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Mutagens and carcinogens in size-classified air particulates of a Northern Italian town

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Abstract

This research was designed to examine the presence of mutagenic/carcinogenic compounds in urban airborne particulate matter in relation to particles aerodynamic size. Inhalable (< 10 μm) airborne particulate (PM-10) was collected at a low traffic site in an industrialized Northern Italian town, using a high volume sampler equipped with a cascade impactor for particles fractionation. The organic extracts of different fractions were examined for mutagenicity in *Salmonella typhimurium* strains TA98 and TA98/1,8-DNP_6 using the microsuspension procedure, and for polycyclic aromatic hydrocarbons (PAHs) content by gas chromatography. Size fractionated particles were also analysed for heavy metals (Fe, Mn, Zn, Pb, Cu, Cd, Cr, Ni, V) using plasma spectrophotometry. The results of mutagenicity and chemical analyses indicate that, at the site investigated, inhalable particulate was largely made of fine ($< 0.5\mu$m) particles, which accounted for most of PAHs and mutagenicity. A similar pattern of distribution was found for heavy metals, which were relatively more abundant in small (< 1.5 μm) particles compared to coarser ones.

Keywords: Airborne particulate; Size-classified particulate; Mutagens; Carcinogens; PAH; Heavy metals

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1. Introduction

Research has shown that urban air, particularly in industrialized areas, contains a variety of known mutagenic and carcinogenic substances, including organic compounds such as benzo[*a*]pyrene and benzene, inorganic compounds such as nickel and chromium and radionuclides (Fishbein, 1993; Lewtas, 1993). Experimental studies have shown that extracts of urban air particles can induce skin cancer in animals (Hueper et al., 1962; Epstein et al., 1979; Sasaki et al., 1987) and are mutagenic in bacteria (Kado et al., 1986; Matsumoto and Inoue, 1987; Crebelli et al., 1988, 1995; De Raat, 1988; Viras et al., 1990, 1991; Fuselli et al., 1995; Pagano et al., 1996) and in mammalian cells (Krishna et al., 1984; Hadnagy and Seemayer, 1986; Hadnagy et al., 1989). Moreover, several epidemiological studies carried out over the last 40 years to investigate the health risk related to air pollution suggested a possible increased lung cancer risk for urban populations (Schwartz, 1991; Summerhays, 1991; Tzonou et al., 1992; Pershagen and Simonato, 1993). Therefore the monitoring of urban air for genotoxic micropollutants, in addition to conventional toxic pollutants (e.g. SO_2, NO_x, CO), is receiving increasing consideration to the aim of evaluating health risks for people living in urban areas.

Air pollution is presently considered a primary health problem in Lombardy, a highly industrialized and urbanized region of North Italy, with lung cancer rates higher than the Italian average. In this region, ambient air quality standards for regulated air pollutants are sometimes exceeded, with consequent endorsement of temporary emergency measures such as traffic restriction in urban areas or prohibition for circulation of uncatalyzed vehicles. However, few if any information is available on local levels of airborne mutagenic/carcinogenic pollutants, especially in the finest, deeply inhalable particulate matter.

The aim of this work was to study the content of mutagenic and carcinogenic compounds in air particles collected at a typical medium-size Lombard town, characterized by high industrial pollution and a relatively moderate traffic intensity, using both bacterial mutation assays and chemical methods. Emphasis was given to the analysis of the distribution of both mutagens and organic (PAHs) and inorganic (heavy metals) carcinogens in air particulate in relation to particle aerodynamic size, in view of the greater ability of smaller particles to reach the bronchial and alveolar portions of the respiratory tract.

2. Material and methods

2.1. Air samplings

Brescia is the second largest town in Lombardy (approx. 200 000 inhabitants; 100 km from Milan). The town is heavily industrialized, featuring mainly foundries and metal industries. From the beginning of the 1970s, air pollution emissions from diffuse heating sources were reduced because of the adoption of a district heating system, the largest in Italy, used by more than 50% of the population.

Samples of inhalable (< 10 μm) airborne particulate matter (PM-10) were collected during the spring of 1991 in a residential area of the town, with moderate car traffic. Particles were collected on preweighted glass fiber filters using a high volume sampler (High Volume PM-10 sampler, Andersen) placed at the street level. In one sampling (A) the total PM-10 was collected, whereas in other samplings (B, C, D and E) a 5-stage cascade impactor (Andersen) was applied downstream of the high-volume sampler, in order to separate particles according to their aerodynamic size in five classes: 7.2–10 μm (stage 1), 3–7.2 μm (stage 2), 1.5–3 μm (stage 3), 0.95–1.5 μm (stage 4), 0.5–0.95 μm (stage 5) and < 0.5 μm (final stage). Filters were stored at -20°C in the dark until sample preparation.

2.2. Sample preparation

Glass-fiber filters were dried at room temperature, weighted and extracted for 300 cycles in a Soxhlet apparatus with 200 ml of dichloromethane (DCM). In order to obtain sufficient amount of extracts for mutagenicity tests and PAH analysis,

filters from samplings B, C and D were pooled together according to the granulometry of particles collected and their extracts combined in two larger samples: 0.5–10 μm (stages 1–5) and < 0.5 μm (final stage). The extracts were evaporated to dryness with a rotating evaporator and the residues stored in the dark at −20°C until analyses for mutagenicity and PAH content. Filters of sampling E were used for metal analysis with no previous extraction.

2.3. Airborne particulate mutagenicity analysis

The mutagenicity of air particulate extracts was analysed in the so-called Kado test, a simple modification of the *Salmonella*/microsome liquid-incubation procedure (Kado et al., 1983). This procedure was previously found to be especially sensitive to airborne mutagens (Kado et al., 1986; Lewtas et al., 1990; Bagley et al., 1992) and it was recommended for the analysis of complex environmental mixtures (Agurell and Stensman, 1992; Claxton et al., 1992). Briefly, air particle extracts were dissolved in dimethyl sulfoxide (DMSO) and analyzed in triplicate plates at three increasing concentrations (50, 100 and 200 μg/plate), with and without exogenous metabolic activation (provided by liver S9 from Aroclor pretreated rats), using the *S. typhimurium* tester strains TA98 and TA98/1,8-DNP_6. The latter strain is deficient in *O*-acetyl transferase and resistant to the mutagenicity of nitropyrenes (McCoy et al., 1983) and it is widely used as an indicator of the presence of nitroarenes in complex mixtures. Positive control substances were 4-nitro-*o*-phenylenediamine for TA98 − S9, 4-nitroquinoline-1-oxide for TA98/1.8-DNP_6 − S9, 2-acetylaminofluorene for TA98 + S9 and benzo[*a*]pyrene for TA98/1,8-DNP_6 + S9. Pure DMSO and extracts of filter blanks were also tested as negative controls.

The results of mutagenicity assays are expressed as specific mutagenic activity, i.e. induced his^+ revertants/mg of tested material, calculated by linear regression analysis of the linear part of the dose–response curves. These values were also used to calculate air mutagenicity levels (i.e. induced revertants/m^3) on the basis of the content of organic extract/m^3.

2.4. Chemical analysis

The PAH concentrations were determined by high-resolution gas chromatography (HRGC) on extracts of the pooled samples B, C and D after a purification process of the samples themselves.

The analysis was carried out using a Hewlett-Packard mod. 5990 gas chromatograph. The operating conditions were the following:

- Column: SE-54 (25 m × 0.32 mm × 0.52 μm)
- Detector: FID
- Detector temperature: 300°C
- Injection system: on-column
- Carrier gas: helium
- Column temperature:
  1. 80°C (initial temperature)
  2. 30°C/min increase up to 120°C
  3. 4°C/min increase up 280°C (final temperature).

The detection limit was 0.1 ng/m^3 for each compounds. The PAH contents were calculated on the basis of recovery tests carried out with a standard mixture.

Recovery percentages, tested in several experiments, were close to 100%. A somewhat lower percentage was, however, detected in the case of low molecular weight compounds. The reference PAH mixture EPA 610 (Supelco Inc., USA) was used as standard.

Filters of the sampling E were analyzed for the content of heavy metals (Fe, Mn, Zn, Pb, Cu, Cd, Cr, Ni, V) by ICP-AES analysis with plasma spectrophotometry JY24 (Jobin-Yvon Instruments, Longjumeau, France). The filters were treated with nitric acid to oxidize the organic matrix and to dissolve the metals. Blanks were also performed. The detection limits were 5.3, 7.2, 6.3, 2.6, 2.8, 23.8 and 3.2 ng/ml for Zn, Pb, Fe, Mn, Cr, Cu and Ni respectively. Multielement solution (MSQ-6, C. Erba, Milan, Italy) was used as standard.

3. Results

Table 1 shows weight values and content of

Table 1
Samplings of airborne particulate matter in the city of Brescia (weight values and gaseous pollutant levels)

Sample (date)	Filtered air	Particulate collected (mg)			Particles ($\mu g/m^3$)	Organic extract (mg)	Organic extract/m^3 (μg)	NO_2 ($\mu g/m^3$)	SO_2 ($\mu g/m^3$)
		Total PM-10	0.5–10 μm	< 0.5 μm					
A (27 March 1991)	1545	105.8			68.5	12.7	8.2	70	32
B (19 March 1991)	1483	144.4	53.2	91.2	97.4			78	31
C (26 March 1991)	1545	54.1	18.3	35.8	35.0			70	33
D (9 April 1991)	1450	185.0	37.8	147.2	127.6			56	18
B–D (pooled)	4478					29.0* 36.0**	6.5* 8.1**		
E (29 November 1991)	1484	193.2	—	—	130.2	—	—	41	14

* 0.5–10 μm.
** < 0.5 μm.

dichloromethane extractable material in airborne particles sampled at a residential site in Brescia. The total concentration of inhalable particles (total PM-10) were in most cases above the Italian air quality standard (60 $\mu g/m^3$), and in one case (sample D) also above the recommended US standard (150 $\mu g/m^3$ as daily mean). The content of organic matter from the unfractionated air particles sample (12% by weight, sample A) was fairly similar to the figures reported from previous studies in other Italian cities (Crebelli et al., 1995; Fuselli et al., 1995). On the other hand, a larger amount of organic extract (13.3–26.4%) was obtained from the pooled filters of samplings B–D, possibly in connection to different meteorological conditions. Interestingly, in these samples

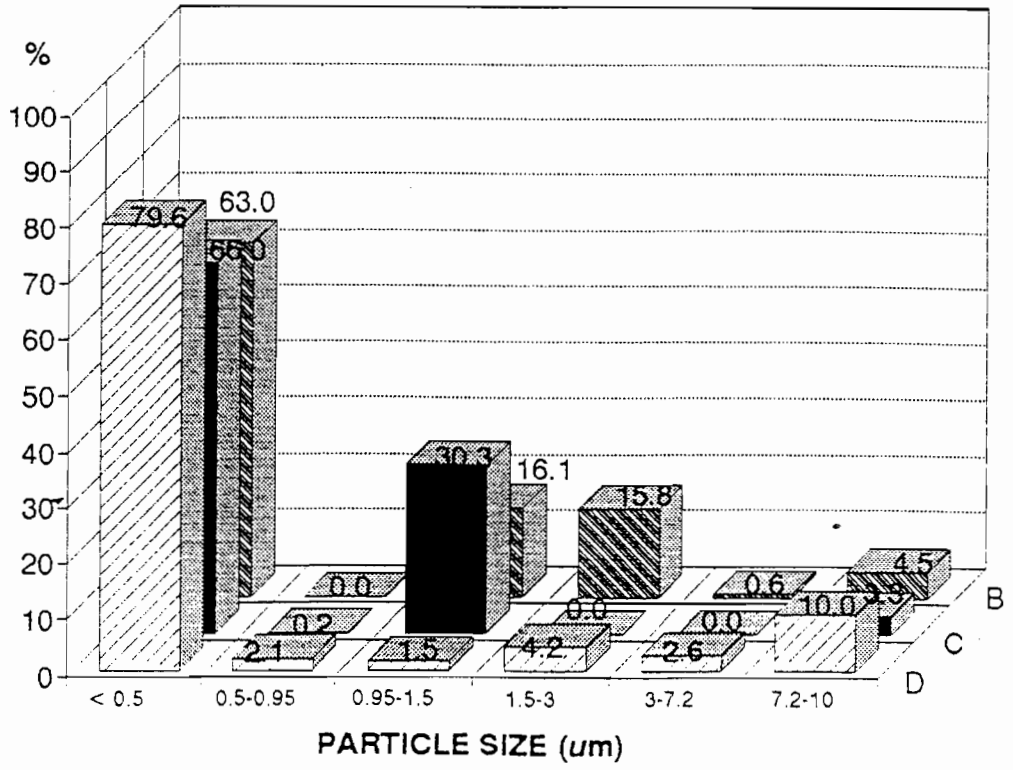

Fig. 1. Distribution of PM-10 particulates collected during samplings B, C and D in Brescia and fractionated according to the aerodynamic size using a 5-stage cascade impactor.

a relatively greater amount of organic matter was obtained from finest particles, with aerodynamic diameter < 0.5 μm.

In the same table, the average levels of SO_2 and NO_2 recorded during samplings are shown: both gaseous pollutants were below the Italian air quality standards (125 μg/m³ for SO_2, mean of 24 h and 200 μg/m³ for NO_2, mean of 1 h) during particles collection.

Fig. 1 shows size distribution of airborne particles fractionated by granulometry using the Andersen 5-stage cascade impactor (samples B–D). The major contribution to the total PM-10 matter (63–80%) was given by the finest fraction (< 0.5 μm), whereas larger particles contributes to a more limited extent.

Table 2 summarizes the results of mutagenicity assays with both non-fractionated and fractionated airborne particulates. All samples were found to be mutagenic in strain TA98 in the absence of exogenous metabolic activation, revealing the presence of direct-acting mutagens. The addition of S9 mix did not significantly increase mutagenic activities. In the fractionated samples, the extract of fine particles (< 0.5 μm) was more mutagenic, on a weight basis, than the extract of coarser particles (0.5–10 μm). From the above data, and from the weight values shown in Table 1, air mutagenicity levels (induced revertants/m³) are calculated, which indicate that finest particles give the prevalent contribution to air mutagenicity.

Parallel experiments with strain TA98/1,8-DNP_6 gave mainly inconclusiveresults with the unfractionated sample, because of the high toxicity elicited by the sample in this strain, especially in experiments without S9. The lower viability of

Table 2
Mutagenicity of airborne particulate matter extracts in *Salmonella typhimurium* tester strains (results from microsuspension test experiments)

Sample	Induced revertants/mg of extract				Induced revertants/m³ of air			
	TA98 −S9	TA98 +S9	TA98/ 1,8-DNP_6 −S9	TA98/ 1,8-DNP_6 +S9	TA98 −S9	TA98 +S9	TA98/ 1,8-DNP_6 −S9	TA98/ 1,8-DNP_6 +S9
A (unfractionated)	520	577	0.0*	116*	4.3	4.7	0.0	0.9
B–D (0.5–10 μm)	310	369	263	313	2.0	2.4	1.7	2.0
B–D (< 0.5 μm)	942	697	860	422	7.6	5.6	7.0	3.4

* Toxic effect.

Table 3
Polycyclic aromatic hydrocarbon (PAH) concentration in samples of airborne particulate matter sampled in the city of Brescia

Sample	PAH* (μg/mg of extract)							
	BaA	Chr + Tri	Pe	BaP	BeP	BghiPe	Py	ΣPAH
A (unfractionated)	0.46	1.15	0.13	0.35	0.96	1.77	5.26	10.08
B–D (0.5–10 μm)	0.04	0.03	0.02	0.02	0.04	0.05	0.26	0.46
B–D (< 0.5 μm)	0.25	0.29	0.07	0.22	0.42	0.53	2.09	3.87
	PAH* (ng/m³ of air)							
A (unfractionated)	3.77	9.41	1.07	2.82	7.83	14.47	42.87	82.24
B–D (0.5–10 μm)	0.25	0.22	0.15	0.16	0.27	0.30	1.72	3.07
B–D (< 0.5 μm)	2.04	2.36	0.58	1.78	3.43	4.34	16.96	31.49

* PAH: polycyclic aromatic hydrocarbon; BaA, benzo[*a*]anthracene; Chr + Tri, chrysene + triphenylene; Pe, perylene; BaP, benzo[*a*]pyrene; BeP, benzo[*e*]pyrene; BghiPe, benzo[*ghi*]perylene; Py, pyrene.

this TA98 derivative, compared to the parent strain, may explain the greater toxicity elicited by the test material in this strain. On the other hand, the results obtained with organic extracts of fractionated particles were basically comparable in the two strains (Table 2, samples B–D), suggesting a negligible contribution of nitroarenes and other nitrogen containing compounds to the mutagenicity of this sample.

Table 3 shows the concentrations of PAH in fractionated and non-fractionated particulate matter. Trace amounts of carcinogenic PAH (benzo[*a*]pyrene, benzo[*a*]anthracene) were found in all samples. The comparison of PAH concentrations in particles of different size shows far larger concentrations of all PAHs identified in finest particles (< 0.5 μm), which alone accounted for approx. 90% of the total PAH content in samples B–D.

The concentrations of carcinogenic and non-carcinogenic heavy metals was measured in another fractionated air particles sample (sample E), which was processed for analysis without previous extraction. Data in Table 4 show heavy metal concentrations in sample E which are comparable to the levels usually found in other European industrialized areas (World Health Organization, 1987), possibly reflecting the proximity to foundries and metal industries to the sampling site. Similarly to what observed for organic pollutants, also in this case the greater concentrations of heavy metals were found in small size particles (< 1.5 μm).

4. Discussion

This study was aimed at evaluating the presence of organic and inorganic mutagens and carcinogens in airborne particulate collected at a typical industrialized Northern Italian town. The sampling site was located in a residential area, where neighbouring industrial activities are supposed to be a major source of atmospheric pollution, even though the contribution of automobile exhausts cannot be dismissed. Mutagenicity testing of dichloromethane extracts of particulate matter, using a very sensitive bacterial assay, showed in all samples the presence of direct-acting mutagens, including species different from nitroaromatics. A similar activity profile was previously reported in studies on the organic extract of airborne particulates collected in another urban area (Crebelli et al., 1988). The fractionation of particles by size, using a cascade impactor, de-

Table 4
Heavy metals concentrations in size-fractioned airborne particles collected in the city of Brescia

Particle size (μm)	Concentration of heavy metals in air particles								
	$\mu g/m^3$ (% distribution by particle size)				ng/m^3 (% distribution by particle size)				
	Fe	Mn	Zn	Pb	Cu	Cd	Cr	Ni	V
10.0–7.2	0.93	0.04	0.03	0.08	0.05	2.6	4.3	17.3	6.9
	(19.3)	(2.8)	(0.7)	(4.2)	(10.9)	(8.6)	(11.6)	(16.7)	(10.8)
7.2–3.0	0.67	0.58	0.70	0.12	0.04	4.4	4.4	11.7	11.7
	(13.9)	(40.6)	(15.3)	(6.3)	(8.7)	(14.5)	(11.9)	(11.3)	(18.3)
3.0–1.5	0.03	0.03	0.28	0.09	0.02	0.8	4.0	24.0	5.3
	(0.6)	(2.1)	(6.1)	(4.7)	(4.3)	(2.6)	(10.8)	(23.1)	(8.3)
1.5–0.95	2.15	0.46	2.58	1.06	0.20	9.9	13.2	23.0	13.2
	(44.5)	(32.2)	(56.3)	(55.5)	(43.5)	(32.6)	(35.7)	(22.1)	(20.7)
0.95–0.5	0.51	0.20	0.65	0.23	0.09	8.5	2.8	11.3	14.2
	(10.6)	(14.0)	(14.2)	(12.0)	(19.6)	(28.0)	(7.6)	(10.9)	(22.3)
< 0.5	0.54	0.12	8.34	0.33	0.06	4.2	8.3	16.6	12.5
	(11.2)	(8.4)	(7.4)	(17.3)	(13.0)	(13.8)	(22.4)	(16.0)	(19.6)
Total PM-10	4.83	1.43	4.58	1.91	0.46	30.4	37.0	103.9	63.8
	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)

monstrated that suspended material was mainly composed of very fine particles (< 0.5 μm). Mutagenicity assays with fractionated samples demonstrated that the organic extract of finest particles was the most mutagenic, also on a weight basis, suggesting a different qualitative composition of the organic matter absorbed on airborne particles in relation to particle size, possibly related to their different deposition rates and atmospheric behaviour. This is conceivable in view of the well known extensive atmospheric transformation of precursor mutagenic compounds (Albrecheinski et al., 1985; Takeda and Teranishi, 1986).

A greater contribution to total air mutagenicity by finest particles was also reported in studies carried out in Greece (Viras et al., 1990, 1991), the Netherlands (De Raat, 1988) and Italy (Pagano et al., 1996), thus indicating that this phenomenon may be a general feature of the distribution of genotoxins in airborne particles. In addition, in this study a similar patter of distribution was also demonstrated for several carcinogenic and non-carcinogenic PAHs and heavy metals. Similar results were reported very recently in an independent work (Schnelle et al., 1996). Obviously PAH, which are proximate mutagens, do not play themselves a significant role in the response elicited by airborne particle extracts, which are typically direct acting mutagens. Rather, PAHs can be regarded as tracers of related compounds (mainly oxygenated derivatives) which play a major role in air particles mutagenicity in bacteria (Matsumoto and Inoue, 1987).

In conclusion, the results of this work suggest that the potential cancer risk associated to air particulate pollution is mainly related to the inhalation of fine particles (< 1.5 μm), which contain the highest amount of both organic and inorganic mutagens/carcinogens. These particles have greater possibilities to exert a detrimental effect, reaching the deep respiratory tract and lung alveoli. These conclusions are in line with the recently proposed US air quality standard for fine particles, which considers PM-2.5 instead of PM-10 as a more significant parameter for the evaluation of health hazards related to air pollution (Reichhardt, 1996).

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References

Agurell E, Stensman C. Salmonella mutagenicity of three complex mixtures assayed with the microsuspension technique. A WHO/IPCS/CSCM study. Mutat Res 1992;276:87–91.

Albrecheinski TM, Michalovic J, Gibson TL. Atmospheric reactions of polynuclear aromatic compounds as measured in a smog chamber. In: Cooke M, Dennis AJ, editors. Polynuclear aromatic hydrocarbons. Columbus, OH: Battelle Press, 1985:69–85.

Bagley ST, Stoltz SL, Becker DM, Keen RE. Characterization of organic extracts from standard reference materials 1649, 'urban dust/organics' and 1650, 'diesel particulate matter', using a microsuspension assay. A WHO/IPCS/CSCM study. Mutat Res 1992;276:81–86.

Claxton LD, Douglas G, Krewski D, Lewtas J, Matsushita H, Rosenkranz H. Overview, conclusions and recommendations of the IPCS collaborative study on complex mixtures. Mutat Res 1992;276:61–80.

Crebelli R, Fuselli S, Meneguz A et al. In vitro and *in vivo* mutagenicity studies with airborne particulate extracts. Mutat Res 1988;204:565–575.

Crebelli R, Fuselli S, Turrio Baldassarri L, Ziemacki G, Carere A, Benigni R. Genotoxicity of urban air particulate matter: correlations between mutagenicity data, airborne micropollutants and meteorological parameters. Int J Environ Health Res 1995;5:19–34.

De Raat WK. Polycyclic aromatic hydrocarbons and mutagens in ambient air particles. Toxicol Environ Chem 1988;16:259–279.

Epstein SS, Fiyi K, Asahina S. Carcinogenicity of a composite organic extract of urban particulate atmospheric pollutants following subcutaneous injection in infant mice. Environ Res 1979;11:163–176.

Fishbein L. Sources, nature and levels of air pollulants. In: Tomatis L, editor. Air pollution and human cancer. Monographs. European School of Oncology. Springer-Verlag, 1993:9–34.

Fuselli S, Benigni R, Conti L, Carere A, Crebelli R. Volatile organic compounds (VOCs) and air mutagenicity: results of one year monitoring at an urban site. Int J Environ Health Res 1995;5:123–132.

Hadnagy W, Seemayer NH. Induction of C-type metaphases and aneuploidy in cultures of V79 cells exposed to extract of automobile exhaust particles. Mutagenesis 1986; 1:445–448.

Hadnagy W, Seemayer NH, Tommingas R, Ivanfy K. Comparative study of sister-chromatid exchanges and chromosomal aberrations induced by airborne particulates from an urban and a highly industrialized location in human lymphocyte cultures. Mutat Res 1989;225:27–32.

Hueper WC, Kotin P, Tabor EC, Payne WW, Falck H, Sawicki E. Carcinogenic bioassay on air pollutants. Arch Pathol 1962;74:89–116.

Kado NY, Langley D, Eisenstadt E. A simple modification of the *Salmonella* liquid-incubation assay. Increased sensitivity for detecting mutagens in human urine. Mutat Res 1983;121:25–32.

Kado NY, Guirguis NG, Flessel CP, Chan RC, Chang K, Wesolowski JJ. Mutagenicity of fine (< 2.5 mm) airborne particles: diurnal variation in community air determined by a *Salmonella* micro-preincubation (microsuspension) procedure. Environ Mutagen 1986;8:53–66.

Krishna GJ, Nath J, Ong T. Correlative genotoxicity studies of airborne particles in *Salmonella typhimurium* and cultured lymphocytes. Environ Mutagen 1984;6:584–592.

Lewtas J. Experimental evidence for the carcinogenicity of air pollutants. In: Tomatis L, editor. Air pollution and human cancer. Monographs, European School of Oncology. Springer-Verlag, 1993:49–61.

Lewtas J, King LC, Williams K, Ball LM, De Marini M. Bioassay-directed fractionation of 1-nitropyrene metabolities: generation of mutagrams by coupling reverse-phase HPLC with microsuspension mutagenicity assays. Mutagenesis 1990;5:481–489.

Matsumoto H, Inoue K. Mutagenicity of a polar portion in the neutral fraction separated from organic extracts of airborne particulates. Arch Environ Contam Toxicol 1987;16:409–416.

McCoy EC, Anders M, Rosenkranz HS. The basis of the insensitivity of *Salmonella typhimurium* strain TA98/1,8-DNP_6 to the mutagenic action of nitroarenes. Mutat Res 1983;121:17–23.

Pagano P, De Zaiacomo T, Scarcella E, Bruni S, Calamosca M. Mutagenic activity of total and particle-sized fractions of urban particulate matter. Environ Sci Technol 1996;30:3512–3516.

Pershagen G, Simonato L. Epidemiological evidence on air pollution and cancer. In: Tomatis L, editor. Air pollution and human cancer. Monographs, European School of Oncology. Springer-Verlag, 1993:64–74.

Reichhardt T. EPA considers standard for airborne fine particles. Environ Sci Technol 1996;30:68A–69A.

Sasaki Y, Kawai T, Ohyama K-I, Nakama A, Endo R. Carcinogenicity of extract of airborne particles using newborn mice and comparative study of carcinogenic and mutagenic effect of the extract. Arch Environ Health 1987;42:14–18.

Schnelle J, Wolf K, Frank G, Hietel B, Gebefügi I, Kettrup A. Particle size-dependent concentrations of polycyclic aromatic hydrocarbons. Analyst 1996;121:1301–1304.

Schwartz J. Particulate air pollution and daily mortality in Detroit. Environ Res 1991;56:204–213.

Summerhays J. Evaluation of risks from urban air pollutants in the southeast Chicago area. J Air Waste Manage Assoc 1991;41:844–850.

Takeda N, Teranishi K. Effects of environmental conditions on polycyclic aromatic hydrocarbons and mutagenicity of airborne particulate matter. Bull Environ Contam Toxicol 1986;36:685–692.

Tzonou A, Maragoudakis G, Trichopoulos D et al. Urban living, tobacco smoking and chronic obstructive pulmonary disease: a study in Athens. Epidemiology 1992;3:57–60.

Viras LG, Athanasiou K, Siskos PA. Determination of mutagenic activity of airborne particulates and of the benzo[*a*]pyrene concentrations in Athens atmosphere. Atmos Environ 1990;24B:267–274.

Viras LG, Siskos PA, Samara C, Kouimtzis T, Athanasiou K, Vavatzandis A. Polycyclic aromatic hydrocarbons and mutagens in ambient air particles sampled in Thessaloniki, Greece. Atmos Environ 1991;10:999–1007.

World Health Organization. Air quality guidelines for Europe. Copenhagen: WHO Regional Publications, European Series No. 23, 1987.