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MUTAGENICITY ASSESSMENT OF AIRBORNE PARTICLES IN MEXICO CITY

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Abstract—The Ames's TA98 strain of Salmonella typhimurium with and without mammalian metabolic activation was used to detect the mutagenic activity of organic chemicals associated with airborne particles. Two kinds of particles: total suspended (TSP) and those particles with an aerodynamic diameter of $10 \mu m$ or smaller (PM10) were collected in glass fiber filters using high-volume samplers during the dry season (December 1989–March 1990) in the Metropolitan Area of Mexico City at five stations of the air quality network belonging to the Ministry of Social Development. Although the highest mass concentrations of particles were obtained from the Northeastern and Southeastern areas, the largest frequency of mutations was found Downtown which indicated that vehicle exhaust was an important source. Contrary to what was expected, the mutagenic responses were higher for PM10 than for TSP samples. On the other hand, the microsome activation increased significantly the mutagenic activity of the complex mixture, which hinted at the presence of higher amounts of indirect (or promutagens) than direct mutagens both for TSP and PM10.

Key word index: Airborne particles, mutagenicity, Mexico City, PM10, TSP, Ames test.

INTRODUCTION

The annual emission of pollutants in México is higher than 16 million tons, of which 65% come from vehicles and the other 35% from industries. 24% is generated in Mexico City (SEDUE, 1986).

The airborne particle size is considered a major factor in determining the toxic effects (Natusch and Wallace, 1974). As only 30-40% of the organic compounds on the airborne particles have been identified, van Houdt *et al.* (1987) considered that the effect of the complex mixture could give a more realistic basis of mutagenic activity than the test of its individual components because of the occurrence of synergisms or antagonisms (Alink *et al.*, 1983).

In order to detect mutagens, a simple and sensitive short-term bioassay was used with histidine-dependent strains of *Salmonella typhimurium* to test chemicals or complex mixtures (Maron and Ames, 1983). This analysis has had a certain predictive value for carcinogenesis because most chemicals classified as carcinogens have also been mutagens (McCann *et al.*, 1975).

Evidence of mutagenicity of airborne particles has been shown in urban, industrial and rural areas (Møller and Alfheim, 1980; Pitts *et al.*, 1982; Alink *et al.*, 1983; de Raat, 1983; Ohsawa *et al.*, 1983; Kado et al., 1986; Butler et al., 1987; van Houdt et al., 1987; Viras et al., 1990; Houk et al., 1992; Nardini and Clonfero, 1992).

Simultaneous analysis of the mutagenic activity was made for total suspended particles (TSP) and for those particles that could be deposited in the tracheobronchial and alveolar regions, the PM10 (with aerodynamic diameter of 10 μ m or less), also called respirable particles (Hileman, 1981; van der Meulen *et al.*, 1987) in order to point out the differences between them in various sites of Mexico City.

MATERIALS AND METHODS

Five stations of the air quality network of the Ministry of Social Development (SEDESOL) located at different sites of the Metropolitan Area of Mexico City (Fig. 1) were selected for the study, namely: in the Northeast (NE), Xalostoc (X); in the Northwest (NW), Tlalnepantla (T); Downtown (DT), Merced (M); in the Southeast (SE), Estrella (E); and in the Southwest (SW), Pedregal (P).

The particles were collected during the dry season from December 1989 to March 1990, in glass fiber filters exposed during 24 h in high volume samplers (Andersen) with an average flow rate of $1.1332 \text{ m}^3 \text{min}^{-1}$ both for total suspended particles (TSP) and particles with an aerodynamic diameter of 10 μ m or smaller (PM10). Each station was equipped with two kinds of samplers so that the data of

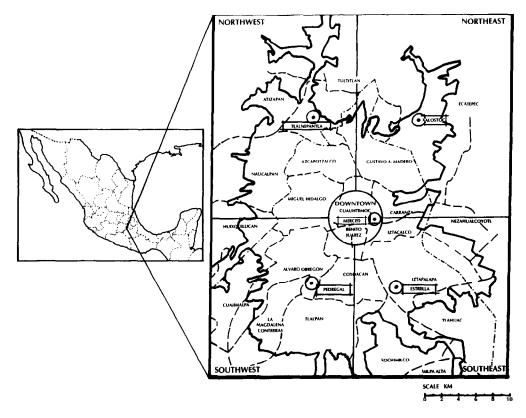


Fig. 1. Map of Mexico showing the Metropolitan Area of Mexico City with five stations of the air quality network of the Ministry of Social Development (SEDESOL) where the particles were collected \odot .

 Table 1. Comparison of TSP and PM10 in five sites of the Metropolitan Area of Mexico City through a portion of the dry season 1989–1990

Area	Sample size n	Mean TSP (μg m ⁻³)	Mean PM10 (μg m ⁻³)	Mean ratio	Standard deviation ratio	Correlation coefficient
NE	15	595.3	284.0	0.425	0.039	0.919
NW	8	_			_	
DT	12	290.8	166.5	0.579	0.045	0.949
SE	15	494.5	182.7	0.372	0.069	0.886
SW	15	144.2	68.5	0.477	0.081	0.691

both, for the same day, could be compared (Table 1). The samplers were installed on roofs 3.5-4 m above the street. Twenty four hours before and after exposure, the filters were maintained in standard conditions (25° C and 35% of relative humidity) until constant weight was reached. The data of particulate matter weight was furnished by SEDESOL.

The organic compounds of half of the filters were extracted with 350 ml of methanol (Baker, spectrophotometric reagent) in a Soxhlet apparatus over 8 h. The extract was left to evaporate until dryness at 45°C in stream bath and the residues were dissolved in 12 ml of dimethyl sulfoxide (DMSO). The samples were sterilized by Swinnex filtration through a high density polypropylene membrane with a pore size of 0.2 μ m and stored in the dark at 4°C. The extracts were then exposed to the standard Salmonella typhimurium microsome procedure, as described by Maron and Ames (1983). After testing the strains TA98 (for frameshift mutations) and TA100 (for base pair substitutions), the former showed higher sensitivity, which agreed with the results of Tokiwa et al. (1980) and Talcott and Harger (1980). De Raat (1983) found TA98 strain more sensitive to the extracts than TA100 when S9 was omitted, but the response of TA100 was higher when S9 was used. Also de Flora et al. (1989) found TA100 more sensitive to fractions containing metabolically activated polycyclic aromatic hydrocarbons than TA98 but that TA98 was more sensitive to unfractioned samples. TA98 strain was thus employed in the subsequent experiments performed, with or without the addition of the S9 mixture which was prepared using the liver of 200 g male rats (Sprague-Dawley) injected with Aroclor 1254 (200 mg ml⁻¹ diluted in corn oil) in a single intraperitoneal dose of 500 mg kg⁻ ¹, five days before being sacrificed (Maron and Ames, 1983).

The spontaneous reversion obtained for the TA98 strain was 30-50 per plate which corresponded quite well with the values described in the literature (Maron and Amcs, 1983). The suspension of 10^8 bacteria (0.1 ml), the extract (0.1 ml) and in the case of the S9 mix 10% (0.5 ml) were added in a 2 ml molten top agar with traces of histidine and biotine in excess. Then they were plated in triplicate over glucose agar plates and incubated at 37° C during 48 h. Picrolonic acid (100 µg per plate) and benzo(a)pyrene (1 µg per plate) were used as positive controls for direct and indirect mutagenicity, respectively, and DMSO was the negative control.

Due to the fact that only half of the filters were used for these studies, the extract obtained was very small. An analysis of concentration-response was made, based on the selection of a point around the middle on the regression line of the mutagenic activity, which also induced a satisfactory result when metabolic activation was involved. The concentration was $68 \text{ m}^3 \text{ ml}^{-1}$, equivalent to one hour of sampling and it was used for all the samples obtained.

RESULTS AND DISCUSSION

Mass concentration of TSP and PM10

The relationship between mass concentration of TSP and PM10 collected in four of the five sites was then calculated since in one of them (Tlalnepantla), only eight pairs of data were obtained (Table 1). The range of mean ratios PM10/TSP in the stations were from 0.372 to 0.579 (Table 1) which corresponded quite well with the data of Rodes and Evans (1985) of 0.381–0.577 from eight locations in U.S.A. In Netherlands, the ratio was higher ≈ 0.7 and according to van der Meulen *et al.* (1987), the difference with U.S.A. was

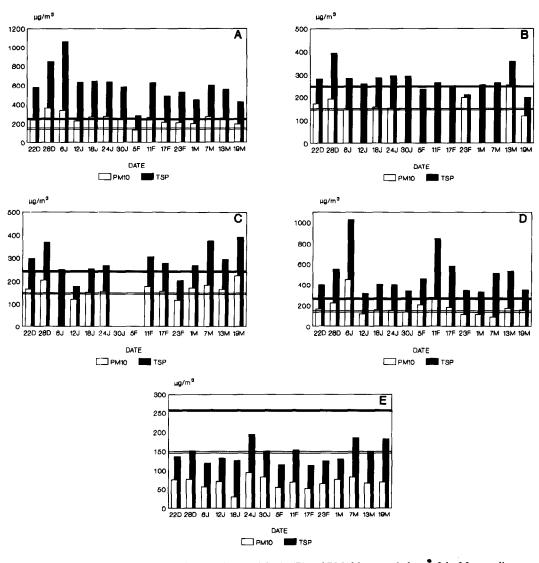


Fig. 2. Mass concentrations of total suspended particles (TSP) and PM10 in several places of the Metropolitan Area of Mexico City: Xalostoc, Northeast (A); Tlalnepantla, Northwest (B); Merced, Downtown (C); Estrella, Southeast (D); and Pedregal, Southwest (E). Data afforded by SEDESOL. The lines show the standard limits for both particles.

due to the relative high humidity in Western Europe. Likewise, correlations between TSP and PM10 were significant showing that PM10 is contained by TSP in the analysis of areas (Table 1) independent of its contribution.

The highest amount of particles (TSP and PM10) was obtained in Xalostoc (NE) (Fig. 2A) where all TSP values went beyond $260 \ \mu g m^{-3}$, considered to be a 24 h standard (Hileman, 1981) and all PM10 values exceeded the 150 $\ \mu g m^{-3}$ standard level (Solomon *et al.*, 1989). Estrella (SE) showed equivalent values to those obtained in Xalostoc (Fig. 2D) but the values were lower for Merced (DT) (Fig. 2C), Tlalnepantla (NW) (Fig. 2B) and Pedregal (SW) (Fig. 2E). Thus, the most important amount of particles resulted to be detected in the Eastern part of the Metropolitan area may be because the erosive and soil-related material of the zones close to the city, influence this area.

Mutagenic activity of TSP and PM10 samples with and without metabolic activation

Figure 3 showed the number of revertants induced by the extracts of TSP without S9 mix (A) and with S9 mix (B). Similar to the results obtained by Barale *et al.* (1991), the values were higher for + S9 and in this work more than half of the cases suggested the presence of more active indirect mutagens than direct. The same was obtained with the extracts of PM10 in which the mutagenic activity was higher when the S9 mixture was added (Fig. 4B) than when it was not (Fig. 4A). In PM10 the expression of the indirect mutagens was actually more effective than in TSP.

Higher mutagenic responses were found in PM10 than in TSP (Figs. 3 and 4); 89% of the total samples of PM10 is higher than the double of the negative control (Fig. 4) and 72% of the TSP (Fig. 3). The θ statistic test of Katz (1979) was used to corroborate these findings and the significant values were 94.5% for PM10 and 87.5% for TSP when compared with negative control. Anyway, both analyses gave the result of a mutagenic response that was the opposite of what was expected. The organic compounds associated to the airborne particles of the respirable fraction $(<10 \,\mu\text{m})$ are in general more active than those obtained by the TSP samples, although the amount of particles obtained of the latter in these analyses were higher. Possibly, when large and small particles were collected together, the chemical reactions could render the smaller particles less mutagenic. This fact should be tested later.

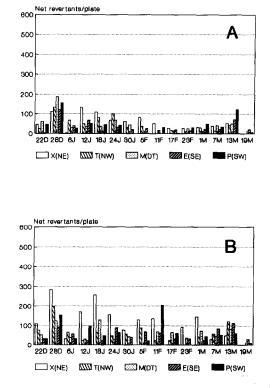


Fig. 3. Net histidine revertants obtained in Salmonella typhimurium strain TA98 without (A) and with (B) mammalian metabolic activation (S9 fraction) exposed to TSP extracts of five places of the Metropolitan Area of Mexico City during some portion of the dry season (D, December; J, January; F February; M, March) of 1989-1990.

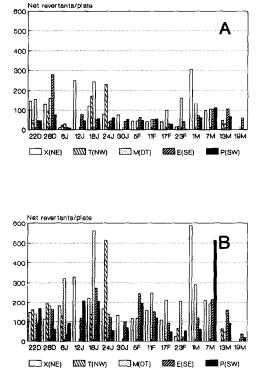


Fig. 4. Net histidine revertants obtained in Salmonella typhimurium strain TA98 without (A) and with (B) mammalian metabolic activation (S9 fraction) exposed to PM10 extracts of five places of the Metropolitan Area of Mexico City during some portion of the dry season (D, December; J, January; F February; M, March) of 1989–1990.

The Spearman ranks correlations between mass particle concentration and mutagenicity were very scarce. Such correlations were only obtained in the TSP analysis of two stations: NW (r = 0.64) without metabolic activation and NW (r = 0.60) and SE (r = 0.57) with metabolic activation.

Exploratory data analysis of global mutagenic activity

As the distribution of the mutagenic activity in the sampling sites was irregular, the medians for -S9 and

+ S9 were calculated by means of the multiple box and whisker plots (Hoaglin *et al.*, 1983) with the highest results for + S9 (Fig. 5). The highest medians of mutagenic response for PM10 appeared in Merced (DT), Xalostoc (NE) and Tlalnepantla (NW), followed by Estrella (SE) and Pedregal (SW), while for TSP, Xalostoc (NE) had the highest median and for the other stations the medians were lower.

The main source of pollution Downtown was the vehicle exhaust because of dense traffic conditions,

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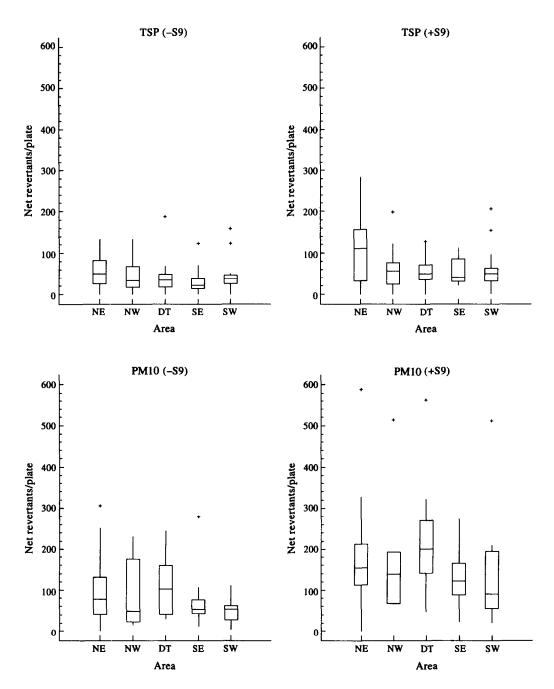


Fig. 5. Schematic boxplots of the mutagenic responses of Salmonella typhimurium strain TA98 obtained in five stations of the Metropolitan Area of Mexico City for extracts of total suspended particles (TSP) and PM10 with and without mammalian microsome activation (S9). + outliers.

while in NE and NW the pollution coming from the combustion of diesel and gas oil from industries was added. Thirty per cent of the total mexican industry is concentrated in this Metropolitan Area, thus a significant contributor of pollution (CGRUPE, 1991). In the findings of Viras *et al.* (1990) the highest mutagenic values for TSP were found Downtown and not in sites located in the industrial areas.

Correlations of mutagenicity among stations

The correlation analysis of the five sites studied (Table 2) for TSP without metabolic activation showed a close association among these with the exception of the Northeastern area which could only be correlated with Downtown but whenever the metabolic activation is involved, the Northern portion could be related with Downtown and with the Southeastern area. For PM10 the correlation was only found in the Southern area with and without metabolic activation. It was observed that the PM10 had very local behavior and effects within a complex dynamic atmosphere, at the same time that the relation among the zones for TSP samples could be a further coincidence of sources of particles having the vehicle exhaust as a common denominator, which probably is the only origin of particles Downtown (DT).

When a lack of difference of mutagen activity between urban and non-urban areas was found, it was interpreted to be a reflection of the largeness of the polluted area (Ohsawa *et al.*, 1983) or due to longrange transportation of the particles (Alink *et al.*, 1983; Masclet *et al.*, 1988).

Correlation of mutagenicity with pollutants and meteorological data

In relation with the concentrations of pollutants, different results were found depending on the presence or absence of metabolic activation. Thus positive correlation appeared without S9 in NW and SO₂ (r=0.7658), DT and NO₂ (r=0.6799) and SW and CO (r=0.6964). With S9 correlations were found in NW (r=0.8712) and SW (r=0.6141) with NO₂. Kado et al. (1986) observed that mutagenicity of fine particles sampled in some cities of the San Francisco Bay Area was highly correlated with lead and much less correlated with NO₂, O₃ and SO₂.

In this study no correlations of mutagenicity were found with relative humidity, temperature, wind speed or direction.

Indirect and direct mutagens

Yamanaka and Maruoka (1984) and Viras *et al.* (1990) observed that the microsomal enzymes of S9 did not produce any effect or reduce the mutagenic activity of the extracts in TA98 Salmonella strain, maybe because the main active agents in the extracts were direct. On the other hand, the results in this work (Figs. 3 and 4) as well as those obtained by de Wiest *et al.* (1982), Ohsawa *et al.* (1983) and Kado *et al.* (1986) showed that mammalian microsome activation significantly increased the mutagenic activity. Therefore, it can be supposed that the presence of indirect mutagens (promutagens) in these sites was higher than the direct acting mutagens.

The direct-indirect mutagen response relation per station showed further associations among all zones, both in TSP and PM10 samples. This proves that in the particulate phase of the atmosphere a series of chemical reactions occur in the conversion of mutagens.

Møller and Alfheim (1980), Tokiwa *et al.* (1980), Funcke *et al.* (1981) and Thrane and Mikalsen (1981) found polycyclic aromatic hydrocarbons (PAH) produced by incomplete combustion of coal, wood, oil and gasoline in the complex organic mixture extracted from the airborne particles and that they could be the

	NE	NW	DT	SE	SW	
TSP						
NE		0.52	0.53*	0.48	0.45	
NW	0.40		0.75*	0.68*	0.58*	
DT	0.66*	0.63*		0.76*	0.82*	— S9
SE	0.00	0.60*	0.19	_	0.81*	
SW	0.44	0.23	0.42	0.28		
			+ S9			
PM10						
NE		0.42	0.48	0.20	0.30	
NW	0.42		0.09	0.01	0.55	
DT	0.42	- 0.15	_	0.21	0.04	— S9
SE	0.07	0.07	0.21		0.51*	
SW	0.12	- 0.26	0.07	0.58*	—	
			+ S 9			

Table 2. Correlation matrix from the mutagenic activity of extracts of TSP and PM10 among the five sites studied in the Metropolitan Area of Mexico City without (-S9) and with (+S9) metabolic activation

* Significant values (p < 0.05).

sponsors of some of the indirect mutagenicity produced. As a result of nitration of PAH in the environment (Nielsen, 1983) evidence hinted at the production of potent frameshift mutagens in TA98 with direct effects (Pitts *et al.*, 1978; Pitts, 1987; Houk *et al.*, 1992; Espinosa *et al.*, 1993).

CONCLUSIONS

The mean ratios PM10/TSP in four stations of the Metropolitan Area of Mexico City agreed quite well with those obtained in eight locations of U.S.A.

Most of the samples of TSP and PM10 of the stations located in the Eastern part exceeded the standard levels. Erosive and soil-related material influenced the mass concentration of particles in this area.

When the S9 mixture was added for both TSP and PM10, the mutagenic response was higher, indicating that the presence of indirect mutagens or promutagens was higher than that of the direct ones.

Higher mutagenic response was found in PM10 than in TSP. Chemical reactions should occur when large and small particles were collected together (TSP samplers) and thereby rendering the smaller particles less mutagenic.

The highest mutagenic activity in PM10, with or without S9, was found Downtown where vehicle exhaust, due to the dense traffic conditions, could be the main source of pollution.

Correlations of mutagenicity among stations for TSP were found in most of the cases without S9 but for PM10, it could only be noted in the Southern part of the Metropolitan Area (with and without S9).

No correlations of mutagenicity were found with relative humidity, temperature, wind speed or direction.

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