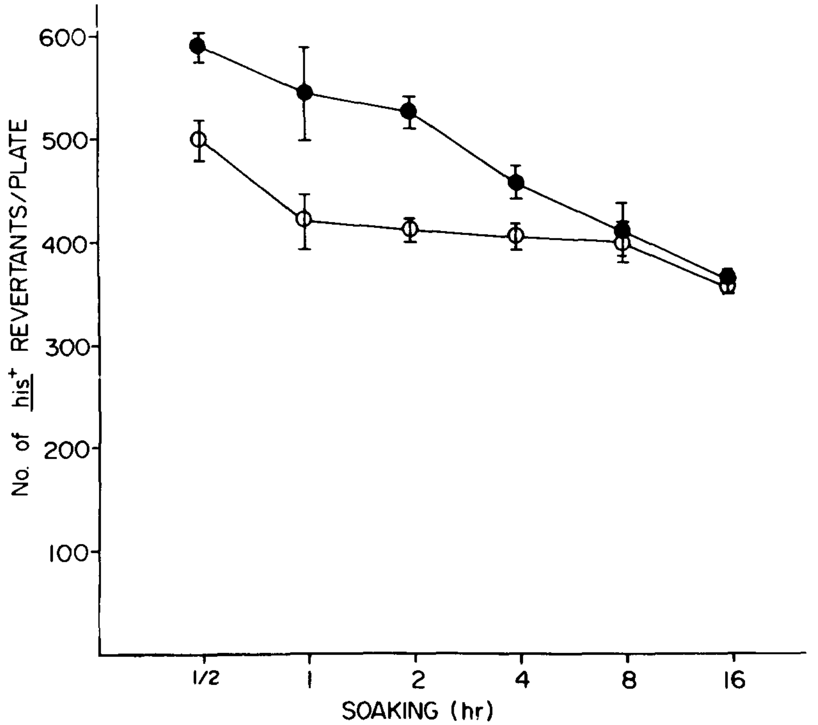

Fig. 1. Effect of longer soaking times on the mutagenic activity of airborne particle extracts in *S. typhimurium* TA98, with S9 (●) and without S9 (o). The concentration of extract at each time point was equivalent to 7.9 mg particles/plate. Bars represent standard error of means.

2.3. Mutagenicity assay

The Ames histidine (his^+) reversion assay (Ames *et al.*, 1975) and the arabinose resistant (Ara^r) forward mutation assay (Ruiz-Vazquez *et al.*, 1978; Whong *et al.*, 1981) in *Salmonella typhimurium* were carried out using the plate incorporation test. The organic extracts of airborne particles were tested in the Ames tester strains TA98 and TA100 and the arabinose tester SV50 with and without S9 activation. The S9 used was prepared from livers of Aroclor-1254 (500 mg/kg body weight) preinduced male Wistar rats. In the Ara^r assay system, SV50 cells (3×10^6) from an overnight culture and 0.1 ml of extract were added to molten soft agar containing 0.2 ml of 20% L-arabinose and were overlayed onto M9 bottom plate. The plates were scored for his^+ revertants in the Ames testers and Ara^r colonies in SV50 after incubation for 2 and 3 days at 37 °C, respectively. The data are means of two experiments, using three plates for each concentration. Even though in each experiment four concentrations were tested, for brevity, only the data from the highest concentration are reported. Positive control compounds, 2-aminoanthracene (2AA) and 2,4,7-trinitro-9-fluorenone (TNF) were included in each experiment. Spontaneous revertants or mutants were determined in plates treated with DMSO.

3. Results and Discussion

The data on the mutagenicity assay for extracts of airborne particles soaked for 5–60 min are shown in Table I. The results indicate comparable mutagenic responses among 5, 15 and 30 min of soaking. The extract from airborne particles soaked for 60 min indicated a decrease in mutagenic activity both with and without S9 activation in comparison with those soaked for shorter times. To obtain information on longer periods of soaking, equal amounts of sample filter were soaked for ½, 1, 2, 4, 8 and 16 hr in a separate set of experiments. The results are presented in Figure 1. Soaking of air particles in acetone for half-hour gave the highest *his*$^+$ revertants. With increased time of soaking, a corresponding decrease in number of revertants was noticed (Table I and Figure 1). Comparable response was observed both with and without S9 activation. With S9 activation the number of *his*$^+$ revertants per plate decreased from 591 with ½ hr to 362 with 16 hr soaking (Figure 1). The blank control filter did not show any mutagenic activity. These results suggest that a short period of soaking in acetone was sufficient to extract most of the mutagens from airborne particles. The decrease in mutagenicity with increase in time of soaking may be indicative of instability or volatilization of mutagens in prolonged presence of organic solvent such as acetone.

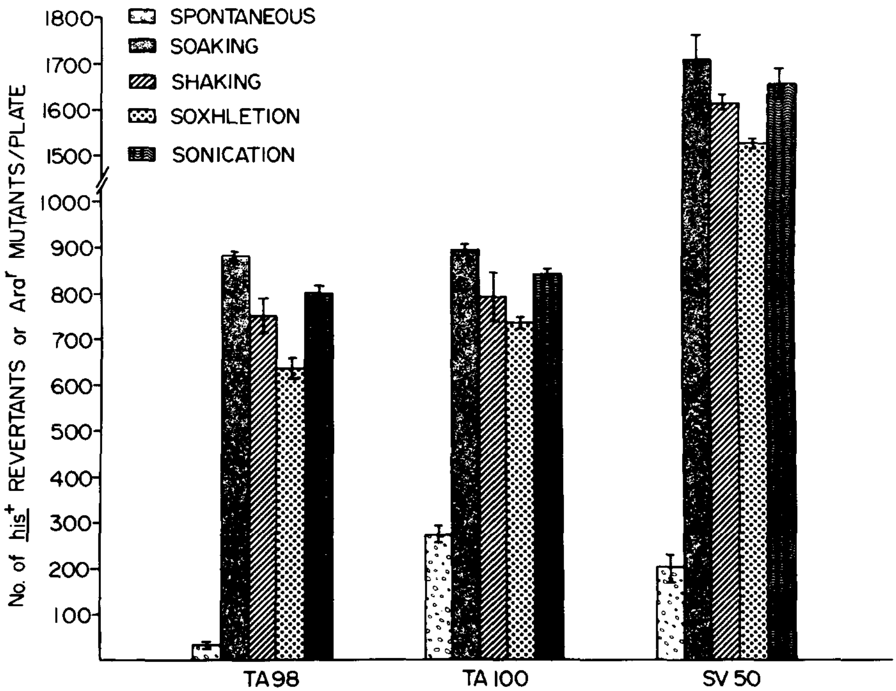

Fig. 2. Comparative mutagenic response of TA98, TA100 and SV50 of *S. typhimurium* to extracts prepared by soaking, shaking, soxhletion, and sonication of airborne particles in acetone with S9 activation. The extract concentrations were: 13.8 mg particles/plate for TA98 and TA100; 10.2 mg particles/plate for SV50 (based on original particle weight). Numbers represent average of two experiments. Bars represent standard error of means. The data on shaking, soxhletion, and sonication have been previously published (Krishna *et al.*, 1983).

Similar response with S9 activation further indicates that this phenomenon manifests for both direct acting mutagens and promutagens.

Comparative data on extraction methods with S9 activation (Figure 2) indicate that the extract from sample filters soaked for 1/2 hr yielded the highest mutagenic activity with all testers. At the concentrations tested with S9, the number of *his*$^+$ revertants per plate in TA98 was 879, 799, 753 and 637; for TA100 was 887, 841, 790 and 737; in SV50 the number of *Ara*^{r} mutants was 1706, 1654, 1614, and 1526 for soaking, sonication, shaking, and soxhletion, respectively. The differences were statistically significant at $p < 0.01$ for TA98 and at $p < 0.05$ for TA100 and SV50 by analysis of variance. Similar response was noticed without S9 activation in TA98 and TA100 as well as in SV50 (Figure 3). However, relatively fewer revertants/mutants were observed in the assays without S9. Although the differences among various methods were not very striking, they were statistically significant for SV50 ($p < 0.01$).

The effectiveness of the soaking method for the extraction of mutagens from air particles may be due to loose adsorption of mutagens on air particles and high solubility of mutagens in acetone. The results of the Ames assay were confirmed by the *Ara*^{r} assay. Similar response observed both with and without S9 activation, confirms the efficiency

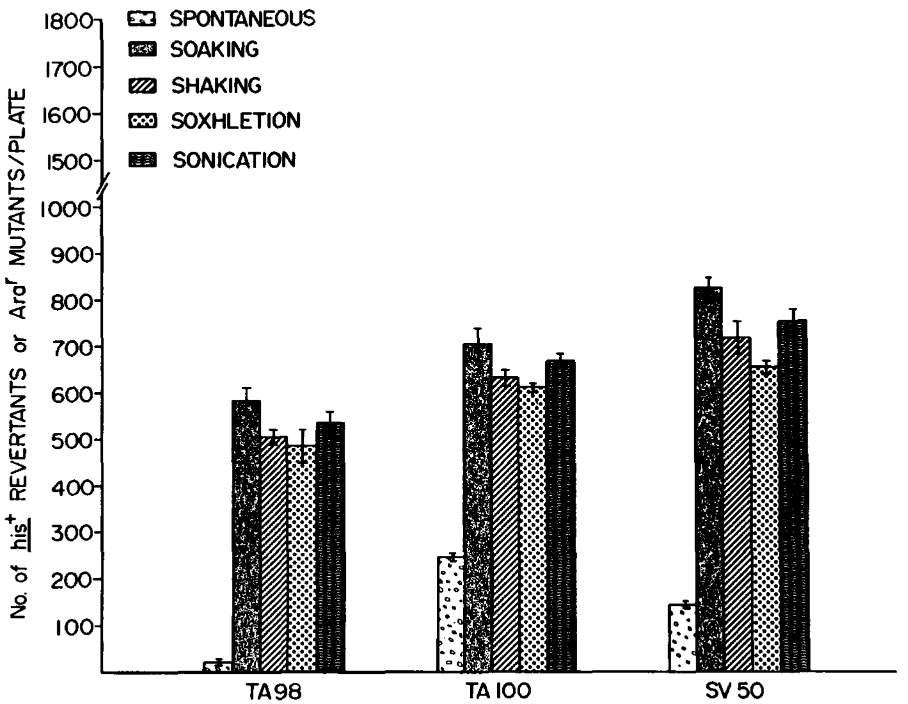

Fig. 3. Comparative mutagenic response of TA98, TA100 and SV50 of *S. typhimurium* to extracts prepared by soaking, shaking, soxhletion, and sonication of airborne particles in acetone without S9 activation. The extract concentrations were: 13.8 mg particles/plate for TA98 and TA100; 1.0 mg particles/plate for SV50 (based on original particle weight). Numbers represent averages of two experiments. Bars represent standard error of means.

of soaking in the extraction of promutagens and direct acting mutagens. However, at this time it is not clear as to how mechanical force (shaking and sonication) inhibits and/or retards the extraction process. The minimal effectiveness of soxhletion in mutagen extraction is in agreement with other studies (Jungers *et al.*, 1981; Krishna *et al.*, 1983). This may be due to the instability of mutagens in the presence of boiling solvent. Temperature of boiling solvent in combination with other physical and chemical factors, perhaps, affected the stability of mutagens (Chrisp and Fisher, 1980; Fisher *et al.*, 1979).

The results reported here indicate that soaking up to 1/2 hr as a method for the extraction of mutagens from airborne particles is promising and needs to be further explored with different samples. If soaking is indeed better or as good as shaking, soxhletion or sonication, then the extraction of mutagens from airborne particles for bioassay will become much easier and simpler.

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