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# Mutagenic Activity and Chemical Analysis of Airborne Particulates Collected in Pisa (Italy)

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In the last few years there has been much concern about the problem connected to the exposure to mutagens present in the environment of industrialized countries. Particularly, the mutagenic activity of airbone particulate matter has been studied by many investigators and correlated with elevated lung cancer mortality rates (Talcott and Harger 1980, Walker et al. 1982, Mumford et al 1987, Mass et al 1987).
In most cases the *Salmonella typhimurium*/microsome test (Ames et al. 1975) has been used for these studies. This short-term test, which is the most validated among the short-term genotoxicity tests, provides an important indication on the carcinogenic potential of environmental pollutants. That are complex mixtures containing a wide variety of compounds potentially capable of causing additive, antagonistic or synergistic genotoxic response in living organisms (Raymond West et al 1988).
Several studies have suggested that diverse factors, such as traffic and metereological conditions, could affect the levels of pollutants in the air (Alfheim and Moller 1979, Tokiwa et al 1983, Lewis et al 1988, Rossi et al 1990).

In our work, we have investigated three different areas in Pisa, where the intensity and the kind of the road traffic were different. Airborne particles have been collected during a year and the genotoxic activity has been studied using TA98 and TA100 strains of *Salmonella typhimurium*.

## MATERIALS AND METHODS

Airborne particles samples were collected from three different urban areas: an area near a street with intense fast moving traffic (site 1), an area near a street with intense and slow traffic (site 2) and an area near the historical centre where road traffic is limited (site 3).

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In these three sites daily samples were drawn for all of 1989. Sampling was performed on glass fiber filters; flow rate was kept about 20 litres per minute and the total volume was accurately measured. The filters collected each month have been weighted and each one was divided into two parts, thus forming two groups. One of these, consisting of the half filters of each month, has been extracted in organic phase and the other one in inorganic phase. Genotoxic studies have been performed on the organic extracts that were obtained by means of three extractions in ultrasonic bath: the first one using 10 ml of ultrapure methylene chloride and the others using 10 ml of ultrapure hexane.
Mutagenicity assay using the *Salmonella typhimurium* TA98 and TA100 strains was performed as described by Maron and Ames (1983).
S9 hepatic fraction for metabolic activation was prepared from male Swiss albino mice, CD1 strain pretreated with sodium phenobarbital (100 mg/Kg) and ß-naphtoflavone (80mg/kg) (Bronzetti et al 1983). Protein concentration of S9 fraction was determined according to Lowry et al (1951) (32mg/ml). The S9 mix was prepared according to the technique previously described (Ames et al 1973b) i.e. 0.5ml of the S9 mix containing 10% S9 were added to the agar surface.
Before performing the biological assay, the organic extracts were evaporated at room temperature. The residues were immediately dissolved in 10ml of dimethylsulfoxide (DMSO) and were kept at 4°C no longer than few weeks before testing.
Three independent tests, all with or without S9 mix were carried out in duplicate plates for each sample.
In table 1 spontaneous and induced reversion frequencies by standard mutagens in TA98 and TA100 Salmonella strain, obtained during the study, are reported.

Differencies among the mutant His+ inducted by samples collected in the three sites have been evaluated by non-parametric anova according to Kruskal-Wallis approach (Armitage 1971), and paired comparisons by Mann-Whitney U test (Armitage 1971).
Association between airborne particles measure and mutant His+ in the presence of S9 mix has been carried out by means of linear regression analysis (Siegel 1956). Regression line and 95% confidence interval for site 1 and site 3 are reported in figure 1.
Monthly distributions of mutagenicity results for each site related to the standard denominators (revertants/m3) were tested by chi-square with 11 degrees of freedom against a theoretically expected even distribution (Siegel 1956).
To estimate and to evaluate the cyclic trend various

methods were available, such as those proposed by Edwards (1961), Walter and Elwood (1975), Hewitt et al (1971). Owing to the use restrictions existing to apply the Edwards method, as to the denominator costancy, the Walter-Elwood test and the non-parametric ranking sum test described by Hewitt, have been employed. The first one supply informations about the time occurence of the peack value and its amplitude as well as the significance against the sinusoidal function, while the second one suggests the six-month period with the maximum rank value. As to the latter, to derive useful indications, the observed data were assumed to be representative of the subsequent year as well.

Table 1. Spontaneous and induced by standard mutagen reverse frequencies in S. typhimurium

| | TA98 | | TA100 | |
|---|---|---|---|---|
| | -S9 | +S9 | -S9 | +S9 |
| Hycantone (20µg/p) | 493±25 | - | 944±44 | - |
| BP (1mg/p) | | 227±22 | | 329±15 |
| DMSO (0.2ml/p) | 32± 4 | 27± 1 | 137±21 | 123±14 |
| Blanks | 33± 3 | 21± 1 | 139±28 | 102±18 |
| Controls | 39± 3 | 29± 1 | 121±15 | 137±16 |

BP: Benzo(a)pyrene; DMSO: dimethyl sulfoxide.

## RESULTS AND DISCUSSION

The dose-response curve of samples of airborne particles collected during the year have been determined and in table 2 is reported that one of May as example. The mutagenicity of such sample was found both with and without metabolic activation.
All samples were tested both in TA98 or in TA100 strains, but the results obtained with TA100 were omitted because no effects or a very low effects were observed.
The results reported in tables 3 represent the number of revertants per cubic meter corresponding to the linear parts of the dose-response curves. In table 3A are reported the sampling and testing results of samples collected from January to December from the site 1. The mutagenic activity was higher during the winter period (October- February) in the presence of metabolic activation; the amount of airborne particles was also higher in this period, the analysis of linear regression between total airborne particulate and mutagenecity in TA98 strain showed in fact a clear-cut

correlation (R=0.807; t=4.315; p<0.01) (fig. 1A) This is not true when the samples were tested without metabolic activation.

Table 2. Dose-response relationship of sample of May mutagenicity in the presence or in the absence of S9mix

| Dose µg/plate | Mutagenicity (His+ revertants ± S.D.) S9 | TA98 | TA100 |
|---|---|---|---|
| 0 | - | 33± 1.2 | 121±14.9 |
| 125 | - | 45± 5.3 | 126± 9.7 |
| 250 | - | 78± 2.1 | 137±11.5 |
| 500 | - | 163±12.4 | 169±17.5 |
| 0 | + | 22± 0.5 | 137±15.7 |
| 125 | + | 35± 1.7 | 200±16.8 |
| 250 | + | 44± 2.3 | 178±13.0 |
| 500 | + | 78± 3.1 | 182±22.3 |

The samples collected from the site 2 shown a peak of mutagenicity in January and February in the presence of S9 mix (table 3B). In this experimental conditions the lower values were found in May and June. The results obtained without metabolic activation showed a peak in January and February and were the half from March to July; mutagenic activity was not evident from August to December. In this site any significant correlation between mutagenicity and amount of particulate was not found. A similar behaviour was observed with samples drawn from the site 3 and tested without metabolic activation, even if, August, September and Dicember sampling were not carry out in this site (table 3C). In the presence of S9 mix, the mutagenicity was observed in this site over the year but the higher values were found in the winter months. Also in this case the degree of correlation between mutagenic activity with S9 and amount of particulate collected was high and significant (r= 0.699; t= 2.583; p< 0.05) (fig 1B).
The results reported in this work show that from January to July the higher mutagenic effect was always found in the site 2 in the presence of S9mix. In fact, the variance analysis showed that the differencies found in the site 2 versus the site 1 or 3 are significant at 95%. In the summer the differences of mutagenic value among the three sites were not significant. During following months the mutagenic effect remained constant in the site 2, while in the site 1 and 3 it increased. In this period the differences in the site are significant at 95% between

Table 3. Summary of sampling results and mutagenicity a year period in sites 1, 2 and 3

| Sampling months | Air sampling volume (m3) | total particles (µg/Nm3) | Mutagenicity (a) TA98 Revertants/m3 -S9 | +S9 |
|---|---|---|---|---|
| SITE 1 (A) | | | | |
| January | 768 | 125 | 25.1 | 21.0 |
| February | 664 | 96 | 30.6 | 20.2 |
| March | 816 | 64 | 8.8 | 15.0 |
| April | 812 | 48 | 11.2 | 12.1 |
| May | 864 | 59 | 10.1 | 4.4 |
| June | 796 | 59 | 11.2 | 3.4 |
| July | 676 | 57 | 8.0 | 3.8 |
| August | 636 | 51 | 0.0 | 7.2 |
| September | 580 | 76 | 0.0 | 12.2 |
| October | 628 | 101 | 7.5 | 19.9 |
| November | 616 | 75 | 2.8 | 21.7 |
| December | 320 | 113 | 0.0 | 29.4 |
| SITE 2 (B) | | | | |
| January | 752 | 117 | 26.7 | 33.0 |
| February | 665 | 113 | 32.8 | 28.8 |
| March | 653 | 100 | 12.0 | 11.2 |
| April | 655 | 90 | 17.7 | 16.9 |
| May | 824 | 69 | 15.8 | 6.8 |
| June | 686 | 82 | 15.4 | 5.4 |
| July | 744 | 75 | 10.5 | 10.3 |
| August | 779 | 62 | 0.1 | 9.5 |
| September | 674 | 87 | 0.0 | 8.5 |
| October | 722 | 96 | 0.0 | 6.0 |
| November | 717 | 69 | 0.1 | 9.9 |
| December | 436 | 131 | 0.0 | 8.9 |
| SITE 3 (C) | | | | |
| January | 759 | 108 | 19.8 | 14.9 |
| February | 555 | 90 | 26.8 | 17.4 |
| March | 750 | 66 | 7.5 | 5.6 |
| April | 634 | 53 | 13.0 | 9.1 |
| May | 831 | 65 | 3.5 | 2.9 |
| June | 712 | 59 | 3.2 | 2.4 |
| July | 716 | 49 | 4.9 | 5.6 |
| August | - | - | n.d. | n.d. |
| September | - | - | n.d. | n.d. |
| October | 671 | 103 | 0.7 | 10.0 |
| November | 715 | 69 | 0.0 | 10.5 |
| December | 161 | 82 | n.d. | n.d. |

a) Mutagenicity is the average value of three independent experiments. Spontaneous revertants have been subtracted.

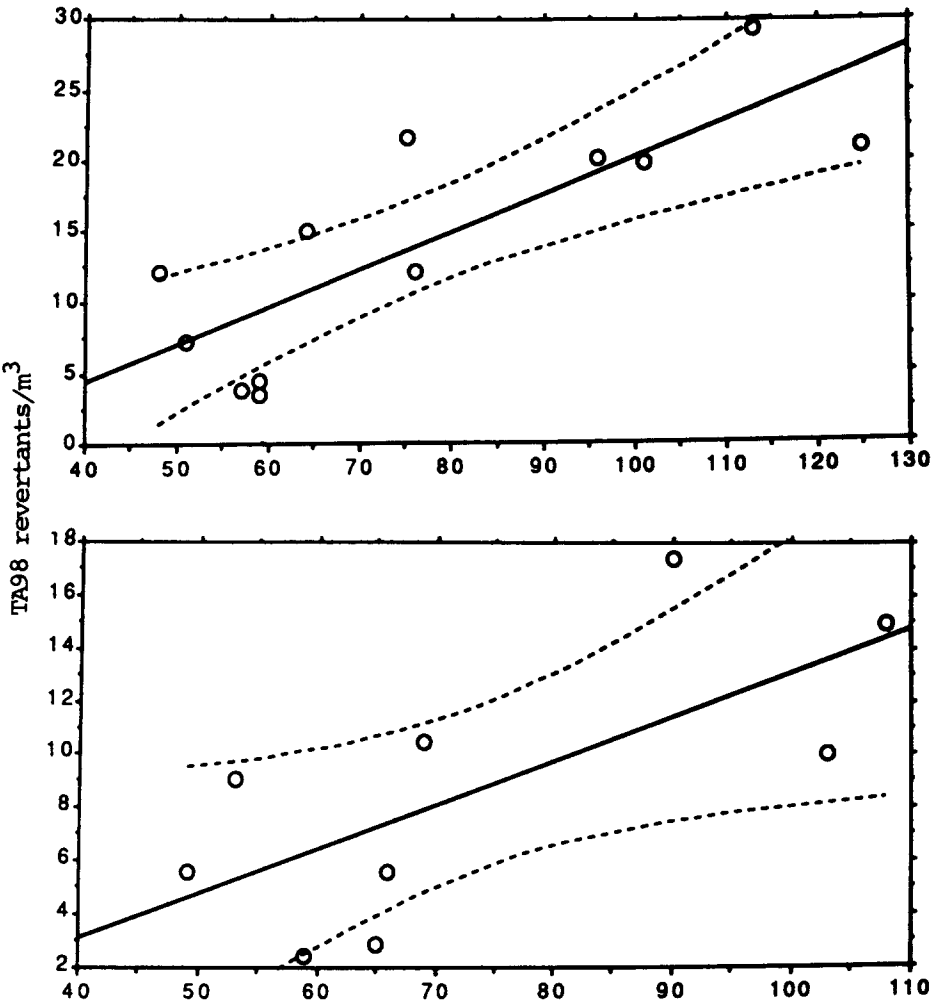


Figure 1. Linear regression between amount of particulate matter collected in site 1 (A) or in site 3 (B) and number of revertants His+ in TA98 strain in the presence of S9 mix.

the site 1 and the site 2 or 3.
Our results show a cyclic trend of mutagenicity, statistically significant, during the year: lower in the summer, higher in the winter. In fact, with Walter-Elwood test a peak on December 12th (amplitude= 0,87; $p<0,001$) in site 1 and a peak on February 6th (amplitude= 0,57; $p<0,001$) in site 2 was observed; with Hewitt test an excess of mutant His+ in the October-

March six-month in site 1 and in the November-April six-month in site 2 was suggested.
These tests have not been employed with mutagenicity results obtained in site 3 because annual sampling were not complete.The results obtained in this study agree with earlier reports showing that mutagenic activity of air samples reached the maximum value during cold months, both with or without metabolic activation (Barale et al 1989, Atherholt 1985).
However, we have hypothesized that qualitative changes have occurred during the sampling period. In fact, whereas in the first semester the differences between mutagenic activity with or without metabolic activation were not evident, it was very clear in the second period of year. This suggests that variations in the kind of mutagens condensed and coated onto the particulate surface was occurred.
These results confirm the importance of periodic repeated sampling during the year to obtain complete information about the pollution and the mutagenicity of an area.

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REFERENCES

Ames BN, Durston WE, Yamasaki E, Lee FD (1973) Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc Natl Acad Sci (USA) 70:2281-2285

Ames BN, McCann J, Yamasaki E (1975) Methods for detecting carcinogens and mutagens with the Salmonella mammalian microsome mutagenicity test. Mutat Res 31:347-364

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

Armitage P (1971) Statistical methods in medical research. Oxford, Blakwell Scientific Pubblications.Mixtures II" New York and London: Plenum Press, p 319

Atherholt TB, McGarrity GJ, Louis JB, McGeorge LJ,Lioly PJ, Daisey JM, Greenberg A, Darack F (1985) Mutagenicity studies of New Jersey ambient air particulate extracts. In Waters MD, Sandhu SS, Lewtas J, Claxton L.,Strauss G, Nesnow S (Eds):

"Short-term Bioassay in the Analysisi of Complex Environmental Mixtures IV" New York and London: Plenum Press, p 211

Barale R, Zucconi D, Giorgelli F, Carducci AL, Tonelli M, Loprieno N. (1989) Mutagenicity of airbone particles from a nonindustrial town in Italy. Environ Mutagen 13:227-233

Bronzetti G, Bauer C, Corsi C, Del Carratore R, Galli A, Nieri R, Paolini M (1983) Genetic Biochemical studies on perchloroethylene "in vitro" and "in vivo". Mutat Res 116:323-331

Edward JH (1961) The recognition and estimation of cyclic trends. Ann Hum Genet 25:83-87.

Hewitt D, Milner J, Csima A, Pakula A (1971) On Edwards criterion of seasonality and a non-parametric alternative. Brit J Prev Soc Med 25:174-176.

Lewis CW, Baumgardner RE, Stevens RK, Claxton LD, Lewtes J (1988) Contribution of woodsmoke and motor vehicle emissions to ambient aerosol mutagenicity. Environ Sci Technol 22:968-971.

Lowry OH, Rosembroug NJ, Far AL, Randal RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275

Mass MJ, Marr CM, Mumford JL (1987): Preneoplastic trasformation of respiratory epithelial cells by complex organic mixtures in a clonal assay. In Gray RH, Chess EK, Mellinger PJ, Riley RG, Springer DL (eds) "Health and Environmental Research on Complex Organic Mixtures" 24th Hanford life Sciences Symposium, CONF 851027 pp 317-327

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

Mumford JL, He XZ, Chapman RS, Cao RS, Harris DB, Li XM, Xian YL, Jiang WZ, Xu CW, Chang JC, Wilson WE, Cooke M (1987) Lung cancer and indoor air pollution in Xuan Wei, China. Science 235: 217-220

Raymond West W, Smith PA, Booth GM, Lee ML (1988) Isolation and detection of genotoxic components in Black River sediment. Environ Sci Technol 22:224-228)

Rossi C, Poli P, Buschini A, Cassoni F, Galli A, Vellosi R, Del Carratore R (1991) Genetic activity of samples collected from a waste incinerator and its neighboring areas. Toxicol Environ Chemistry 30:51-61

Siegel S (1956) Non-parametric statistics for the behavioural sciences. McGraw-Hill (ed), Series in Psycology, New York

Talcott R, Harger W (1980) Airborne mutagens extracted from particles of respirable size. Mutat Res 79:177-180

Tokiwa H, Kitamori S, Horikawa K, Nakagawa R (1983) Some findings on mutagenicity in airborne particulate pollutants. Environ Mutagen 5:87-100

Walker RD, Connor TH, MacDonald EJ, Trieff NM, Legator MS, MacKenzie KW Jr, Dobbings JG (1982) Correlation of mutagenic assessment of Huston air particulate extracts in relation to lung cancer mortality rates. Environ Res 28:303-31