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Genotoxicity of Air Borne Particulates Assessed by Comet and the Salmonella Mutagenicity Test in Jeddah, Saudi Arabia

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Abstract: Fine airborne respirable particulates less than 10 micrometer (PM10) are considered one of the top environmental public health concerns, since they contain polycyclic aromatic hydrocarbons (PAHs) which are among the major carcinogenic compounds found in urban air. The objective of this study is to assess the genotoxicity of the ambient PM10 collected at 11 urban sites in Jeddah, Saudi Arabia. The PM10 extractable organic matter (EOM) was examined for its genotoxicity by the single cell gel electrophoresis (SCGE) comet assay and the Salmonella mutagenicity (Ames) test .Gas chromatography-mass spectrometry was used to quantify 16 PAH compounds in four sites. Samples from oil refinery and heavy diesel vehicles traffic sites showed significant DNA damage causing comet in 20-44% of the cells with tail moments ranging from 0.5-2.0 compared to samples from petrol driven cars and residential area, with comet in less than 2% of the cells and tail moments of < 0.02.In the Ames test, polluted sites showed indirect mutagenic response and caused 20-56 rev/ m^3, mean while residential and reference sites caused 2-15 rev /m3. The genotoxicity of the EOM in both tests directly correlated with the amount of organic particulate and the PAHs concentrations in the air samples. The PAHs concentrations ranged between 0.83 ng/m^3 in industrial and heavy diesel vehicles traffic sites to 0.18 ng /m^3 in the residential area. Benzo(ghi)pyrene was the major PAH components and at one site it represented 65.4 % of the total PAHs. Samples of the oil refinery site were more genotoxic in the SCGE assay than samples from the heavy diesel vehicles traffic site, despite the fact that both sites contain almost similar amount of PAHs. The opposite was true for the mutagenicity in the Ames test. This could be due to the nature of the EOM in both sites. These findings confirm the genotoxic potency of the PM10 organic extracts to which urban populations are exposed.

Keywords*:* Genotoxicity, PM10 air particulats, comet, salmonella mutagenicity, PAHs.

Introduction

Over the last decade, there has been an increasing concern about the adverse health effects caused by human exposure to airborne chemical pollutants. These concerns have been reinforced by laboratory studies that confirmed the carcinogenic potential of airborne particulate matter in animal experiments [1, 2] and by epidemiological studies that supported a possible correlation between lung cancer and the mutagenic activity of air organic matter in urban areas [3, 4, 5]. Also, air pollutants are potentially significant reproductive and developmental toxicants [6, 7]. Ambient air genotoxicants can originate from emissions of fuel combustion (motor vehicle exhaust, space heating, power generation); waste incineration and industrial processing [8, 9]. Exhaust from diesel engines is considered to be a major contributor to the mutagenicity of urban air [2], and its particulates is characterized by a carbonic mixture composed of approximately 18,000 different high-molecular-weight organic compounds [10]. Diesel engines release 10 times the amount of breathable PM compared to unleaded gasoline engines. Diesel exhaust particulate PM acts particularly through polyaromatic hydrocarbons (PAHs), which can easily pass through epithelial cells membranes, bind themselves to cytosolic receptors and then affect cell growth and differentiation [11, 12]. PAHs are human mutagens and carcinogens [2] and are potentially significant

reproductive and developmental toxicants [6, 13]. In a study in New Jersey, USA, the risk of fetal death, premature birth, and low birth weight was significantly higher for those with a high prenatal polycyclic organic matter exposure [14]. Mutagenicity and genotoxicity of organic complex mixture of urban airborne particles have been demonstrated with different short-term tests on prokaryotic and eukaryotic organisms [15-18]; including mammalian cells cultured in vitro [19, 20] and laboratory animals [21]. Of the short term in vitro bioassays for detecting mutagens is the salmonella/mammalian microsome mutagenicity test [22, 23]. The salmonella test developed by Ames is about 90% accurate in detecting a wide variety of carcinogens as mutagens. The test is based on the fact that essential properties of most carcinogens are their ability to damage DNA. The test uses bacteria as a sensitive indicator of DNA damage and uses mammalian liver extract for metabolic conversion of chemicals to their active mutagenic forms. The bacterial strains are a set of histidine operon mutants. The standard tester strains contain other mutation that greatly increases their ability to detect mutagens. Another short term bioassay system for detecting DNA damaging agents is the single cell gel electrophoresis (SCGE) test or comet test [24]. This is one of the most sensitive methods for detecting DNA damage; it detects DNA single/double strand breaks and alkali-labile sites, in individual eukaryotic cells. It is reliable and rapid Therefore, this method has been widely used for studying mutagenic environmental chemicals [25-27]. The objective of this study is to assess the genotoxicity of the ambient PM10 fraction of outdoor airborne particulates in Jeddah, Saudi Arabia. The acetone extracts of these PM10 particulates were examined for its genotoxicity by the single cell gel electrophoresis (comet assay) and the Salmonella mutagenicity Ames test.

Materials and Methods

Air Sampling Sites

Ambient Air particulates matter (PM10) were collected uniformly using Staplex high volume air sampler at an average flow rate of 1.4 m^3/min. Sampling lasted 24 hrs and was carried out between Jan 17, 2004 and Feb 9, 2004 at 11 urban sites (Table 1) within a radius of 15 km in Jeddah city which is the second largest city in the Kingdom of Saudi Arabia. It is in the western region of Saudi Arabia, at the coast of the Red Sea with nearly 2.5 of permanent inhabitants. The territory of the city is well ventilated. Air collection sites were selected to be representative of industrial area which is impacted by emissions of chemicals (sites 1, 2, 8); Business area with heavy traffic of petrol and diesel driven motor vehicle (sites 3, 4, 5, 6) in addition to diesel driven trucks (site 7); residential area with petrol driven vehicle (sites 9); high-way (site 10) and reference site which is the roof of King Fahd Medical Research Center at a quiet neighborhood (site 11). All these sites are assigned on a map for Jeddah area. Air sampler was placed 8 feet above the ground with electric supplies as a source of energy to avoid creating interference when using petrol generator. Following sampling, all the glass fiber filters, 8" x 10" (TFAGF 810) were immediately placed in plastic bag and stored at –20ºC until processed. The amount of air particulate matters was determined gravimetrically by weighing the filters before and after air sampling.

Table 1: Sampling sites in Jeddah city and the amount of EOM of particle matter (PM10) collected in 24 hours on glass fiber filters.

Location	*Description*	*PM10 (EOM) (µg/ m3)*
Alnujoom square	Oil refinery, industrial operations, south periphery of the city.	14.8
King Abduaziz sea port	Crowded with commercial ships, Sugar factory.	25.4
Altahlea	Water treatment plant, west periphery of the city, close to the red sea coast.	20.4
Bab Mekkah	Downtown area and a heavy traffic zone.	12.0
Albaeaah square	Cross road, part of the city center.	17.2
Palestine street	Main road in the city.	24.0
Kubry Almurabah	Cross road bridge in the city	31.0
Breeman	Burning of garbage, truck routes heavily polluted by transport emission.	40.0
Khafar Alswahel	Red sea costal area, periphery of the city.	10.0
Alshmessy	Highway, outside the city.	13.3
Reference site	Roof of three stories building (King Fahd Medical Res. Center) in a quiet residential neighborhood. North eastern part of the city.	06.0

Extraction of the Filters

The materials collected onto or absorbed to the glass fiber filters used in the PM10 air sampler were extracted with acetone in Soxhlet apparatus. The glass fiber filter was cut into small strips and placed in a Soxhlet tube and immersed in 300 ml of acetone. The mixture was refluxed

for 18-24 h. The extract was then concentrated using rotary evaporator. The condensate was weighed and dissolved in appropriate amount of dimethylsulfoxide (DMSO). All processes were carried out without direct exposure to light. This extract was used for the assay of genotoxicity.

Quantification of PAH

Gas chromatography-mass spectrometry (GC-MS) was used to quantify 16 PAH compounds in the PM10 extractable organic matter (EOM). For the GC/MS analysis, the filters were extracted by ultrasonication with tetrahydrofuran. The PAHs were separated on a reverse-phase column (C18-RP-MZ – PAH) and analyzed with GC-MS using RTX-XLB column 30 m, 0.25 mm i.d., 25 um ft.

Single Cell Gel Electrophoresis (Comet Assay)

DNA strand breaks were determined by way of the alkaline comet assay [24, 27], were 1 ml of peripheral blood were exposed to 250 μg of the filter extracts for different periods of time. After treatment, $1x10^5$ cells combined with 100 μl of molten low melting agarose (LMA) and immediately pipetted 75 μl onto comet slide (Trevigen, USA), then the slides were immersed in freshly prepared cold lysing solution (2.5M NaC1, 100mM Na2 EDTA, 10mM Tris, pH 10, 1% sodium sarcosinate), 1% Triton X-100 and 10% DMSO was added just before use for a minimum of 1 h at 4°C. Slides then were laid in horizontal electrophoresis apparatus filled with freshly prepared alkaline solution, pH ≥ 13, the slides were immersed in the lysing solution for 20 minutes, then electrophoreses was performed at 1 Volt/cm for 20 minutes. Slides were rinsed for 5 minutes three times and then immersed in 70% ethanol for 5 minutes. After air drying slides were stained with 50μl ethedium bromide (20 μg/ml), covered and placed in a humidified air-tight container to prevent drying of the gel and were analyzed within 3-4 hours.

Comet Scoring

A total of 50 cells from each of the duplicated slides were examined randomly under fluorescence microscope (Olympus BX-51, Japan). The extent of DNA damage was measured quantitatively using comet analysis system (Loats, USA) based on extended dynamic range imaging (EDRI) technology, which expressed the comet tail moment automatically. Parallel to each experiment a series of negative controls were done in order to determine any non-specific formation or reduction in the comet.

Salmonella Mutagenicity Test (AmesTest)

Mutagenicity test was performed by the plate-incorporation technique using the Salmonella typhimirium strain TA98 as described by Maron and Ames [22] with and without metabolic activation S9 fraction. The liver S9 fraction was prepared as described by Ames [28], were male Sprague-Dawley rat was injected intraperitoneal with sodium Phenobarbital, dissolved in saline at doses of 30 mg/kg for the first 4 days and 60 mg/kg on each of the following 3 days. Prior to scarifying the animal, β-naphthoflavone, suspended in corn oil, was given by the same route at a dose of 80 mg/kg for 2 days. The rat was sacrificed by decapitation. Liver was homogenized in ice-cold 0.15 M KCI solution in a Teflon homogenizer, centrifuged at 9000 x g at 4ºC for 10 min. The supernatant (S9) was stored at -86ºC until used. Sterility of the preparation was determined by plating 0.1 ml of the (S9) preparation on bacto-pepton agar plate. EOM was tested with and without the metabolic activation (S9) fraction. Each PM10 OEM sample was assayed in three doses 25, 50, and 100 μg of condensate/plate using two replica plates per dose. DMSO (100 μ1) was used as a negative control. Revertants colonies were scored after 48 h incubation at 37°C using Image pro-plus software. Standard mutagens were used as positive controls including 4-nitroquinoline-1-oxide, 0.5 pg/plate; sodium azide, 5 pg/plate (without S9); 2-aminoanthracene, 2.5 pg/plate (with S9). Positive response was defined by at least a two fold increase in revertants over the negative control. Results were expressed as the number of adjusted revertant colonies per cubic meter of air (revertants/m^3).

Results

Airborne Organic Materials

Table 1 shows the amount of acetone extractable organic materials on filters at different sites per cubic meter of air. The EOM is ranging from 40 μg/m^3 in the highly polluted locations to 6 μg/m^3 in the reference site (11). It is clear that 'Breeman' (site 8) has the highest amount of organic materials (40 μg/m^3). The major contributors to this pollution at this site are diesel emissions and the municipal incinerator of all Jeddah dumps which is located east of this road. Second highest EOM was found at 'Kubry Almurabah' (site 7) which has 31 μg/m^3. This site is road intersection in a business area with a lot of petrol driven cars and diesel vehicles which are allowed to move during night time. As for site 2 which has 25.4 μg/m^3, it is considered to be in an industrial area with sugar factory nearby beside being near to Jeddah main sea port with all the emission coming from the ships and from trucks loading and unloading these ships. Results for the remaining sites are in agreement with their traffic status with the lowest level of organic particulates found near the sea coast 'Khafar Alsawahel' (site 9) 10 μg/m3 and the reference (site 11) with 6.0 μg/m^3.

Ambient levels of PAHs

The results of chemical analysis of the EOM in 4 places are given in Table 2. A sum of 16 PAHs collected on individual filters was analyzed. The PAHs varied from 0.83 ng/m^3 in the oil refinery (site 1), in the garbage

burning, and heavy diesel- powered motor vehicles traffic location (site 8) to 0.18 ng/m^3 in residential areas (site 3) and 0.18 in the reference (site 11). Six of these PAHs compounds are known to be carcinogens and to induce reversions to histidine independence in strain TA98 of Salmonella [29]. At site 8 the benzo(ghi)perylene represent 65.4% (0.53 ng/m^3) of the total PAHs, this was followed by the known carcinogens indeno (123-cd) pyrene and Benzo(a)pyrene at concentrations of 0.12 ng/m^3 and 0.03 ng/m^3 respectively. At the oil refinery location (site 1), benzo(ghi)perylene was highest (0.17 ng/m^3) followed by Phenanthrene (0.13 ng/m^3), then fluoranthene and Benzo(a)pyrene at a concentrations of 0.08 and 0.07 ng/m^3 respectively. The other PAHs compounds occurred as minor components.

Table 2: PAHs content in the PM10 EOM samples from four monitoring sites in Jeddah, Saudi Arabia.

PAHs EOM	*Site 11 (ng/m³)*	*Site 3 (ng/m³)*	*Site8 (ng/m³)*	*Site1 (ng/m³)*
Naphthalene	0.01	0.01	0.02	0.03
Acenaphthylene	<0.01	<0.01	<0.01	<0.01
Acenaphthene	<0.01	<0.01	<0.01	0.01
Fluorene	<0.01	<0.01	<0.01	0.02
Phenanthrene	0.05	0.03	0.04	0.13
Anthracene	<0.01	<0.01	<0.01	0.01
Fluoranthene	0.02	0.01	<0.01	0.08
Pyrene	0.02	0.02	0.02	0.06
Benzo(a)anthracen	0.01	0.01	<0.01	0.04
Chrysene	0.01	0.01	<0.01	0.04
Benzo(b)fluoranthene	0.01	0.01	0.02	0.06
Benzo(k)fluoranthene	0.01	<0.01	0.01	0.02
Benzo(a)pyrene	0.01	0.02	0.03	0.07
Dibenz(ah)anthracene	<0.01	<0.01	0.02	0.01
Benzo(ghi)perylene	0.02	0.05	0.53	0.17
Indeno(123-cd)pyrene	0.01	0.02	0.12	0.08

Genotoxicity in SCGE (Comet) Assay

In single cell gel electrophoresis assay, cells with increased DNA strands breaks display, increase migration of the DNA from the nucleus toward the anode. Comets formed as broken ends of the negatively charged DNA molecules become free to migrate in the electric field toward the anode. Two main principles are believed to determine the pattern of the comet formation. The ability of DNA to migrate, which is a function of both size of the DNA fragment and the number of broken ends that may be attached to a larger pieces can migrate a short distance from the comet head. Results of the EOM genotoxicity in the comet assay are shown in Table 3 and are expressed as comet tail moment. EOM at 'Breeman' (site 8) with heavy diesel emission in addition to the garbage burning presented the strongest genotoxic responses with 44% of the cells exhibited comet with an average tail moment of 2.0. Next highest genotoxicant sample was from 'Alnujoom Square' (site 1) it caused comet in 20% of the cell with an average tail moment of 0.5. The remaining locations of heavy petrol driven cars of city main road or residential areas showed very weak DNA damaging effects with less than 2% of the cells exhibited comet with an average tail moment of less than 0.02. There was a good correlation between DNA damage in the comet test and the amounts of pollutants and PAHs sequestered by the filters (Table 1). The genotoxicants in the comet assay does not seem to be the direct toxicants, since the toxicity appeared only in the presence of the metabolic activation system (S9) and there was no comet in absence of (S9).

Table 3: Air particulate PM10 extractable organic matter genotoxicty in peripheral blood cells determined by single cell gel electrophoresis (Cells were treated with 250 µg EOM / ml of blood)

Location	*Tail Moment*	*Cells with comet (%)*
Alnujoom square	0.0-0.01	2
King Abduaziz sea port	0.0-0.01	1-2
Altahlea	0.0-0.02	1-2
Bab Mekkah	0.0-0.02	1-2
Albaeaah square	0.0-0.01	1-2
Palestine street	0.0-0.01	1
Kubry Almurabah	0.0-0.01	2
Breeman	0.5 ± 0.2	20
Khafar Alswahel	02 ± 0.5	44
Alshmessy	0.0-0.02	1-2
Reference,Roof of Kingh Fahd Medical Research Center	0.00-0.01	1-2

Mutageicity in the Ames Test

Mutagenicity of the acetone extractable matters in the TA-98 Ames test is shown in Table 4. Out of the 11 sites investigated, 3 exhibited moderate mutagenic activities ranging from 20rev/m^3 to 56rev/m^3. The highest mutagenic response was found at the garbage burning in addition to heavy diesel- powered motor vehicles traffic location 'Breeman' (8) with 56 rev/ m^3. This was followed by extracts from heavy traffic intersection 'Kubry

Almurabah' (7) with 34rev/m^3, then the sugar factory (site 2) with 20 rev/ m^3. Three sites exhibited weak mutagenic activities ranging from 9 to 15 rev/m^3 corresponding to area of oil burning toursh 'Alnujoom square' (1) and city main roads (5, 6). Four open areas or sea shore have 4 to 6 rev/ m^3. The lowest mutagenic activity corresponding to 2rev/m^3 was measured at the background location 'KFMRC' (11).All filter extracts required the metabolic activation fraction (S9) for their mutagenic response which indicates that ambient PM10 particulates extracts are indirect mutagens.

Table 4: Mutagenicity of PM10 extractable organic matter in Salmonella typhimurium (TA98). The plate incorporation assay was conducted with S9.

Sampling	*Site*	*Adjusted no. of revertant/m^3*
1		09
2		20
3		04
4		06
5		13
6		15
7		34
8		56
9		06
10		04
11		02

Discussion

Several studies have pointed to the relationship between ambient air pollution and adverse health effect. The biological mechanisms that could be responsible for these effects are revealed by bioassay-directed chemical analysis of the crude organic extracts. Several studies suggested that polycyclic aromatic hydrocarbons might be responsible for most of the mutagenic and genotoxic activity [30]. Several in vitro studies with fractionated crude organic complex mixtures demonstrated that the highest mutagenic and DNA damaging activities is associated with the fractions, which contain most of the PAHs and nitro-PAHs derivatives [18, 31, 32]. Chemical analyses of PAHs provide specific information about a number of known mutagenic and carcinogenic PAHs [33-36]. Levels of 16 PAHs measured in this study are ranging from 0.18 ng/m^3 for non polluted and residential locations (n=2) to 0.82 ng/m^3 for the industrial and polluted locations (n=2). These concentrations are much lower compared to those measured in other large cities around the world, including Montreal, Canada with a level of 22.9 ng/m^3 in a polluted and 1.9 ng/m^3 in non-polluted areas; [37], in Athens, Greece the level of PAHs was 10.87 ng/m^3 measured outdoors [38], in Cairo (Egypt) concentrations of PAHs was 14.79 ng/m^3 during the winter season and 7.53 ng/m^3 in summer [39] and in Kuwait PAHs concentrations measured at 14 sites ranged from 5 to 13 ng/m^3 with phenanthrene representing 35% of the total PAHs. The proportion of five and six-ring PAHs increased around the oil lakes [40]. In Jeddah, the high-molecular-weight PAHs such as benzo(ghi)perylene and B[a]P was the most abundant among the 16 PAHs analyzed, measuring respectively 0.53 ng/m^3 and 0.05 ng/m^3 in polluted areas compared to 0.035 ng/m^3 and 0.02 ng/m^3 in non polluted areas. The lower molecular-weight PAHs were only present as a minor fraction. The high-molecular-weight PAHs are, however, of greater concern because of their significant mutagenic/carcinogenic potency, [35]. B[a]P in the present study is significantly lower than that reported in Birmingham (UK) where the concentrations ranged between 0.81 ± 0.65 ng/m^3 in winter and 0.25 ± 0.20 ng/m^3 in summer [41] and in Heraklion (Greece) the B[a]P concentrations ranged between 0.14 and 3.47 with an average of 1.16 ng/m^3 during the period 2000–2002 (42).

The genotoxicity of the PM10 EOM was also assessed using the SCGE assay. This assay is a rapid and sensitive method to examine single and double-strand DNA breaks [43], and it has been used to evaluate ambient air genotoxicity in several studies [44-46]. In the present study, SCGE assay demonstrated good correlations between PAHs concentrations and DNA damageas shown in tables 2 and 3. Extractable organic matters from sites 1 and 8 which are relatively more polluted caused comet formation in 20 and 44 % of the cells with a tail moment of 5 (± 0.2) and 2 (± 0.5). Meanwhile in the less polluted areas, cells with comet were < 2% with a tail moment of 0.01-0.02. Other studies found similar result where the genotoxicity of organic extracts of particles collected from dense traffic area were greater than in limited traffic area according to the same comet assay [47].

Results of the Salmonella mutagenicity assay provides reproducible results that correlate with measured PAH concentrations. Samples taken at sea port in addition to sugar factory (site2), at diesel driven trucks (site 7) and at garbage burning in addition to truck routes (site 8) showed higher mutagenic activities in the Salmonella test with 20, 34, and 56 adjustable rev/m3 respectively, meanwhile samples from the residential or petrol driven motor vehicle sites (sites 3, 4, 5, 9, 10, 11) showed an average of 6 rev/m^3. Similar studies using Ames assay, showed that revertants number of TA98 strain was higher in dense traffic sectors than in sectors where traffic was more limited [44, 47]. It worth noticing that EOM from site 1 was more genotoxic in comet assay than in the mutagenicity Ames test .This could be due to the nature of the comopounds at the two sites; this emphasizes the need to use more than one test for genotoxicity screening of air pollutants. The EOM were also proven to be indirect mutagens in both tests since it require activation system (S9). This may imply that the organic materials in the air filter extracts could be polyaromatic hydrocarbon

which is very well known carcinogen and not nitro-polycyclic aromatic compounds which is known to be direct mutagen in the TA-98 Ames [48]. Our results are in accordance with other studies showing greater mutagenic and genotoxic responses of particles' organic extracts from dense diesel traffic areas compare to residential areas. Results of these studies can play a central role in a long term program of cancer prevention aim to identify and minimize human exposure to environmental carcinogens and to aid in the development of preventive program to avoid future human exposure

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References

1. Oftedal, B.; Nafstad, P.; Magnus, P.; Bjorkly, S.; Skondal, A.: Traffic-related air pollution and hospital admission for respiratory diseases in Drammen. Norway 1995–2000, *Eur. J. Epidemiol*., **2003**, *7*, 671–675.
2. Bostrom, C. E.; Gerde, P.; Hanberg, A.; Jernstrom, B.; Johansson, C.; Kyrklund, K.*et al*.: Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environ Health Perspect.,* **2002**, *110*, 451–488.
3. Nyberg, F.; Gustavsson, P.; Jarup, L.; Bellander,T.; Berglind, N.; Jakobsson, R.; Pershagen, G.: Urban air pollution and lung cancer in Stockholm. Epidemiology. **2000**, *11*, 487–495.
4. Cury, P. M.; Lichtenfels, A. J.; Reymao, M. S.; Conceicao, G. M.; Capelozzi, V. L.; Saldiva, P. H.: Urban levels of air pollution modifies the progression of urethane-induced lung tumors in mice. *Pathol. Res. Pract*. **2000**, *196,* 627–633.
5. Zhao, X. S.; Wan, Z.; Zhu, H. G.; Chen, R. P.: The carcinogenic potential of extractable organic matter from urban airborne particles in Shanghai. China. *Mutat. Res.***, 2003**, *540*, 107–117.
6. Dejmek, J.; Solansky, I.; Beneš, I.; Lenicek, J.; Šrám, R. J.: The impact of polycyclic aromatic hydrocarbons and fine particles on pregnancy outcome. *Environ Health Perspect*. **2000**, *108,* 1159-1164.
7. Choi, H.; Jedrychowski, W.; Spengler, J.; Camann, D. E.; Whyatt, R. M.; Rauh, V.; Tsai, W.; Perera, F.: International studies of prenatal exposure to polycyclic aromatic hydrocarbons and fetal growth. *Environ Health Perspect.,* **2006**, *114,* 1744–1750.
8. Kavouras, I. G.; Koutrakis, P.; Tsapakis, M.; Lagoudaki, E.; Stephanou, E. G.; Baer, D. V.; Oyola, P.: Source apportionment of urban particulate aliphatic and polynuclear aromatic hydrocarbons (PAHs) using multivariate methods. *Environ Sci Technol.,* **2001**, *35,* 2288–2294.
9. Liu, Y.; Zhu, L.; Shen, X.: Polycyclic aromatic hydrocarbons (PAHs) in indoor and outdoor air of Hangzhou, China. *Environ Sci Technol.* **2001**, *35,* 840–844.
10. United Nations Environment Programme and World Health Organisation Report, Air pollution in the world's megacities, *A report from the United Nations Environment Programme and World Health Organisation, Environment.* **1994**, *36*, 5–37.
11. Bayram, H.; Devalia, J. L.; Sapsford, R. J. *et al.:* The effects of diesel exhaust particles on cell function and release of inflammatory mediators from human bronchial epithelial cells *in vitro*. *Am J. Respir. Cell Mol Biol*., **1998**, *18,* 441–448.
12. Sydbom, A.; Blomberg, A.; Parnia, S.; Stenfors, N.; Sandstrom, T.; Dahlén, S. E.: Health effects of diesel exhaust emissions. *Eur. Respir. J.* **2001***, 17,* 733–746.
13. Perera, F. P.; Rauh, V.; Tsai, W. Y.; Kinney, P.; Camann, D.; Barr, D. *et al*.: Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. *Environ Health Perspect.,* **2003**, *111*, 201–205.
14. Vassilev, Z. P.; Robson, M. G.; Klotz, J. B.: Associations of polycyclic organic matter in outdoor air with decreased birth weight: a pilot cross-sectional analysis, *J. Toxicol Environ Health Part A.,* **2001**, *64,* 595–605.
15. Binkova, B.; Cerna, M.; Pastorkova, A.; Jelinek, R.; Benes, I.; Novak, J.; Sram, R. J.: Biological activities of organic compounds adsorbed onto ambient air particles: comparison between the cities of Teplice and Prague during the summer and winter seasons 2000–2001, *Mutat. Res.* **2003**, *525,* 43-59.
16. Zhao, X.; Wan, Z., Chen, G.; Zhu, H.; Jiang, S.; Yao, J. Genotoxic activity of extractable organic matter from urban airborne particles in Shanghai, China. *Mutat. Res.* **2002**, *514*, 177–192.
17. Cerna, M.; Pastorkova, A.; Vrbikova, V.; Smid, J.; Rossner,P. Mutagenicity monitoring of airborne particulate matter (PM10) in the Czech Republic. *Mutat. Res.* **1999***, 444*, 373–386.
18. Buschini, A.; Cassoni, F.; Anceschi, E.; Pasini, L.; Poli, P.; Rossi, C.: Urban airborne particulate: genotoxicity evaluation of different size fractions by mutagenesis tests on microorganisms and comet assay. *Chemosphere.* **2001***, 44,* 1723–1736.
19. Hsiao, W. L.; Mo, Z. Y.; Fang, M.; Shi, X. M.; Wang, F.: Cytotoxicity of PM (2.5) and PM (2.5-10) ambient air pollutants assessed by the MTT and the Comet assays. *Mutat. Res.* **2000**, *471*, 45–55.
20. Topinka, J.; Schwarz, L. R.; Wiebel, F. J.; Cerna, M.; Wolff, T.: Genotoxicity of urban air pollutants in the Czech Republic. Part II. DNA adduct formation in mammalian cells by extractable organic matter. *Mutat. Res.* **2000**, *469*, 83–93.
21. Cury, P. M.; Lichtenfels, A. J.; Reymao, M. S.; Conceicao, G. M.; Capelozzi, V. L.; Saldiva, P. H.

Urban levels of air pollution modifies the progression of urethane-induced lung tumors in mice. *Pathol. Res. Pract.* **2000**, *196*, 27–633.
22. Maron, D. M.; Ames, B. N.: Revised methods for the Salmonella mutagenicity test. *Mutation Res.* **1983**, *113,* 73-215.
23. Sugimura, T.; Nagao, M.; Kawachi, T.; Honda, M.; Yahagi, T.; Seino, Y.; Matusushima, T.; Shirai, A.; Sawamura, M.; Sato, S.; Matsumoto, H.; Matsukura, N.: in: Origins of Human Cancer, H. Hiatt, J.D. Watson, J. A. Winsten (Eds.), *(Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.)* **1977**.
24. Singh, N. P.; Stephen, R. E.: Microgel electrophoresis: sensitivity, mechanisms and DNA electrostretching. *Mutat. Res.* **1997**, *383*, 167–175.
25. Mitchelmore, C. L.; Chipman, J. K.: DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring. *Mutat. Res.* **1998**, *399*, 35–147.
26. Kassie, F.; Parzefall, W.; Knasmiiller, S.: Single cell gel electrophoresis assay: a new technique for human biomonitoring studies. *Mutat. Res.* **2000**, *463,* 13–31.
27. Sasaki, Y. F.; Sekihashi, K.; Izumiyama, F.; Nishidate, E.; Saga, A.; Ishida, K.; Tsuda, S.: The comet assay results and carcinogenicity with 208 chemicals selected from the IARC monographs and U.S. NTP Carcinogenicity Database. *Crit. Rev. Toxicol.*, **2000**, *30,* 629–799.
28. Ames, B. N.; McCann, J.; Yamasaki, E.: Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test, *Mutat. Res.*, **1975**, *31,* 347-364.
29. World Health Organization International Agency for Research on Cancer (IARC) Monographs on the evaluation of carcinogenic risks to humans, volume 32, polynuclear aromatic compounds, Part 1, *Chemical. Environmental and Experimental Data*.
30. DeMarini, D. M.: Mutation spectra of complex mixtures, *Mutat. Res.*, **1998**, *411*, 11–18.
31. Lazarova, M.; Slamenova; D.: Genotoxic effects of a complex mixture adsorbed onto ambient air particles on human cells in vitro; the effects of vitamins E and C. *Mutat. Res.,* **2004**, *557,* 167–175.
32. Ducatti, A.; Vargas, V. M. F.: Mutagenic activity of airborne particulate matter as an indicative measure of atmospheric pollution. *Mutat. Res.*, **2003**, *540,* 67–77.
33. IARC, Overall Evaluation of Carcinogenicity: an Updating of IARC Monograms 1 to 42, IARC monographs on the evaluation of the carcinogenic risk to humans, Supplement 7, *International Agency for Research on Cancer, Lyon*, **1987**.
34. Eide, I.; Neverdal, G.; Thorvaldsen, B.; Grung, B.; Kvalheim, O. M.: Toxicological evaluation of complex mixtures by pattern recognition: correlating chemical fingerprints to mutagenicity. *Environ. Health Perspect.*, **2002**, *110,* 985–988.
35. 35. Bartsch, H.; Malaveille, C.; Camus, A. M.; Martel Planche, G.; Brun, G.; Hautefeuille, A.; Sabadie, N.; Barbin, A.: Validation and comparative studies on 180 chemicals with S. typhimurium strains and V79 Chinese hamster cells in the presence of various metabolizing systems. *Mutat. Res*., **1980**, *76,* 1–50.
36. Sakai, M.; Yoshida, D.; Mizusaki, S.: Mutagenicity of polycyclic aromatic hydrocarbons and quinines on Salmonella typhimurium TA97. *Mutat. Res*. **1985**, *156*, 61–67.
37. Vyskocil, Z.; Fiala, V.; Che'nier, L.; Krajak, E.; Ettlerova, J.; Bukac, C.; Viau, S. Emminger. Assessment of multipathway exposure of small children to PAH. Environ. Toxicol. *Pharmacol.,* **2000**, *8,* 111–118.
38. Kyrtopoulos, S. A.; Georgiadis, P.; Autrup, H.; Demopoulos, N. A.; Farmer, P.; Haugen, A.; Katsouyanni, K.; Lambert, B.; Ovrebo, S.; Sram, R.; Stephanou, G.; Topinka, J.: Biomarkers of genotoxicity of urban air pollution. Overview and descriptive data from a molecular epidemiology study of populations exposed to moderate-to-low levels of polycyclic aromatic hydrocarbons: the AULIS project, *Mutat. Res*., **2001**, *496,* 207–228.
39. Hassanien, M.A. ; Rieuwerts, J. ; Shakour, A.A. ; Bitto, A.: Seasonal and annual variations in air concentrations of Pb, Cd and PAHs in Cairo, Egypt. *Int J Environ Health Res.,* **2001**, *11,* 13-27.
40. Gevao, B.; Al-Omair, A.; Sweetman, A.; Al-Ali, L.; Al-Bahloul, M.; Helaleh, M.; Zafar, J.: Passive sampler-derived air concentrations for polybrominated diphenyl ethers and polycyclic aromatic hydrocarbons in Kuwait. *Environ Toxicol Chem.*, **2006***, 6,* 1496-1502.
41. Smith, D. J.; Harrison, R. M.: Concentrations, trends and vehicle source profile of polynuclear aromatic hydrocarbons in the UK atmosphere, *Atmos. Environ*. **1996**, *30*, 2513–2525.
42. Tsapakis, M; Stephanou, E. G.: Occurrence of gaseous and particulate polycyclic aromatic hydrocarbons in the urban atmosphere: study of sources and ambient temperature effect on gas/particle concentration and distribution. *Environ. Pollut.***, 2005**, *133,* 147–156.
43. Anderson, D.; Yu, T.W.; McGregor, D. B.: Comet assay responses as indicators of carcinogen exposure. Mutagenesi*s,* **1998**, *13,* 539–555.
44. Monarca, S.; Feretti, D.; Zanardini, A.; Moretti, M.; Villarini, M.; Spiegelhalder, B.; Zerbini, I.; Gelatti, U.; Lebbolo, E.: Monitoring airborne genotoxicants in the rubber industry using genotoxicity tests and chemical analyses. *Mutat. Res.* **2001**, *490,* 159–169.
45. Alfaro-Moreno, E.; Martinez, L.; Garcia-Cuellar, C.; Bonner, J. C.; Murray, J. C.; Rosas, I.; Rosales, S. P.; Osornio-Vargas, A. R.: Biologic effects induced in vitro by PM10 from three different zones of Mexico City. *Environmental Health Perspectives,* **2002**, *110,* 715–720.
46. Gabelova, A.; Valovicova, Z.; Horvathova, E.; Slamenova, D.; Binkova, B.; Sram, R. J.; Farmer, P. B.: Genotoxicity of environmental air pollution in

three European cities: Prague, Kosice and Sofia. *Mutation Res.,* **2004**, *563,* 49–59.

47. Vellosi, R.; Vannucchi, C.; Bianchi, F.; Fiorio, R.; Rosellini, D.; Ciacchini, G.; Giaconi, V.; Bronzetti, G.: Mutagenic activity and chemical analysis of airborne particulates collected in Pisa (Italy). *Bulletin of Environmental Contamination and Toxicology.* **1994***, 52,* 465–473.
48. Cerna, M.; Pochmanova, D.; Pastorkova, A.; Benes, I.; Lenicek, J.; Topinka, J.; Binkova, B.: Genotoxicity of urban air pollutants in the Czech Republic. Part I. Bacterial mutagenic potencies of organic compounds adsorbed on PM10 particulates. *Mutat Res.*, **2000**, *469*, 71-82.