Chemical and Mutagenic Patterns of Airborne Particulate Matter Collected in 17 Italian Towns

R. Barale,1 L. Giromini,2 S. Del Ry,2 B. Barnini,2 M. Bulleri,1 I. Barrai,1 F. Valerio,3 M. Pala,3 and J. He4

1 Dipartimento di Biologia Evolutiva, University of Ferrara, Ferrara, Italy; 2 Dipartimento di Scienze dell’Ambiente e del Territorio, University of Pisa; 3 Istituto Tumori, Genova, Italy; 4 Department of Environmental Health, Zhejiang Medical University, The People’s Republic of China

The mutagenicity of airborne particulate matter collected in 17 towns of Italy in 1990 was assessed using the Ames test. The mutagenicity of crude extract correlated with amount of lead, suggesting the direct contribution of gasoline car exhausts. Moreover, the mutagenicity correlated with particulate matter amounts. An inverse correlation with temperature was observed. The crude extracts were fractionated in acid, basic, and neutral fractions. The latter was further separated into polycyclic aromatic hydrocarbon (PAH), polar, and nonpolar fractions. Acid and polar fractions showed the higher mutagenicity. Average recovery of mutagenicity was about 60%. — Environ Health Perspect 102(Suppl 4):67–73 (1994).

Key words: airborne mutagens, chemical fractions, Ames test, Italian towns

Introduction

Several studies on urban-air mutagenicity have been performed in different countries for assessing the potential mutagenic exposure of the human population in towns (1–8). The studies of correlation between mutagenicity and chemical pollutants have been carried out to possibly identify either the major sources of mutagenic materials or reliable mutagenicity descriptors (9–12). Recently, we reported the results of an extensive mutagenicity study with the Ames test on particulate matter collected in 11 Italian towns (13). In spite of the large differences of geographical location and size among the towns, we found that mutagenicity of crude extract of airborne particles from all towns correlated significantly with particulate matter and nonmethanic hydrocarbons. During 1990, we repeated the investigation with 17 towns from the north and south of Italy. We wanted to examine in detail the possible correlations between chemical-physical variables and mutagenicity of particulate matter crude extract and its fractions.

Material and Methods

Sampling

A train was available from Italian Railway Agency during January to April of 1990. It carried mobile monitoring stations with equipment for the collection of samples of airborne particulate matter, the continuous monitoring of the principal gaseous pollutants (SO2, NO2, nitrogen oxides [NOx]), CO, CO2, O3, nonmethanic hydrocarbons, [NMHC]) as well as equipment for recording meteorological variables (temperature, pressure, wind direction and velocity, lightness, humidity, and precipitation).

In each city, the mobile station was placed downtown at street level, 4 to 8 m from the street, and air pollutants were monitored for 3 consecutive days. Particulate matter (<10 μm diameter) was sampled by an Andersen 2000 High Volume sampler on precleaned fiberglass filters (Gelman type A/E, 23 x 25 cm) with a constant air flow (70 m3/hr). To reduce possible artifact build-up on the filter during prolonged sampling time (as well as the possible blow-off of the more volatile compounds), we limited sampling to 7 hr (7 A.M. to 2 P.M.) since we found that this sampling period was highly representative of 24 hr (13) (Table 1).

Material collected on the filter was sonicated 20 min in dichloromethane (DCM) and then soxhlet extracted, always in DCM, for 16 hr. The organic material was dried with rotavapor under nitrogen stream, weighted and then resuspended in dimethylsulfoxide (DMSO). Part of the crude extract was also separated into acid, basic, and neutral fractions by standard methods elsewhere described (8). The neutral fraction was applied onto a preparative thin-layer chromatography (TLC) plate (PSC Fertigplatten Kieselgel 60, Merck). Elution of TLC plate was carried out with a mixture of n-hexane:benzene (1:1, vol:vol), and its components were separated by their polarity. Bands containing polycyclic aromatic hydrocarbons (PAH), polar, and nonpolar (high molecular weight oxygenated compounds) fractions were detected by their fluorescence under UV light (365 nm). The bands’ identification was assigned by comparing their retention factor (RF) to suitable standards applied simultaneously onto the same TLC.

| Time periods, hr | Particles | NO2 | SO2 | CO | NMHC | Temperature |
|------------------|-----------|-----|-----|----|------|-------------|
| 7–24             | b         | 1.08| 1.17| 1.39| 1.17 | 1.13        |
|                  | r²        | 0.92| 0.90| 0.94| 0.79 | 0.79        |

NHMC, nonmethanic hydrocarbons. The value of r² significant at the 0.1% level with 16 degrees of freedom is r² = 0.71

Table 1. Coefficient regressions (b) of 7-hr sampling values on 24-hr ones and related correlation values (r²). Seventeen different cities for a total of 51 determinations.
Table 2. Correlations (r) between variables sampled for 7 hr in the air of 17 Italian towns in 1990 (51 determinations).

| Variable | NO | NO<sub>2</sub> | SO<sub>2</sub> | CO | O<sub>3</sub> | NMHC | Temperature |
|----------|----|-------------|------------|----|-----------|-------|-------------|
| Particles | 0.49 | 0.58 | 0.53 | 0.45 | -0.36 | 21 | -0.39 |
| NO | 0.67 | 0.72 | 0.77 | -0.08 | 0.43 | -0.02 |
| NO<sub>2</sub> | 0.69 | 0.59 | -0.05 | 0.42 | -0.08 |
| CO | -0.07 | 0.27 | -0.12 |
| O<sub>3</sub> | -0.05 | 0.56 |
| NMHC | -0.02 |

NMHC, nonmethanichydrocarbons.

Bands were scraped out and extracted into toluene and then the eluate was concentrated to dryness with nitrogen. No gravimetric determination was performed because of the small amounts of recovered material. PAH analysis has been described elsewhere (14).

Mutagenicity Test

Crude extracts and fractions were tested in the Salmonella/microsome assay using the standard plate incorporation test as described by Maron and Ames (15). The TA98 and its nitroreductase and O-acetyltransferase-deficient strains (TA98NR, TA98-l, and 8DNP<sub>5</sub>) were routinely used. The metabolic system was provided by S9 mix containing 4% of liver S9 fraction prepared from male Sprague-Dawley rats pretreated with Aroclor. Each crude organic extract was tested at four doses of DMSO solution corresponding to 0, 0.5, 1, 2, and 4 m<sup>3</sup> of air. Whereas, fractions at doses corresponding to 2.5, 5, 10, and 20 m<sup>3</sup>, eight samples out of a total 54, were not processed or tested for mutagenicity because of problems involved in sampling or extraction and fractionation. 2-Aminofluorene (Sigma, St. Louis, MO), 2 μg/plate with S9, and hycanthone (Winthrop, Spain), 20 μg/plate, were used as positive controls for checking both TA98 and S9 efficiency. The TA98NR and TA98-l, 8DNP<sub>5</sub> strains were controlled for their resistances to 8 ng/plate of 1-nitropropene (Aldrich, purified by AM Belisario, Napoli, Italy) and to 4 ng/plate 1,8-dinitropropene (Sigma).

Statistical Analysis

At the tested doses, no gross toxicity was observed even at the highest ones; however the model proposed by Margolin (16):

\[ Y = (a + b \times X) \exp(-T \times X), \]  

was applied to evaluate the coefficient (b) of the linear regression jointly to the toxicity parameter T. Moreover, to make the more reliable comparisons among extracts and fractions often showing different toxicity, we used the specific average reversion activity (SARA), which is the ratio between the response (Y), obtained with Margolin equation at the average dose, and the average dose. Regression coefficient b and SARA indicate essentially the same phenomenon, but SARA also includes toxic effects (13). For example, one polar fraction from the city of Aosta was characterized by a b value equal to 25.24, whereas the SARA value, calculated at the average dose tested in that experiment (9 m<sup>3</sup> equivalent of air) and including the effect of T parameter (T = 0.0305) was 22.00. In the case of negligible toxicity (for example, one Pisa polar fraction, T = 0.00063) the b value, 10.31, was very close to the SARA one (i.e., 10.27). Because toxicity was in general absent or moderate, SARA values were very close to b coefficients. SARA values had been also regressed on b values, and very tight correlations were obtained (r<sub>S9</sub> = 0.91; r<sup>2</sup> = 0.996; + S9, r<sub>S9</sub> = 0.96; r<sup>2</sup> = 0.998). With S9, b (0.96) was close to 1 and suggested that the presence of metabolic activation substantially reduced the toxicity of crude extracts.

Results and Discussion

Correlations between Meteorological and Chemical Variables

Table 2 showed the correlations between particles, SO<sub>2</sub>, NO, NO<sub>2</sub>, CO, O<sub>3</sub>, NMHC, and temperature. All variables

Figure 1. Regression of particulate matter amounts (μg/m<sup>3</sup>) on temperature sampled in 17 Italian towns in 1990.

Figure 2. Mutagenicity of crude extract from particulate matter collected in 17 Italian towns with and without metabolic activation (S9). BZ, Bolzano; AO, Asta; TO, Torino; MI, Milano; GE, Genova; UD, Udine; VR, Verona, PR, Parma; PI, Pisa; SS, Sassari; RO, Roma; AQ, Aquila; AP, Ascoli Piceno; NA, Napoli; CS, Cosenza; CT, Catania; and TA, Taranto.
except for $O_3$ correlated negatively with temperature. In particular, we observed an exponential negative correlation between temperature and particulate matter (Figure 1). Such temperature effects could be due to much more industries, domestic heating, and atmospheric inversions in northern Italy. Meanwhile, we only observed a positive correlation between temperature and $O_3$ as was expected.

**Mutagenicity Patterns**

The mutagenic responses of the strain TA98 (+S9) to crude extracts from particles of the 17 cities (Bolzano [BZ], Aosta [AO], Torino [TO], Milano [MI], Genova [GE], Udine [UD], Verona [VR], Parma [PR], Pisa [PI], Sassari [SS], Roma [RO], Aquila [AQ], Ascoli Piceno [AP], Napoli [NA], Cosenza [CS], Catania [CT], and Taranto [TA]) were given in Figure 2. No particular toxic effect was observed and the $T$ coefficients were often negligible. The responses were expressed as average of three SARA values. In general, the addition of S9 increased mutagenicity of extracts as easily seen in Figure 3, which showed the regression of mutagenicity responses with S9 on those without S9. The mutagenic increase of about 30% because of metabolic activation, suggested the presence of an appreciable amount of indirectly acting mutagens in the air. The mutagenicity (±S9) of crude extract increased linearly with particle amount/m$^3$ of air (Figure 4), and decreased exponentially with temperature (Figure 5). This means that in the colder cities there are larger amounts of mutagenic particles. However, when considering the mutagenic potency of particles, i.e., the mutagenicity per microgram of matter, we did not observe significant variation of mutagenicity (±S9) with temperature ($-\log_2$, $b \approx 0.01$, $p = 0.33$, and $r^2 = 0.00019$; $+\log_2$, $b \approx 0.03$, $p = 0.8$, and $r^2 = -0.0013$) (Figure 6). These data indicate that location or temperature did not influence the direct and indirect mutagenicity per microgram of particle.

Particles were analyzed for the presence of metals such as Pb, Mn, Fe, Cr, V, Zn, Ti, and also studied for the possible correla-
tion with mutagenicity (±S9). A good correlation between SARA without S9 and Pb (Figure 7) and V (r² = 0.52 and r² = 0.61, respectively) and even better in the presence of S9 (r² = 0.67 and r² = 0.81, respectively) was found. These metals appeared to be good indicators of mutagenicity of urban air particles and tracers of pollutants sources, for example, Pb from gasoline exhausts, and V from diesel exhausts and domestic heating with kerosene. The amounts of 15 PAH in our particulate samples have been analyzed and the ratio benzo(α)pyrene/ benzo(g,h,i)perylene ranged 0.2 to 0.9 (Figure 8), a ratio very close to the one (0.2-0.6) that indicated air pollutants from car exhausts (17). No significant variation of the ratio was associated with temperature. Also, we could conclude that the majority of particulate matter collected in downtowns derived from motor vehicle exhaust because of the prevalence of using methane for domestic heating in Italy.

The responses of TA98NR and TA98-1, 8DNP₆, strains to the mutagenicity of crude extracts were about 20 and 40% less than that of TA98, respectively (Figure 9). This result suggested that approximately 20% of the mutagenic activity of the extract required nitroreduction to form direct-acting mutagens, and that approximately 40% of the activity in the extract required transacetylation to form direct-acting mutagens. They appeared to be almost proportionally distributed in all samples, because the correlation coefficients of regressions on TA98 responses were highly significant (b = 0.83, p<0.001, r² = 0.86; b = 0.63, p<0.001, r² = 0.69, for TA98NR and TA98-1, 8DNP₆, respectively).

### Table 3. Relative and total contribution (%) of five fractions to crude extract mutagenicity toward TA98 strain.

| City | S9 | Acid | Basic | PAH | Polar | Nonpolar | Recovery |
|------|----|------|-------|-----|-------|---------|----------|
| MI   | 20 | 8    | 10    | 47  | 5     | 33      | 34       |
| +    | 22 | 12   | 41    | 22  | 3     | 38      |          |
| AO   | 18 | 9    | 6     | 62  | 5     | 70      |          |
| +    | 18 | 12   | 22    | 46  | 2     | 40      |          |
| TO   | 24 | 16   | 5     | 54  | 1     | 43      |          |
| +    | 20 | 18   | 17    | 44  | 1     | 42      |          |
| GE   | 27 | 7    | 4     | 58  | 2     | 71      |          |
| +    | 17 | 10   | 24    | 45  | 4     | 68      |          |
| VR   | 41 | 17   | 13    | 26  | 3     | 45      |          |
| +    | 42 | 14   | 27    | 13  | 4     | 40      |          |
| UD   | 15 | 11   | 2     | 70  | 2     | 97      |          |
| +    | 20 | 4    | 15    | 60  | 1     | 63      |          |
| PR   | 14 | 10   | 8     | 67  | 1     | 100     |          |
| +    | 15 | 15   | 13    | 56  | 1     | 90      |          |
| PI   | 25 | 18   | 9     | 47  | 1     | 71      |          |
| +    | 23 | 11   | 22    | 43  | 1     | 67      |          |
| SS   | 32 | 30   | 11    | 21  | 6     | 39      |          |
| +    | 26 | 20   | 36    | 10  | 8     | 50      |          |
| RO   | 34 | 5    | 3     | 36  | 22    | 50      |          |
| +    | 12 | 37   | 21    | 26  | 4     | 100     |          |
| AO   | 31 | 9    | 15    | 40  | 5     | 68      |          |
| +    | 29 | 13   | 23    | 31  | 4     | 70      |          |
| AP   | 38 | 16   | 3     | 31  | 12    | 91      |          |
| +    | 44 | 17   | 10    | 27  | 2     | 73      |          |
| TA   | 54 | 20   | 4     | 17  | 5     | 66      |          |
| +    | 66 | 15   | 3     | 7   | 9     | 83      |          |
| CT   | 28 | 9    | 1     | 59  | 3     | 71      |          |
| +    | 27 | 7    | 21    | 38  | 7     | 38      |          |
| CS   | 40 | 30   | 2     | 27  | 7     | 68      |          |
| +    | 29 | 30   | 8     | 16  | 7     | 66      |          |
| NA   | 26 | 26   | 5     | 42  | 1     | 60      |          |
| +    | 20 | 28   | 23    | 22  | 7     | 59      |          |
| Average | 30 | 15 | 6 | 44 | 5 | 64 | +S9 27 16 20 32 5 62 |

Abbreviations: PAH, polycyclic aromatic hydrocarbons; MI, Milano; AO, Aosta; TO, Torino; GE, Genova; UD, Udine; PR, Parma; PI, Pisa; SS, Sassari; RO, Roma; AQ, Aquila; AP, Ascoli Piceno; TA, Taranto; CT, Catania; CS, Cosenza; NA, Napoli.

### Fractions

The relative contribution of five fractions to the crude extract mutagenicity with and without S9 was reported in detail in Table 3. The average recoveries of direct and nondirect mutagenicity were 64% and 62%, respectively. Polar-neutral and acid fractions showed higher mutagenic contribution (44 and 30%, respectively, −S9). Fairly similar results have been recently obtained with particulate matter sampled in a street with heavy traffic in the center of Rome (18). As it was expected, the addition of S9 increased the proportion of the mutagenic activity of PAH fractions from 6 to 20%. These results are in general agreement with these obtained from the study of a standard airborne particulate matter sample (National Institute of Standards and Technology Standard Reference Material 1649) collected over a period of over 12 months in the Washington, DC, area (19). There were two exceptions. In our samples, the contribution of the basic fraction was constantly present (about 15%). The higher contribution of the polar-neutral fraction was more typical of street canyon particulate extract, directly affected by point sources, particularly gasoline and diesel engine emissions (19,20). Among all the variables considered, temperature regressed better with the fractions’ mutagenicity (Figures 10,11). In particular, we found...
that the acid fraction's contribution to mutagenicity (+S9) was positively associated (p = 0.011) with temperature, but polar fraction correlated negatively (p = 0.004). Meanwhile, basic, nonpolar, and PAH contribution to mutagenicity was not associated with temperature variations, except that PAH +S9 showed a moderate negative correlation (p = 0.057).

Acid, basic, and polar-neutral fractions were tested with TA98NR and TA98, DNP6 in order to assess the proportional contribution to total mutagenicity of compounds that required nitrroreduction and transacetylation to form direct-acting mutagens. The contribution of the former class of mutagens observed in acid, polar-neutral, and basic fractions was 38, 35, and 20%, respectively; whereas transacetylation-dependent mutagens contributed much more: 60, 77, and 48%, respectively (Table 4). In particular, similar proportions were observed in all polar-neutral fractions tested, since the regression coefficients of TA98 responses on TA98NR and TA98, DNP6 were 0.96 and 0.93, respectively. These findings further revealed a large homogeneity in the mutagenic patterns of airborne particles. These particles were collected from many different towns, but probably all directly affected by the same point sources. Moreover, similar results have been obtained in a study performed on particulate matter collected at street level near a heavily used four-lane street in Rome, Italy (8).

**Conclusions**

The present paper showed that the air mutagenicity associated with particles collected in 17 Italian cities at street level possessed some interesting features. Regardless of the level of industrialization, size, and number of inhabitants, mutagenicity seemed to be correlated mostly with auto vehicle exhaust pollutants. The amount of particulate matter, besides Pb, appeared to be a quick and dirty indicator for mutagenicity.

Because Pb emissions are continuously being reduced in several countries, it is our opinion that particulate matter remains the most relevant indicator, particularly when lacking complex instruments and facilities.

Moreover, the temperature seemed to be the physical variable most closely associated with significant quantitative and qualitative modification of mutagenicity patterns. One may observe that temperature is a variable associated with mutagenicity variations without any causal relationships. For instance, the North is more industrialized than the South; however, some industrial towns such as Genova in the north (but beside the sea), Taranto and Catania in the south, showed low mutagenicity levels when high temperatures were recorded during sampling. Therefore, temperature must have some influence on air mutagenicity, perhaps facilitating the volatilization or dispersion of mutagens. Moreover, cold temperature inversions might keep the air pollution more concentrated near ground level (21). Other variables, such as O3 and lighting (which co-variate with temperature), might be involved in the observed mutagenicity pattern variations, but properly designed experiments under way in our laboratory reduced the likelihood of light playing an important role in modifying mutagenicity at street level.

At the moment, we have no plausible explanations for the observed inverse relative contribution to mutagenicity displayed by
acid and polar fractions, except for the observation that variations in this pattern were associated with temperature. We can conclude that, in spite of the relatively few of samples (three) collected in each town during a somewhat limited time (five months), interesting models of urban air mutagenicity patterns emerged. The models were created through accurate recording of a large number of variables. With the support of environmental chemists, this will allow more detailed statistical analysis and more accurate interpretations of the patterns.

REFERENCES

1. Pitts JN, Grosjean D, Mischke TM. Mutagenic activity of airborne particulate organic pollutants. Toxicol Lett 1:65–70 (1977).

2. Alheim I, Lofroth G, Moller M. Bioassay of extracts of ambient particulate matter. Environ Health Perspect 47:227–238 (1983).

3. Fukino H, Mimura S, Inoue K, Yamane Y. Mutagenicity of airborne particles. Mutat Res 102:257–247 (1982).

4. Courtois YA, Min S, Boizard-Callais F, Lachenal C, Jacot-Deschamps JM, Jolly P, Fesy B. Genotoxicity of atmospheric particles in Paris. Mutat Res 130:238 (1984).

5. de Raat WK. Genotoxicity of aerosol extracts. Some methodological aspects and the contribution of urban and industrial locations. Mutat Res 116:47–63 (1983).

6. Butler JP, Kneip TJ, Mukai F, Daisey JM. Intertown variations in the mutagenic activity of the ambient aerosol and their relations to fuel use patterns. In: Short-term Bioassay in the Analysis of Complex Environmental Mixtures, IV (Waters MD, Shandu SS, Lewtas J, Izzo AJ, eds). New York: Plenum, 1985;233–246.

7. Miguel AG, Daisey JM, Sousa JA. Comparative study of the mutagenic and genotoxic activity associated with inhalable particulate matter in Rio de Janeiro air. Environ Mol Mutagen 15:36–43 (1990).

8. Creeboli R, Fuselli S, Meneguez A, Aquilina G, Conti L, Leopard P, Zinno A, Baris F, Carere A. In vitro and in vivo mutagenicity studies with airborne particulate extracts. Mutat Res 204:565–575 (1988).

9. Tokiwa H, Kitamori S, Takahashi K, Ohnishi Y. Mutagenic and chemical assay extracts of airborne particulates. Mutat Res 77:99–108 (1980).

10. Reali D, Shlitz H, Lohse C, Barale R, Loprieno N. Mutagenicity and chemical analysis of airborne particulates from a rural area in Italy. Environ Mol Mutagen 6:813–823 (1984).

11. van Houdt JJ, Alink GM, Boleij JSM. Mutagenicity of airborne particles related to meteorological and air pollution parameters. Sci Total Environ 61:23–36 (1987).

12. De Flora S, Bagnasco M, Izzotti A, D’Agostini F, Pala M, Valerio F. Mutagenicity of polycyclic hydrocarbon fractions extracted from urban air particles. Mutat Res 224:305–318 (1989).

13. Barale R, Loprieno N, Giorgelli F, Scarpato R, Scapolli C, Barrai I. Correlation between mutagenicity of airborne particles and air pollution parameters in eleven Italian towns. Int J Environ Health Res 1:37–53 (1991).

14. Barale R, Giromini L, Ghelardini G, Scapolli C, Loprieno N, Pala M, Valerio F, Barrai I. Correlations between 15 polycyclic aromatic hydrocarbons (PAH) and the mutagenicity of the total PAH fraction in ambient air particles in La Spezia (Italy). Mutat Res 249:227–241 (1991).

15. Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutat Res 113:173–215 (1983).

16. Margolin HB, Kaplan N, Zeiger E. Statistical analysis of the Ames Salmonella/microsome test. Proc Natl Acad Sci USA 78:3779–3783 (1981).

17. Alheim I, Moller M. Mutagenicity of airborne particulate matter in relation to traffic and meteorological conditions. In: Short-term Bioassay in the Analysis of Complex Environmental Mixtures, II (Waters MD, Sandhu SS, Lewtas J, Claxton L, Nesnow S, eds). New York: Plenum Press, 1980;319–336.

18. Creeboli R, Fuselli S, Conti G, Conti L, Carere A. Mutagenicity spectra in bacterial strains of airborne and engine exhaust particulate extracts. Mutat Res 261:237–248 (1991).

19. Lewtas J, Chuang J, Nishioka M, Petersen B. Bioassay-directed fractionation of the organic extract of SRM 1649 urban air particulate matter. Int J Environ Anal Chem 39:245–256.

20. Lewtas J. Genotoxicity of complex mixtures: strategies for the identification and comparative assessment of airborne mutagens and
carcinogens from combustion sources. Fundam Appl Toxicol 10:571–589 (1988).
21. Watts RR, Drago RJ, Merrill RG, Williams RW, Perry E, Lewtas J. Wood smoke impacted air: mutagenicity and chemical analysis of ambient air in a residential area of Juneau, Alaska. JAPCA 38:652–660 (1988).