Environmental Mutagenesis 6:585–592 (1984)

Correlative Genotoxicity Studies of Airborne Particles in *Salmonella typhimurium* and Cultured Human Lymphocytes

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The acetone extracts of ambient air particulates collected locally were tested for their capacity to induce sister chromatid exchanges (SCEs) and chromosomal aberrations (CAs) in human lymphocytes, and to induce gene mutations (GMs) in *Salmonella typhimurium*. The extracts caused dose-related clastogenic/mutagenic responses in all three assay systems. With the same concentration, it seems that the Ames *Salmonella*/microsomal assay with TA98 gave the highest, and the chromosomal aberration assay with human lymphocytes the lowest, mutagenic/clastogenic responses, respectively. Because high frequencies of SCEs were induced by solvent extracts of airborne particles, this study further indicated the usefulness of SCE assay in human lymphocytes for genotoxicity studies of airborne particles.

Key words: airborne particles, SCEs, chromosome aberrations, gene mutations, genotoxicity

INTRODUCTION

In recent years many studies have been conducted to determine the potential toxicological properties of air pollutants. Solvent extracts of airborne particles from many locations have been shown to be mutagenic in the Ames *Salmonella*/microsomal assay system [Chrisp and Fisher, 1980; Hughes et al, 1980; Epler, 1980; Holmberg and Ahlborg, 1983]. Recently, using the Ames assay, Walker and co-workers [1982] found that the mutagenicity level of Houston air particulate extracts from seven sites is correlated with the lung cancer mortality.

Results of cooperative investigation of West Virginia Agriculture and Forestry Experimental Station and NIOSH. Published with the approval of the Director of the West Virginia Agriculture and Forestry Experimental Station as Scientific paper No. 1882.

Received January 30, 1984; revised and accepted March 30, 1984.

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Since our main concern regarding air pollution is its potential health hazard to the exposed population, it would be of interest to determine whether solvent extracts of airborne particles can cause genetic damages/alterations in human cells. To date, only a few studies with human cells have been carried out. Solvent extracts of city smog from the heavily industrialized Rhine-Ruhr area of West Germany [Schurer et al, 1980; Seemayer et al, 1982], and airborne particles from Lexington, Kentucky [Lockard et al, 1981] were found to induce sister chromatid exchanges (SCEs) in human peripheral lymphocytes. Whether the solvent extracts of airborne particles can also induce chromosomal aberrations or other genetic changes has not yet been reported.

In earlier studies conducted in our laboratories, the solvent extracts of airborne particles collected locally were found to be mutagenic in the Ames *Salmonella*/microsomal assay [Whong et al, 1981]. Present studies were designed to determine whether solvent extracts of airborne particles could induce SCEs and chromosomal aberrations (CAs) in human peripheral lymphocytes, and to compare the genotoxicity responses of SCEs, the chromosomal aberrations, and the Ames assay system to the same concentration of solvent extracts.

MATERIALS AND METHODS

Sample Collection and Preparation

Airborne particles were collected, extracted, and prepared as described in previous studies [Krishna et al, 1983]. In brief, the airborne particles were collected with a Hi-Vol air sampler during Sept–Oct 1982 on high-purity glass microfiber filters. The sample filters were soaked in acetone for 30 min and then filtered through Whatman number 2 filter paper. The acetone extract was concentrated 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 dimethylsulfoxide (DMSO) and were either used immediately or stored at −20°C until used. The amount of DMSO in different concentrations of air particles tested was the same, ie, 0.1 ml/plate for the Ames test or 0.1 ml/10 ml culture medium for the SCE and chromosome aberration assays.

Assay Systems

Sister chromatid exchange assay in human peripheral lymphocytes. Heparinized human peripheral lymphocytes from two normal unrelated male and female donors (0.6 ml/10 ml culture) were cultured in the dark at 37°C for 72 hr in RPMI-1640 culture medium supplemented with 15% fetal bovine serum (FBS), l-glutamine (final concentration 2 mM), 1% pencillin-streptomycin (GIBCO), 0.1 ml phytohemagglutinin (PHA, GIBCO), and 25 μM 5-bromo-2′-deoxyuridine (BrdU). The airborne particulate extract and the positive and negative control compounds were added 24 hr after the cultures were initiated. Mitomycin C (5 ng/ml) and DMSO (0.1 ml) were used as positive and negative controls, respectively. Colcemid (0.2 μg/ml, GIBCO) was added for the last 3 hr of incubation. At the end of the culture period, cells were collected, treated for 7–8 min with 0.075 M KCl, fixed in methanol-glacial acetic acid (3:1), spread on cold wet slides, and air dried. The preparations were stained with Hoechst 33258 (5 μg/ml) in phosphate buffer (pH 6.8), exposed to "black" light (55–60°C) for 6 min at a distance of 1 cm, and stained

with Geimsa for 10–15 min as described by Perry and Wolff [1974] and Goto et al [1978]. The slides were coded, and 25 cells per dose were scored from each person.

Chromosomal aberration assay in human peripheral lymphocytes. Human peripheral blood was cultured as described for SCEs with the exception that the culture duration was 52 hr. The clastogenic activity of organic extracts of airborne particles was tested during S and G_2 phases of the cell cycle [Preston et al, 1981]. The airborne particle extract as well as the positive and negative control compounds were added to the culture at 42 hr after culture initiation and allowed to remain in culture throughout the course of the culture period. BrdU was included in the culture media to insure that only first-division cells were scored. Staining was achieved as described for SCEs. The slides were coded and 50 cells were scored from each person.

Ames *Salmonella* assay. The same concentrations of organic extracts of airborne particles used in SCE and chromosome aberration assays were tested in *Salmonella typhimurium* as described by Ames et al [1975] in strains TA98 and TA100 without metabolic activation. The data are means of two experiments, in which two plates were used for each concentration. Spontaneous revertants were determined in plates treated with DMSO. Positive controls of 2,4,7-trinitro-9-fluorenone (TNF) and N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) were included in each experiment.

Statistical analysis. The Student t-test was used to compare the SCE frequency and histidine reversion frequency with the negative controls. The chi-square test was used to analyze chromosomal aberration frequency. The correlation coefficient (R) was calculated between dose response and the observed and predicted values in all assays. General linear regression curves using quadratic equation were also drawn.

RESULTS

The results of the SCE assay on the airborne particulate extract with different concentrations are shown in Table I. The data indicate a clear dose response (R = 0.98, Fig. 1). At the highest concentration of 10.93 mg particulate per 10 ml culture tested, the average SCE frequency was 20.74 per cell. This frequency is about three times greater than the background level. Comparable responses were obtained for both blood donors at all concentrations.

The frequency of chromosomal aberrations with different concentrations of the airborne particulate extract is presented in Table II. Although the frequency and type of chromosomal aberrations varied somewhat between donors, the combined aberration frequency showed a dose-related response (R = 0.58, Fig. 1). At the highest concentration tested (10.93 mg per 10 ml culture), 13 chromosomal aberrations per 100 cells were observed, which is significantly greater than that of the spontaneous level (1 in 100 cells). A similar response is evident even without chromosomal gaps.

The data on gene mutations induced by the airborne particle extract in the Ames assay with TA98 and TA100 are shown in Table III. With the same concentrations used in the cytogenetic assays, the Ames test yielded a relatively higher number of revertants compared to the cytogenetic assays. Dose-related response was observed in both tester strains TA98 and TA100 (R = 0.99 in TA100, and R = 0.97 in TA98, Fig. 1). The highest concentration tested (10.93 mg per plate) yielded 607 and 727 *his*$^+$ revertants in TA98 and TA100, respectively.

TABLE I. Sister Chromatid Exchanges Induced by Solvent Extract of Airborne Particles in Human Lymphocytes

Airborne particles[a]	Donor	SCEs/chromosome	SCEs/cell ± SE
Negative control[b]	A	0.14	5.88 ± 0.54
	B	0.16	7.20 ± 0.49
Positive control[c]	A	0.27	12.32†† ± 0.84
	B	0.21	9.28** ± 0.79
0.36	A	0.24	10.60†† ± 0.74
	B	0.18	8.00* ± 0.55
1.09	A	0.26	11.28†† ± 0.56
	B	0.22	9.80† ± 0.56
3.64	A	0.38	17.04†† ± 1.21
	B	0.34	15.64†† ± 1.03
10.93	A	0.47	21.44†† ± 1.27
	B	0.44	20.04†† ± 0.95

[a]mg/10 ml culture.
[b]0.1 ml DMSO/10 ml culture.
[c]50 ng mitomycin C/10 ml culture.
*$P > 0.05$.
**$P < 0.05$.
†$P < 0.01$.
††$P < 0.001$.

Table IV shows a comparative clastogenic/mutagenic response of different doses with different assay systems on the basis of per cell or plate per mg of particulate. In general, it is evident that there is a decrease in clastogenic/mutagenic response with an increase in the dose of airborne particulate extract. The data show a negative correlation between dose and response in each assay studied.

DISCUSSION

The results reported here show that the solvent extracts of ambient air particles collected from the top of a local building induced SCEs and chromosome aberrations in human peripheral lymphocytes, in addition to *his*$^+$ revertants in the Ames assay with strains TA98 and TA100. None of the genetic end points used in this study required metabolic activation. The direct-acting mutagenicity of airborne particles found in this study is in agreement with those of other reports [Talcott and Wei, 1977; Pitts et al, 1977; Wang et al, 1978; Lockard et al, 1981; Whong et al, 1981]. The common mutagenic components of the solvent extracts of ambient air particles may be the nitroderivatives of polycyclic aromatic hydrocarbons, which do not require metabolic activation for their mutagenic activity.

It may be observed that most of the concentrations of airborne particles that are positive in the Ames assay are also positive in SCE and chromosome aberration assays. The Ames assay with TA98 appears most sensitive and the chromosomal aberration assay in human lymphocytes seems the least sensitive. It should be noted that assigning equivalence to cytogenetic assays and bacterial assays presents some difficulty, since the genetic end points, the background frequencies, and the scoring of data are different in these assays. Nevertheless, the results indicate differences in gene mutation and cytogenetic effects. Alink et al [1983] have demonstrated good

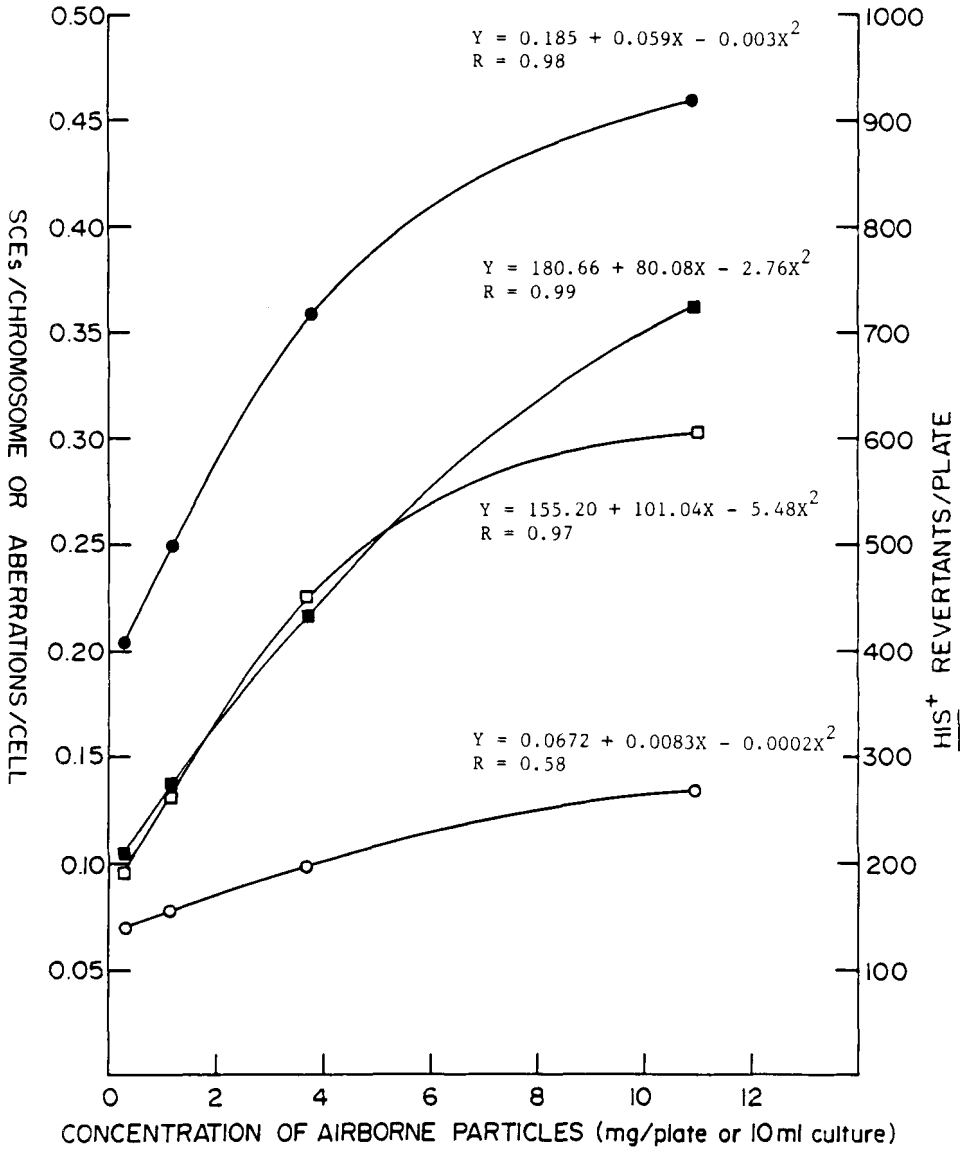

Fig. 1. General linear regression curves, expressing correlation of the clastogenic/mutagenic activity of airborne particle extract in different assays at different concentrations of air particles. Predicted values at the concentrations tested are plotted as follows: sister chromatid exchanges (●), chromosomal aberrations (with gaps) (○), gene mutations in TA100 (■), and gene mutations in TA98 (□).

correlations between dose response of *his*$^+$ revertants in Ames test and frequency of SCEs in Chinese hamster ovary cells after treatment with air particle extract.

The negative correlation between dose and mutagenic/clastogenic response per plate/mg or per cell/mg in different assays indicates a dose-effect compensation. That is, with the same cell number exposed, on per mg basis, higher concentrations of air particles yielded relatively lower numbers of SCEs, chromosomal aberrations, or gene mutations than at lower concentrations. This correlation could be due to toxicity

TABLE II. Chromosome Aberrations Induced by Solvent Extract of Airborne Particles in Human Lymphocytes

Airborne particles[a]	No. of chromosome aberrations in 100 cells		Types of chromosome aberrations[b]							
	−Gaps	+Gaps	G	B	TD	D	F	TR	R	DM
Negative control[c]	0	1	1							
Positive control[d]	4*	7*	3		2					2
0.36	3**	6**	3	2			1			
1.09	5*	9†	4		2	3				
3.64	6*	9†	3		1	1	3		1	
10.93	11†	13†	2			5	1	1	4	

[a]mg/10 ml culture.
[b]Abbreviations used: G, gaps; B, breaks; F, fragments and acentric fragments; TD, chromatid deletions; R, rings; D, dicentrics; DM, double minutes; TR, triradials.
[c]0.1 ml DMSO/10 ml culture.
[d]200 ng mitomycin C/10 ml culture.
*$P < 0.05$.
**$P > 0.05$.
†$P < 0.01$.

TABLE III. Mutagenic Response of *Salmonella typhimurium* to Solvent Extract of Airborne Particles

Airborne particles (mg/plate)	His^+ revertants per plate[a] ± SE	
	TA98	TA100
Negative control (DMSO 0.1 ml/plate)	26 ± 3.84	135 ± 8.37
Positive control[b]	865** ± 31.98	2,327** ± 67.10
0.36	163** ± 4.79	198* ± 1.97
1.09	297** ± 20.90	281** ± 8.55
3.64	439** ± 32.77	431** ± 18.54
10.93	607** ± 17.74	727** ± 26.05

[a]Average of two experiments in duplicate.
[b]0.1 μg 2,4,7- trinitro-9-fluorenone for TA98 and 2.5 μg MNNG for TA100/plate.
*$P < 0.01$.
**$P < 0.001$.

of the airborne particulate extract at higher concentrations that can cause cell killing, due to limited solubility of the active compounds associated with the air particles, or due to other unknown factors. It should be noted that in the cultures treated with higher concentrations the relative proportions of first, second, and third divisions were essentially similar to those treated with lower concentrations.

The increase in the SCE frequency and the dose response in the SCE assay system in the present study further indicate that the SCE analysis using human peripheral lymphocytes can be a valuable method in genotoxicity studies of ambient air particles and other complex mixtures in our environment.

TABLE IV. Induction of SCEs and Chromosomal Aberrations (CAs) in Human Lymphocytes and Gene Mutations (GMs) in the Ames Test by Solvent Extract of Airborne Particles on per mg Basis*

Airborne particles (mg)	Mean SCEs/ cell/mg	Mean CAs/ cell/mg	Mean GMs (TA98) /plate/mg	Mean GMs (TA100) /plate/mg
0.36	7.67	0.14	380.56	175.00
1.09	3.67	0.07	248.62	133.95
3.64	2.69	0.03	113.46	81.32
10.93	1.30	0.01	53.18	54.16

*The spontaneous level has been subtracted and average numbers of each assay were used for calculation (chromosomal gaps were included).

ACKNOWLEDGMENTS

The authors thank Dr. J.D. Tucker for technical suggestions and Professor B.N. Ames for generously providing the *his* tester strains.

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