

RESEARCH ARTICLE

Effects of air pollution on human lung cells

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ABSTRACT

Background. PM_{2.5} are components of the atmosphere of Mexico City and contain polycyclic aromatic hydrocarbons (PAH), which induce toxic effects. Due to different compositions of the PM_{2.5} in all zones of Mexico City and the lack of information about their effects, the main purpose of this study was to evaluate the cytotoxicity and genotoxicity due to soluble organic fractions (SOFs), which contain PAH isolated from the PM_{2.5} collected from several monitoring stations in Mexico City (northeast, central, and southwest) in a human line cell culture NL-20 during a 24-h period.

Methods. We extracted the SOF of PM_{2.5} filters from the different monitoring stations. Human bronchial cells were cultured and assays were subsequently performed on the exposure of SOFs to evaluate the effect on the viability and induction of genotoxicity.

Results. Results show that 0.1 µg/µl of SOF from the central station was more cytotoxic, reducing cell viability to 52.4% and 54.2% during both dry and rainy periods, respectively. Also, cellular anomalies such as multinucleation and nuclear atypia were induced. These percentages of cytotoxicity contrasted against those obtained from SOFs from the northeast area that were 91.2% and 85% at the same concentration during both dry and rainy periods, respectively ($p < 0.05$). Only at 0.1 µg/µl SOF were the results genotoxic from the northeast and central areas ($p < 0.05$).

Conclusions. SOFs from the downtown (central) zone were the most cytotoxic due to the high concentration of automobiles as the main sources of PAH.

Key words: pollution, air, polycyclic aromatic hydrocarbons, lung, bronchial cells.

INTRODUCTION

The lifestyle in big cities demands the use of a large number of motor vehicles that burn large amounts of fossil fuels such as gasoline and diesel.¹ It is known that 3 million vehicles circulate in Mexico City. In addition to the nearly 33,000 industries located in the northeast (NE) and northwest (NW) of the metropolitan area of Mexico City (MAMC), which includes Mexico City geographically, as well as a large number of municipalities in the State of Mexico (north of Mexico City) and some portions of the state of Hidalgo.² Therefore, the atmosphere contains a complex mixture of contaminants, among which are

gases, fumes, vapors, organic particles, and inorganic air particles of biological origin.³ For different reasons, registered matter <2.5 µm (PM_{2.5}) has attracted the attention of this study because it represents a complex mixture that can contain both natural compounds and the product of anthropogenic activities. It can also contain organic compounds (both cyclic and non-cyclic) which contain some potential carcinogens.⁴ Since 2004, Mexico City has been running monitoring stations measuring PM_{2.5} concentrations. Currently there are both automatic and manual networks that perform these measurements in the atmosphere of the MAMC.⁵ Clinical and epidemiological studies show that there is a greater risk of developing lung⁶ and heart^{7,8}

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diseases due to exposure to $PM_{2.5}$. Early descriptive studies of the toxic effects in human bronchial cells were performed analyzing different extractable fractions of filters retaining PM_{10} from the atmosphere of the MAMC. These demonstrated genotoxic,⁹ cytotoxic and proinflammatory effects.¹⁰⁻¹² However, toxic potential of the $PM_{2.5}$ in human bronchial cells is not known. One way of analyzing this effect is evaluating extractable fractions of filters that retain these particles. In the Laboratory of Gas Chromatography and Mass Spectrometry of the Centro de Ciencias de la Atmósfera (CCA)—Universidad Nacional Autónoma de México (UNAM), analysis of the chemical composition and spatial and temporal distribution of the major components of $PM_{2.5}$ in the atmosphere of Mexico City was performed. This is the first comprehensive effort to describe the main compounds contained in these particles. The results revealed that the organic compounds are distributed in accordance with industrial characteristics and activities of each area. For example, $PM_{2.5}$ from NW and southeastern (SE) are associated with primary sources, whereas in NE, central (C) and southwest (SW) areas the origin of $PM_{2.5}$ is associated with secondary sources, i.e., incomplete combustion. The NE region is characterized by compounds from incomplete combustion of burning diesel in the industrial area. Area C is characterized by the vehicular concentration and incomplete combustion of gasoline, which is the main source of $PM_{2.5}$. SW area, due to the wind direction, is characterized by compounds displaced from the NE and C zones modified by the action of sunlight, resulting in oxidized compounds. It also contains compounds from biogenic sources, mainly from trees and other plant species.⁴

This study was carried out in order to determine the effect of soluble organic fractions (SOFs) extracted from the filters with $PM_{2.5}$ of Agustín (NE), Merced (C) and Coyoacán (SW) stations in NL-20 human cells.

MATERIALS AND METHODS

Extraction of the SOF of Filters with $PM_{2.5}$ from the different monitoring stations

The extraction was carried out at the Laboratory of Gas Chromatography and Mass Spectrometry of CCA-UNAM. Filters for the months of April (dry season/warm, D/W) and August (rainy season/warm, R/W) of 2006 were located from the three sampling stations in Mexico City that

will be identified as NE, C and SW, which corresponded to San Agustín, Merced and Coyoacán, respectively. All material was previously washed with acetone to avoid contamination.

Subsequently, the filters were placed in a 100-ml flask and 50 ml of dichloromethane (DCM) was added. The flask was placed in an ultrasonicator at 60°C with coolant to -5.4°C for 30 min. After this period, the DCM of the first extraction was transferred to a new flask and a second extraction was performed with the same conditions. It was then cooled in order to proceed with reduction, which consisted of placing the flask with the last 100 ml of product extraction in a rotary evaporator at 30°C under a pressure of 5" of Hg and 120 rpm. From this reduction, a concentrate was obtained, which was filtered with acrodiscs (MILLEX-NH, nylon 33 mm diameter and 0.45 μ m pore size) in 1-ml flasks, rinsing several times with DCM. When the sample exceeded 1 ml, excess was evaporated with a soft stream of liquid nitrogen to the 1-ml mark. Finally, the extracts were transferred to labeled vials and stored at -4°C. SOF determination was performed by weight difference. Empty vials were weighed on an analytical balance and then 200 μ l was taken from the above-mentioned extract separately from each month and season and placed in heavy vials in order to be reduced with a gentle stream of liquid nitrogen and were again weighed (Table 1).

Cultivation of human bronchial cells

Human bronchial cell line NL-20 (ATCC) was used. Cells were grown in F12 culture supplemented with 4% fetal bovine serum, 2.0 mM L-glutamine, 0.1 mM nonessential aminoacids, 5 μ g/ml insulin, 10 ng/ml epidermal growth factor, 1 μ g/ml transferrin, 500 ng/ml hydrocortisone, 100 μ g/ml streptomycin sulfate, and 100 U/ μ l penicillin-G. Cells were grown to confluence and detached with 0.05% trypsin and 0.01% versene in 0.9% saline solution. Subsequently, exposure microtests were performed for the SOF to evaluate the effect on the viability and inducement of genotoxicity by comet assay.

Cell viability assay by crystal violet

Then, 8000 cells were seeded per well into a 96-well plate and were allowed to adhere for 24 h. The next day, SOFs were exposed for 24 h under the following experimental conditions: control, control with alcohol (96%) (SOF ve-

hicle) and different concentrations of SOF according to two schemes:

- Scheme A or logarithmic
- Scheme B or CCA (Centro de Ciencias de la Atmósfera)

All were performed in triplicate. Concentrations of Scheme A were 0.1, 0.01, 0.001 and 0.0001 $\mu\text{g}/\mu\text{l}$ of SOF corresponding to typical test of log dilutions.

Schedule B consisted of 5, 8, 13, 17 and 20.5 $\mu\text{g}/\text{ml}$ of SOF. It is known that in this scheme mutations were induced in the *Salmonella* system and histidine operon due to SOFs obtained from PM_{10} . Therefore, evaluation in bronchial cells was considered. Thus, scheme A represents high concentrations and scheme B low concentrations of exposure to SOFs.

Subsequently, the wells were washed twice with 100 μl of NaCl 0.9% solution. This solution was removed and cells were fixed by adding 100 μl of formalin at 10% and incubated overnight at 4°C. Subsequently, the plates were washed with tap water and allowed to dry; 100 μl of crystal violet was added to each well for 10 min. Finally, excess colorant was collected and the plate was washed with tap water to remove all residues and allowed to dry for later analysis.

To determine cell viability, 100 μl of 33% acetic acid was added to each well. Dye was collected and placed in a cell and reached 500 μl volume with 33% acetic acid and was read at 595 nm in the spectrophotometer. Viability of 100% was considered in the control group (no exposure) and from this the percentage of viability in each case presented to the SOF was calculated. Finally, cells were restained using the previously mentioned procedure to obtain photomicrographs and subsequently evaluate corresponding morphologic changes in an inverted optical microscope.

Table 1. Determination of the mass of SOF for each station of the sample

Station	SOF obtained (mg)
NE, San Agustín, April	0.89
NE, San Agustín, August	1.31
CENTER, Merced, April	0.60
CENTER, Merced, August	0.40
SW, Coyoacán, April	0.92
SW, Coyoacán, August	0.85

SOF, soluble organic fraction; NE, northeast; SW, southwest.

Evaluation of genotoxicity using the comet assay

First, the culture medium was removed from the cells and detached with 100 μl of a solution of trypsin-versene at 10% diluted 1:5 in NaCl 0.9% and then incubated at 37°C for 10 min. The detached cells were collected in 0.6-ml microtubes (one tube per well) and centrifuged at 2000 rpm for 5 min. The solution was carefully removed without touching the cell pellet, and 150 μl of complete medium was added.

Comet assay preparations were performed on precoated slides with low melting point agarose. The cell suspension was mixed with low melting point agarose and then introduced into cold lysis solution (2.5 M NaCl, EDTA 100 mM, Tris 10 mM pH 10, Triton X-100 1%, DMSO 10%, the latter two components were added prior to use) for at least 1 h. Later, they were placed in a horizontal electrophoresis chamber with buffer at pH 13 for 20 min in order to carry out the DNA denaturation. Finally, electrophoresis was performed at 25 V and 300 mA for 20 min to allow separation of fragmented DNA. Excess of alkali was then removed with a neutral buffer (Tris 4 M) and fixed with 100% ethanol, stained with ethidium bromide (2 mg/mL) and observed under a fluorescence microscope equipped with an excitation filter of 515-560 nm and a 590 nm barrier filter. Using CometAssay IV program, 25 nuclei of three preparations per group were analyzed. This program measures the length of the head, intensity of the head, length of the tail, intensity of the tail and tail moment of each of the nuclei. Of the above parameters, the tail moment was considered because it takes into account both the length and intensity of the comet tail, which is the amount of fragmented DNA due to genotoxic effect (Figure 1). This measurement is referred to as the genotoxicity index (GI).

Statistical analysis

Differences in the percentages of viability and induction of SOF were evaluated by ANOVA; $p < 0.05$ was considered significant.

RESULTS

Cell viability due to SOF effect

Concentration-response assays were performed for cell viability using schemes A and B described above. These results showed an increase in viability, which is interpreted as cell proliferation (SOF effect) in NE and SW

stations. This was not observed in cells exposed to SOF in the C area. In the first instance it was always <100% and, at greater concentrations of SOF, cell viability decreased, mainly in the SOF obtained from the dry season. We can infer that the organic components of the filters with PM_{2.5} from C zone contain toxic compounds that induce the decrease in bronchial cell viability with a median lethal concentration (LC₅₀) of 0.1 µg/µl for scheme A and 17 µg/ml for scheme B (Table 2). It can be observed that during both seasons SOF compounds from the NE station showed little toxicity; the lowest percentage of viability was observed at 17 µg/ml and corresponded to 85%. However, these differences were significant ($p < 0.05$). This can also be corroborated in Figure 2, which exemplifies minimal cellular changes in morphology and NL-20 cell response. There is a similar cell number between the control (Figure 2A) and all others exposed to SOF in the NE station (Figures 2B-2E). A similar response was observed with the effect of SOF from the SW station. However, during the rainy season there was a slight increase in viability for both concentrations of scheme B, which indicates cell proliferation. Concentrations of 0.1 µg/µl and 17 µg/µl were more toxic as they induced viabilities of 74.38% and 78.17%, respectively. This can be corroborated by observing a decrease of cells per field (Figures 2K and 2M, respectively). Significant differences were shown at all concentrations ($p < 0.05$) compared with the control. SOF of station C, as previously mentioned, was the most toxic because LC₅₀ 0.1 µg/µl in both seasons were observed and 17 µg/ml only during the dry season. At these concentrations, decrease in cells per field was observed and some with signs of

cell death such as significant vacuolation in the cytoplasm (Figure 2G and amplification). Striking changes were observed in multinucleated cell groups and atypical colonies in cells exposed to 0.0001 µg/ml and 17 µg/ml (Figures 2F and 2I). It is evident in the SOF that the rainy season produces a decrease of toxic compounds and viability percentages increase with respect to those observed in the dry season. From these results it can be concluded that the compounds contained in SOF of the three stations are different from each other because bronchial cells responded in different ways. Organic SOF compounds in station C are the most toxic, perhaps because of their content (Table 3) and ability of inducing atypical cellular changes. The rainy season causes a reduction of these toxic compounds because viability is increased relative to that observed in the dry season. In relation to the toxic potential of SOF removed from PM_{2.5}, the following pattern of response was revealed: CENTER>SW>NE.

Genotoxicity induced by SOF

Once the toxic potential of the different SOF of the three stations was characterized, the next step was to evaluate the genotoxic potential (Table 4). Comet assays were performed to measure genotoxicity induced at the highest concentrations tested. It can be seen that, in the dry season, GI 0.1 µg/µl was reported twice in NE and CENTER stations and slightly higher in SW compared with the control. Only these were significant ($p < 0.05$). In the rainy season, GI induced by these SOF in stations was not reported.

In summary, only the SOFs during the dry season were genotoxic in bronchial cells. During the rainy season the genotoxic potential of SOF is reduced.

Concentrations of organic compounds in the NE, CENTER and SW Stations

Finally, it was decided to compare the predominant types of compounds in each of the stations and during both seasons. Table 4 shows, in general, the concentrations of PM_{2.5}, benzo-a, benzo-g,h,i, PAH-heavy, 7-PAH-car and nitro-PAH were always higher in the dry season than in the rainy season. This fact correlates with the decrease in NL-20 cell viability only at 0.0001 µg/µl and 17 µg/µl. Results reveal that there is no tendency to consider that higher concentrations of any scheme have the prop-

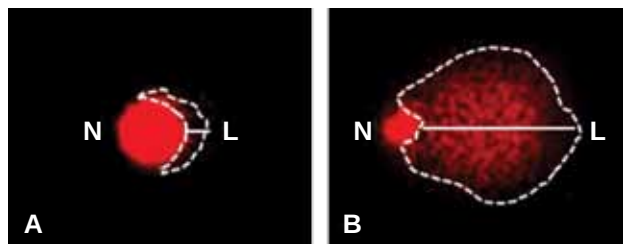
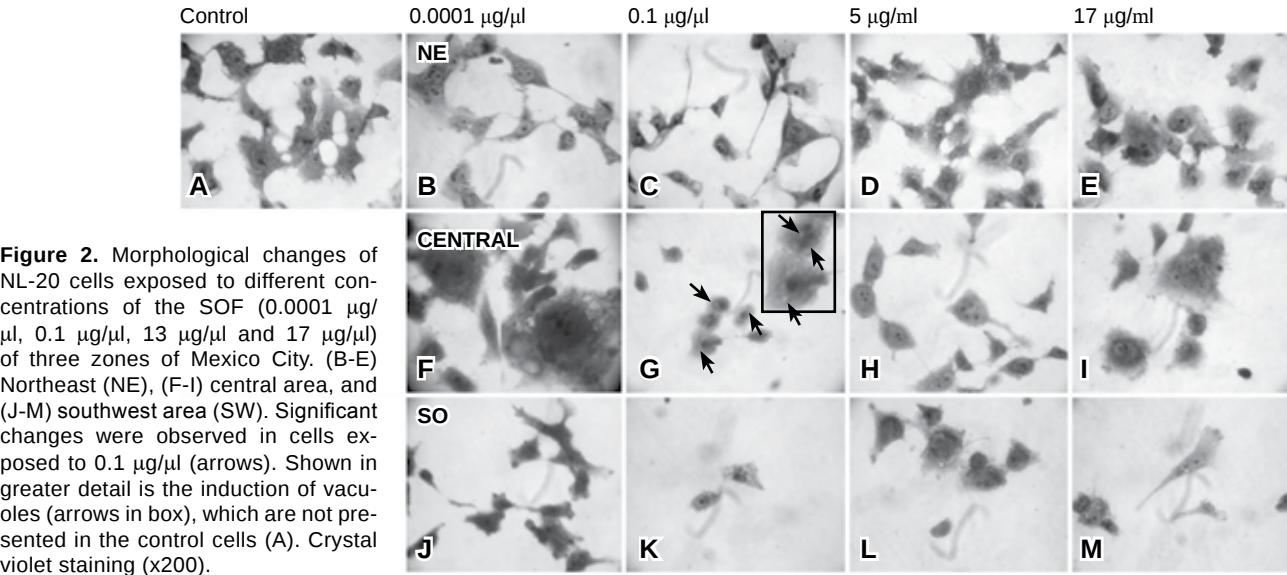


Figure 1. Induction of comets due to the soluble organic fraction (SOF) of the filters of Mexico City. The principle of reducing the cell nucleus (N) at the expense of increase in the length (L) of the comet's tail (stippled area) is used. A) Cell control. B) Cell with nuclear damage. Ethidium bromide staining and fluorescence microscopy (x200).

Table 2. Effect of NL-20 cell viability according to the different SOF of the NE, Central and SE stations of Mexico City

Monitoring station	NE		Central		SE	
Season	Dry	Rainy	Dry	Rainy	Dry	Rainy
Scheme A						
0.0001 µg/µl	98.4 ± 0.002	98.7 ± 0.0	88.4 ± 0.001	97 ± 0.002	84.2 ± 0.001	107.7* ± 0.005
0.1 µg/µl	91.2 ± 0.001	74 ± 0.003	52.4* ± 0.002	54.2* ± 0.003	74.3* ± 0.004	78.1 ± 0.001
Scheme B						
5 µg/ml	98.7 ± 0.001	90 ± 0.001	85.9 ± 0.003	74.6 ± 0.004	107.7 ± 0.006	102.0 ± 0.007
17 µg/ml	84.9* ± 0.006	122.4* ± 0.007	51.3* ± 0.002	96.1 ± 0.001	50.9* ± 0.003	103.6* ± 0.002

Rates represent percentage of viability.
NE, northeast; SE, southeast.
**p* <0.05



erty of being more toxic. What is clear is that at least for $PM_{2.5}$, benzo-g,h,i, PAH-heavy and nitro-PAH contained in fractions from station C were always higher than those obtained from the NE and SW stations. This is generally related to both the reduced viability (Table 2) and cellular alterations observed in cells exposed to SOFs obtained from this station (Figure 2).

DISCUSSION

The objective of this study was to evaluate the biological effect of the SOF extracted from the filters of three monitoring stations: NE, C and SW of Mexico City in

cultures of human bronchial cells. The results showed, at first, that due to the chemical composition of the SOF of the three stations there are certain differences in the content of organic compounds analyzed as well as the amount of the total $PM_{2.5}$. An elevation was observed at concentrations of the $PM_{2.5}$ as well as in benzo(g,h,i), PAH-heavy and nitro-PAH in SOFs obtained from the filter of station C. This additionally correlated with percentages of lower viability that were observed mainly in cells exposed to the SOF of this station and accounted for concentrations close to LC_{50} . In response to what the damage mechanisms of PAH would be on cells that induce toxicity, it was suggested that PAH are hydrophobic

compounds and can enter cells through the plasma membrane. Plant et al. suggested three media system that can explain the input of PAHs.¹³ The first is the existence of a carrier of PAHs, which can be lipoproteins or phospholipid vesicles including albumin. This results from the observation that the addition of serum increases the metabolism of PAHs. PAH transport to the second media (cell membrane) can be fast as reported by Penn et al.¹⁵ However, transport to the third media, which is either intracellular—including the nuclear membrane—has not been proven. PAHs cannot diffuse through an aqueous medium¹⁶ and will always require a lipid vehicle/carrier. An effect caused by PAHs upon interaction with the cytoplasmic membrane is to break the balance to intercalate into the lipid bilayer. Although it is difficult to demonstrate this in the cell, there are some models such as differential scanning calorimetry of the lipid bilayer.¹⁷ This technique detects the disorder of lipid structure due to the presence of foreign molecules. Using this tech-

nique, Librando and colleagues demonstrated that fluorene, fluoranthene and indeno(1,2,3-cd) pyrene PAHs dispersed in liposomes induce disorder of the bilayer.¹⁷ The three PAHs are unable to migrate through the aqueous medium, but when liposomes loaded with the three PAHs are in contact and interact with empty liposomes, the fluorene is able to migrate from a liposome membrane to another without it. We do not know which of the PAH content in the analyzed SOF may be causing the disorder of the membrane and consequently initiate cell lysis. Benzo(g,h,i)perylene and nitro-PAH are found at higher concentrations in the filters of station C with respect to organic compounds of the other two stations. At this time it is difficult to postulate that one or both are heavily involved in the induction of cell death and initiate it at membrane level in bronchial cells. However, it is an important antecedent for initiating a study of membrane disorganization and to compare the ability to do either of these or a mixture of both. Also, this is a quick

Table 3. Content of the components in the filters of the three monitoring stations

	NE		CENTRAL		SE	
	Dry	Rainy	Dry	Rainy	Dry	Rainy
PM _{2.5} (µg/m ³)	25.56 ± 5.0	11.25 ± 5.50	27.0 ± 7.20	14.80 ± 7.0	22.61 ± 5.50	12.2 ± 5.27
Benzo-α (ng/m ³)	0.41 ± 0.18	0.28 ± 0.12	0.35 ± 0.44	0.32 ± 0.12	0.27 ± 0.14	0.19 ± 0.09
Benzo-ghi (ng/m ³)	1.21 ± 0.41	0.82 ± 0.27	1.70 ± 0.42	1.25 ± 0.36	1.04 ± 0.40	0.78 ± 0.27
PAH-heavy (ng/m ³)	5.12 ± 2.01	3.35 ± 1.3	5.64 ± 1.85	3.74 ± 1.30	3.5 ± 1.5	2.34 ± 0.95
7-PAH-car (ng/m ³)	2.7 ± 0.95	1.76 ± 0.74	2.70 ± 0.88	1.70 ± 0.56	1.70 ± 0.70	1.06 ± 0.40
Nitro-HAP (ng/m ³)	135.6 ± 66.0	132.0 ± 68.0	150.4 ± 65.0	132.3 ± 70.3	98.4 ± 46.3	95.7 ± 38.1
Alkanes C ₁₉₋₂₂ (ng/m ³)	7.76 ± 3.0	7.05 ± 3.0	3.62 ± 2.0	3.5 ± 1.80	2.10 ± 1.10	2.405 ± 0.88
Alkanes C ₂₃₋₂₆ (ng/m ³)	34.0 ± 13.8	30.1 ± 7.20	29.1 ± 11.36	23.3 ± 5.82	12.1 ± 4.44	13.5 ± 5.70
Alkanes C ₂₇₋₃₃ (ng/m ³)	30.1 ± 16.4	8.64 ± 3.11	31.55 ± 20.0	9.06 ± 3.36	26.0 ± 17.10	8.82 ± 2.73

NE, northeast; SE, southeast; PAH, polycyclic aromatic hydrocarbons.

Table 4. Induced genotoxicity in NL-20 cells according to different SOF from the NE, CENTRAL and SE stations of Mexico City

Monitoring station	NE		CENTRAL		SE	
	Dry	Rainy	Dry	Rainy	Dry	Rainy
Season						
Control	0.7 ± 0.14	1.6 ± 0.12	1 ± 0.18	1 ± 0.16	1.4 ± 0.17	1.8 ± 0.26
Scheme A						
0.1 µg/µl	1.5 ± 0.18	1 ± 0.12	1.9* ± 0.15	1.2* ± 0.16	1.3* ± 0.13	1.8 ± 0.16
Scheme B						
17 µg/ml	0.85* ± 0.24	1.2 ± 0.12	0.87* ± 0.12	1* ± 0.17	1.8 ± 0.23	1.6* ± 0.18

Values are expressed in accordance with the genotoxicity index (GI).

NE, northeast; SE, southeast.

*p < 0.05.

response recorded at 24 h of exposure. Another method is that PAHs can be transferred directly from the ultrafine particles to the plasma membrane¹⁵ by vesicle trafficking. Analysis by light microscopy revealed that the particles produced by diesel combustion containing higher concentrations of PAH can enter the cell surrounded by a membrane within a vacuole.¹⁸

The results of this study with the crystal violet technique that measured viability show an effect at the level of the cell membrane: undamaged cells retain the dye after strong washes. There are other effects such as the activation pathway of the aryl hydrocarbon (AhR), which uses a membrane protein that is the AhR receptor. A series of signals are then activated among which are the activation/expression of phase I genes of xenobiotic compounds such as cytochromes, the activation/gene expression of phase II detoxification of these compounds such as glutathione-sulphydryltransferase modifying enzymes in order to increase its water solubility.

Finally, other target genes related to such general processes as apoptosis, necrosis and autophagy, which may be involved in the biological response but were not evaluated in this study.¹⁹

Less toxic effects were observed due to SOF from the NE and SW stations. A similar situation was also reported analyzing the PM_{2.5} of various U.S. cities.²⁰ For example, Chirino et al. proved that PM₁₀ produced damage and a decrease in antioxidant defense systems but does not affect cell viability.²¹ Reduction of the toxic effect of SOFs mainly in the rainy season is a fact that has been proven *in vitro* in samples taken in Paris²² and the effect is “washed” and decrease of concentration of various substances including organic compounds of SOF. However, sometimes the discrete toxic effect of PM_{2.5} or of SOF is associated with the generation of free radicals or superoxide and peroxide ions, which may significantly damage the nucleus and the cell membrane.²³ SOFs from the dry season reduced viability compared with the effect of the rainy season SOF (Table 2). However, less toxic samples that discretely reduce viability such as SOF of NE and SW stations do not produce significant genotoxicity. SOF may activate genes involved in the initiation of inflammatory responses rather than in inducing cell death.^{22,23} What is apparent is the response due to the SOF rather than the water-soluble fractions or previously washed PM_{2.5}.²⁴

Genotoxicity due to SOFs has been reported during other times due to PM₁₀ of Mexico City^{11,24,25} and PM_{2.5}.²⁶ For genotoxic induction, it is proposed that PAH contained in SOF can induce the formation of superoxide radicals and metabolic activation, forming toxic compounds that can interact directly with nucleophilic centers such as nitrogenous bases of DNA that is interspersed and forms adducts or indirectly by DNA damage via the formation of superoxide radicals. However, all these studies point to the fact that the genotoxic effect also depends on the composition of PAH²⁷ and, in our case, of PAH contained in the SOF. Such is the case of the SOF of station C, which contains a higher concentration of total PM_{2.5} and benzo(g,h,i), heavy PAH and nitro-PAH. However, these SOF do not induce genotoxic effects as significant as that produced by SOF obtained from wood burning and soot (Villeda-Cuevas 2012, unpublished results).

We conclude that SOF of NE, C and SW stations during the dry season had cytotoxic effects. SOFs from the C station were more cytotoxic and also genotoxic at a concentration of 0.1 µg/µl in both NE and C stations. This is the first study to evaluate the toxic effect of SOFs obtained from PM_{2.5} filters from Mexico City in human bronchial line cells in Mexico. The effect of exposure to SOFs in the induction/AhR activation pathway and activation of other pathways such as apoptosis, autophagy and micro-RNAs of the environmental response of PAH and which regulate other processes such as cell viability and differentiation will be evaluated.

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