



Composition and mutagenicity of PAHs associated with urban airborne particles in Córdoba, Argentina



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ARTICLE INFO

Article history:

Received 7 November 2012

Received in revised form

14 February 2013

Accepted 12 March 2013

Keywords:

Airborne particles

PAHs

Comet assay

MCN test

Argentina

ABSTRACT

The comet assay and micronucleous test were used to assess the genotoxicity of organic compounds associated with particulate material collected in the city of Córdoba, Argentina. Samples were collected on fiber glass filters and their organic extracts were analyzed by GC-MS. These extracts were used for the comet assay on human lymphocytes and for the MCN test with *Tradescantia pallida*. The concentrations of polycyclic aromatic hydrocarbons as well as some of their nitro derivatives were higher during winter. Their composition suggested that their main emission sources were gasoline and diesel vehicles. We observed genotoxic effects of these organic extracts due to the presence of both direct and indirect acting mutagens. We found a good agreement between the two test systems employed, which encourages the further use of plant bioassays for air pollution monitoring, especially in developing countries, due to their flexibility, low cost and efficiency.

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1. Introduction

Airborne particles are a worldwide problem, with most being generated as a result of industrial activities and motor vehicle exhausts. Exposure to airborne particles can result in a wide range of effects such as increased daily mortality and morbidity in adults (Schwartz et al., 1996; Pope and Kalkstein, 1996; Ostro et al., 2000), breathing difficulties and development of lung cancer (Boffetta and Nyberg, 2003; Vineis and Husgafvel-Pursiainen, 2005). In addition to the respiratory tract, vital functions of other organs may be affected as well (Cohen et al., 2005). However, the quantification of the relationship between the above-mentioned harmful effects and particles is complicated by the fact that ambient air is a complex mixture of components with variable chemical compositions and physicochemical properties, long-range transport potential, toxicity and carcinogenicity. Although toxicity may be due to a direct action of particles on the respiratory tissue, the particle composition varies extensively, and toxicological effects may also be mediated by compounds present in or associated with ambient particles (Lei et al., 2004).

In urban environments, airborne particles are usually composed of an inert carbonaceous core covered by layers of adsorbed

pollutant molecules such as metals, acid salts, sulfur compounds, small organic components, and other trace materials (US EPA, 2004). This complex mixture varies depending on the season, geography, meteorological conditions and pollution sources. The organic chemicals associated with airborne particles and measured in the extractable organic matter include hundreds of compounds, among which the polycyclic aromatic hydrocarbons (PAHs) have been the most widely investigated in studies exploring the mutagenic and potentially carcinogenic activity of ambient particulate matter (Atkinson and Arey, 1994; Arey, 1998; International Agency for Research on Cancer (IARC), 1998). As only 30–40% of the organic compounds of the airborne particles have been identified, Van Houtdt et al. (1987) considered that the effects of the complex mixture could give a more realistic basis of mutagenic activity than any test of its individual components because of the occurrence of synergisms or antagonisms (Alink et al., 1983).

In order to assess the genotoxicity of urban air pollution, several studies have been conducted with the majority of them using the Salmonella mutagenicity assay or Ames test. Some others have used mammalian cells by measuring DNA adducts, DNA damage, micronuclei (MN) or chromosomal aberrations. However, very few of these have measured the frequency of micronuclei in *Tradescantia*.

Córdoba is the second largest city in Argentina and several studies have shown that increases in its airborne particulate pollution can cause greater morbidity due to respiratory diseases

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(Carreras et al., 2008). Indeed, previous studies have found that, in the urban area, the average PM₁₀ and PM_{2.5} values are almost 2.5 and 2.8 times higher respectively than the corresponding EU limit values for air quality (Lopez et al., 2011). A few research studies have revealed the mutagenic activity of air pollutants in Córdoba in a biomonitoring experimental system with *Tradescantia* (Carreras et al., 2006), but there is no other information on the components of PM that might have been responsible for this observed genotoxicity. Therefore, it is essential to clarify the nature of organic extracts obtained from PM in Córdoba.

Since many organic air pollutants are adsorbed on the surface of airborne particles, and some of the components are known or suspected human carcinogens, we collected air samples during different seasons in Córdoba city. Here, we report on the results from the comet bioassay in human lymphocytes and on the micronucleous assay in *Tradescantia* pollen mother cells, which identify the genotoxic potency of airborne particles in Córdoba.

2. Materials and methods

2.1. Sampling

Total suspended particles (TSP) were collected on glass fiber filters using a medium volume sampler with a flow rate of 0.2 m³ min⁻¹. The collector was placed 7 m high on the roof of the Chemistry Department at the FCEyN, Córdoba University during the following periods March–April, June–July and November–December, 2008. Each sample was obtained in 24 h sampling periods which were spaced from 7 to 12 days apart from each season. Before sampling, the filters were baked at 180 °C for at least 24 h, after which they were transferred to a chamber with constant humidity at 20–23 °C for another 24 h for conditioning. Afterwards, the filters with particles were equilibrated in the chamber for an additional 24 h. The concentration of particles (mg m⁻³) was determined by differences in the filter weights before and after the 24 h exposures divided by the filtered air volume.

2.2. Organic matter extraction

The solvent extracted organic matter (SEOM) was obtained using an ultrasound bath at 60 °C immersed in 30 mL methylene chloride (MC) (HPLC grade, Chroma-norm), for two 30-min periods, to ensure that all compounds of interest had been extracted. In order to avoid MC evaporation (likely to take place during extraction, as it has a relatively low boiling point), and the subsequent possibility of losing the compounds of interest in the process, a cooling device was fitted over the flask's mouth containing both the sample and MC. The extracts were concentrated with a rotavapor, at 30 °C, and then evaporated under a soft nitrogen flow. The concentrates were filtered with syringe filters, brought to 1 mL, and finally stored at -4 °C until SEOM determination, fractioning and analyses were carried out.

Four 24 h extracts from the same season were pooled and put in a vial in order to form the seasonal organic mixtures. The SEOM mass concentration contained in the particles was determined according to Villalobos-Pietrini et al. (2006), using 1 mL vials previously baked at 400 °C for 24 h, until achieving constant weight. A 200 µL aliquot of the seasonal organic extract was added, and later reduced to fully dried by means of a soft nitrogen flow. Finally, the vial was weighed again on a microbalance, until attaining constant weight. The mass contained in this dried fraction was diluted in 1 mL DMSO (5%) to obtain the total SEOM in mg mL⁻¹. Then, considering the air volume sampled and the filter area used, we expressed the SEOM in µg m⁻³.

2.3. Chemical analysis

Each pooled seasonal extract was passed through a glass column packed with 10 g of silica gel which had been previously cleaned with methylene chloride for 16 h in a Soxhlet system, before being dried and deactivated with 5% distilled water. Fractionation was carried out with 25 mL of each of the following solvents: hexane, hexane:methylene chloride (6:4), methylene chloride and methanol, with each fraction containing the following five deuterated PAHs as internal standards: naphthalene-d8 (136), acenaphthene-d10 (164), phenanthrene-d10 (188), chrysene-d12 (240), and perylene-d12 (264). All fractions were analyzed using a gas chromatograph-mass spectrometer (GC-MS) (Agilent Technologies, model 6890-5973N) with a quadrupole mass filter and an autosampler model 7683. In this system, electron impact mode (70 eV) was used with selected ion monitoring in order to identify and quantify PAHs in a 30-m HP5-MS capillary column (0.25 mm i.d., 0.25 mm film thickness). The oven temperature program was operated as follows: 80 °C for 2 min followed by increases of 5 °C min⁻¹ until reaching 300 °C. Helium was used as carrier gas at a flow rate of 1 mL min⁻¹. Splitless injection was applied for 1 min at 300 °C. The following PAH were analyzed: FA: fluoranthene (FLAN), pyrene (PYR), benzo(a)anthracene (BaA), the sum of chrysene and triphenylene (CHR + TPH), benzo(k,j)fluoranthene (BkF), benzo(a)

pyrene (BaPY), indeno(1,2,3-cd)pyrene (IPY), dibenzo(a,h)anthracene (DBaA) and benzo(ghi)perylene (BghiP). In addition, the following nitro-PAHs were also analyzed: 1-Nitronaftaleno, 2-Nitronaftaleno, 2-Nitrobifenilo, 3-Nitrobifenil, 9-Nitronaftaleno, 9-Nitrofenantreno, 3-Nitrofenantreno, 1,8-Dinitronaftaleno, 2-Nitrofluoranteno, 3-Nitrofluoranteno, 4-Nitropireno, 1-Nitropireno, 2-Nitropireno, 7-Nitrobenzo[a]antraceno, 6-Nitrocriseno, 3-Nitrobenzantrona, 6-Nitrobenzo[a]pireno, 1-Nitrobenzo[e]pireno and 3-Nitrobenzo[e]pireno. Relative response factors were determined for standard compounds in each class and used for quantitative analysis, on the basis of the same target ions (*m/z*). Eight-point calibration curves were obtained for all PAHs, ranging from 8 to 4500 pg mL⁻¹, (*R* = 40.99, *p* < 0.001). PAH detection limits were found to be between 3 and 39 pg m⁻³.

2.4. Meteorological parameters

Meteorological data were obtained from the meteorological station of the National Meteorological Service located at the airport of the city of Córdoba, 7 km north from the city center (-31.31° S, -64.21° W, altitude 484 masl). Thus we investigated the influence of mean temperature, relative humidity (*H*), atmospheric pressure (*P*) and wind speed (*W*).

2.5. Genotoxic analysis

2.5.1. *Tradescantia*-micronucleus assay

The Trad-MCN assay was conducted as described by Ma et al. (1994) with slight modifications. We employed the species *Tradescantia pallida* (Rose) Hunt. var. purpurea Boom, due to its natural resistance and its easy propagation, as well as its demonstrated sensitivity to environmental mutagens (Suyama et al., 2002; Carvalho-Oliveira et al., 2005). Briefly, 12–15 cuttings bearing young inflorescence were exposed over 8 h to the seasonal organic extracts resuspended in dimethyl sulfoxide (DMSO) and tap water. As a positive control, we exposed inflorescences in solutions of trichloromethane (20 mM) and as a negative control inflorescences were exposed in DMSO and in tap water. After the exposition inflorescences were rinsed with distilled water and recovered in tap water for 24 h. Over the entire course of exposure and recovery (32 h), aeration was provided to avoid possible oxygen depletion in the solution. After recovery, the inflorescences were fixed overnight in a 1:3 glacial acetic acid–ethanol solution and then stored in 70% ethanol. The flowers were dissected and young anthers were squashed on a microslide in a solution of acetocarmine stain, with only preparations containing early tetrads being considered. At least ten slides were examined for each treatment with three-hundred tetrads being examined per slide at a magnification of 400X. Micronuclei frequencies were calculated by dividing the total number of micronuclei (MCN) by the total number of tetrads, and expressed as MCN/100 tetrads. Micronuclei were counted on coded slides and the codes were only revealed after completing the entire experiment.

2.5.2. Comet assay

These experiments were carried out using human peripheral lymphocytes which had been isolated from whole blood samples. Twenty milliliters of heparinized venous blood, obtained from a healthy volunteer donor, was centrifuged at 2500 rpm for 20 min. The cellular layer was diluted at 1:1 with HBSS, before being placed over a Ficoll–Paque layer and centrifuged at 1500 rpm for 10 min. The lymphocytes were subsequently collected and washed twice in RPMI 1640 medium by centrifugation at 1500 rpm for 10 min, with the lymphocyte pellet being kept in RPMI 1640 medium (37 °C) supplemented with 1% penicillin/streptomycin and the cellular viability being immediately quantified in a Neubauer chamber. After this stage, cells were immediately used for the mutagenicity experiments.

The lymphocyte viability for all experimental groups before and after treatment was estimated by the trypan blue exclusion test (Altman et al., 1993). A mix of 10 µL cell pellet plus 10 µL trypan blue was incubated for 3 min and then the number of dead cells among live ones was quantified for 100 consecutive cells.

The isolated cells ($1 \cdot 10^6$ for each treatment) were exposed to a mixture of RPMI 1640 medium and the SEOM from each sampling period, for three different concentrations: 20 µL, 40 µL and 80 µL, for 24 h at 37 °C. As a negative control, lymphocytes were exposed in culture medium plus DMSO (0.5%), while as a positive control, cells were exposed in culture medium, DMSO (5%) and nitrofluorene (80 µM).

In order to test the mutagenic activity of the metabolic derivatives of the SEOM we placed these extracts in presence of liver enzymes in order to mimic the normal metabolic process of metabolism (metabolic activation system). Thus, we prepared the same set of treatments with the addition of 100 µL S9 enzymatic mixture from rat liver (S9 fraction, 1 M G6P, 0.4 M MgCl₂, 1.65 M KCl, 0.1 M NADP, 0.2 M Na₂HPO₄/NaH₂PO₄, pH 7.5) to a final volume of 1 mL at 37 °C for 24 h. The negative control was human peripheral lymphocytes (1×10^6 cells) plus DMSO (0.5%) and 100 µL S9 enzymatic mixture in RPMI 1640 medium. The positive control was human peripheral lymphocytes plus 80 µM BaPY and 100 µL S9 in RPMI 1640 medium under the same conditions. After the treatments, the cell pellet was washed twice with RPMI 1640 medium, before performing the alkaline comet assay.

The alkaline comet assay was performed according to Speit and Hartmann (2005) and Tice et al. (2000). The lymphocytes were mixed with 90 µL low

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