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*Full Length Research Paper*

# A study on the use of the comet assay in environmental biomonitoring studies in Saudi Arabia

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**Airborne particulate matter (PM10) contains a large number of genotoxic and carcinogenic substances. Ambient air is reported to be mutagenic in many areas worldwide. Particulate matter has been linked to premature mortality, lung cancer, respiratory and cardiovascular health problems. In the present study, we investigated the genotoxicity of the ambient air PM10 extractable organic matter (EOM) collected at Arafat and Muzdalifa in Makkah, Saudi Arabia. The amounts of sixteen polycyclic aromatic hydrocarbons (PAHs) in the EOM were quantified. The PM10 averages in Arafat and Muzdalifa were 158 and 444.5 µg/m$^3$, respectively which exceeded the U.S. maximum daily of 150 µg/m$^3$. The reference site PM10 was 6.1µg/m$^3$. The EOM averages in Arafat, Muzdalifa and the reference site were respectively 46.4, 151.6 and 6.15 µg/m$^3$ and the PAH averages in the EOM were 2.8, 5.0 and 0.19 ng/m$^3$. The EOM samples were mutagenic in the salmonella TA98 test and damaged human blood cells DNA in the comet assay in a dose related response. The bacterial revertants and the comet tail moment means were higher in Muzdalifa than in Arafat. Regression analyses of both showed a positive relation between each and the EOM concentrations tested (P < 0.001). For routine ambient air genotoxicity monitoring, the use of salmonella TA98 and the comet tests are recommendable. This study provides information for the first time on the PM10 air pollutants and its genotoxic activity in Arafat and Muzdalifa.**

**Key words:** Ambient air PM10, polycyclic aromatic, hydrocarbons, salmonella mutagenicity, comet tests.

## INTRODUCTION

Chemical compounds present in the atmosphere are considered ambient air pollutants when they occur in unnaturally high concentrations and have the potential to cause harm to the environment and human health. Air pollutants particulate matter includes: organic compounds, such as aldehydes, aromatic and polycyclic aromatic hydrocarbons, and dioxins (Brauer et al., 2003).

**Abbreviations: PM10,** Airborne particulate matter ≤10 µm; **EOM,** extractable organic matter; **PAHs,** polycyclic aromatic hydrocarbons; **GC-MS,** gas chromatography-mass spectrometry; **DMSO,** dimethylsulfoxide; **EDRI,** extended dynamic range imaging.

Epidemiological studies provide fairly convincing evidence for an association between ambient particulate matter concentration and increased mortality and morbidity (Krewski et al., 2004; Dominici et al., 2005). However, there is no clear agreement on a biologically plausible mechanism to explain the mortality/morbidity associated with PM exposure (Schwarze et al., 2006).

Genotoxic effects attributed to PM may relate to the content of organic compounds and to the oxidative DNA damage generated by transition metals like iron (Knaapen et al., 2002). However, the exact physiochemical mechanism by which PM produces adverse effects is not clear (Gilli et al., 2007). A number of studies at urban sites have indicated that mobile sources (automobiles,

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trucks, etc.) made a significant contribution to the level of airborne mutagens (Marino et al., 2000, DeMarini et al., 2004). Airborne mutagenic activities have been correlated with polycyclic aromatic hydrocarbons (PAHs) emitted from diesel and gasoline engine exhausts (Kuo et al., 1999). By examining the amounts of individual PAHs and nitro-PAHs and the ratios of benzo(a)pyrene (BaP) to benzo[g,h,i]perylene and pyrene to benzo(a)pyrene, airborne mutagens measured in a nonindustrial town were attributed to emissions from motor vehicle traffic (Lee et al., 1994). The mutagenic and genotoxic potential of extractable organic matter from airborne particles has been demonstrated with several short-term tests on prokaryotic and eukaryotic organisms (Buschini et al., 2001; Baulig et al., 2003).

A battery of short term assays exists for the detection of different genotoxic compounds. Since the late 1970s, it has been recognized that bacterial mutagenicity tests (Claxton et al., 1998) and the single cell gel electrophoresis (comet) assay (Moller, 2006) are the most versatile and adaptable systems for examining and comparing the mutagenicity of multiple environmental systems. The comet assay was described in middle 1980s. The salmonella test developed by Ames is about 90% accurate in detecting a wide variety of carcinogens as mutagens (Bartsch et al., 1980). Animal studies indicate that the comet assay was able to detect genotoxic effects of diesel exhaust particles in lung tissue, colon epithelial cells, bone marrow cells and liver tissue (Moller, 2006, Gilli et al., 2007).

The aims of this study were to quantify the concentrations of airborne particulate matter (PM10) and 16 PAHs in the PM10 extractable organic matter in Arafat and Muzdalifa pilgrimage sites, north of Makkah, Saudi Arabia, none of the 16 PAHs monitored were reported in any air samples. The second part of this study focuses on assessing the genotoxic activities of the PM10 organic extracts. Identification of pollutants and their source are essential for developing effective control strategies to improve air quality and therefore reduce the health impacts associated with fine particulate matter and PAHs. Studies indicated that the comet assay is a sensitive and reliable method for detecting DNA damage single and double-strand breaks in individual cells and it has applications in areas of biomedical and environmental health science (Dusinska and Collins, 2008).

The use of the comet assay in environmental biomonitoring studies has increased as a result of its sensitivity and small sample requirement (Valverde and Rojas, 2009).

## MATERIALS AND METHODS

### Air sampling sites

Samples of PM 10 with aerodynamic diameters ≤10 µm were collected using fixed-site samplers at two pilgrimage locations, Arafat and Muzdalifa in Makkah region, Saudi Arabia during the annual Islamic pilgrimage seasons of 2004 and 2006 which corresponded to the Islamic lunar year of 1424 and 1427 respectively. Hajj is a yearly gathering of Muslims from all over the world to Makkah for religious pilgrimage. Every year over two million Muslims make this journey. The territory of Arafat and Muzdalifa are about 13.6 and 6.8 $km^2$ respectively and they are 10 km apart (Figure 1). It is an open, level area, surrounded by mountains with almost no inhabitants except during the Hajj days. The pilgrims travel to Arafat by many types of motor vehicles and stay there for one day from sunrise to sunset. After sunset, pilgrims are transported to Muzdalifa where they stay overnight.The pilgrims spend the night at Muzdalifah, often sleeping in the open air, before leaving the next morning. As a result, the two sites are impacted by heavy traffic emissions from petroleum and diesel vehicles. Air samples were collected on glass fiber filters , 8 x 10 in (Staplex, product number TFAGF 810) using Staplex high volume PM10 air sampler (Brooklyn, New York) at an average flow rate of 1.4 $m^3$/min. The air sampler was placed in a truck raised 8 feet above ground level.

Electricity was used to operate the air sampler in Arafat. When electricity was not available in Muzdalifa a petroleum-fueled generator was used and was placed 50 m away from the air sampler in the downwind direction to minimize the contribution from the petroleum generator. Each year one sample was collected at each site and the sampling duration was 11 h. Sampling took place during the last week of January 2004 (temperature17-31°C, partly cloudy, winds in Arafat was moving south-westerly with a force of 10-35 km/h) and during the last week of December 2006 (temp 18-28°C, sunny). Following sampling, filters were immediately placed in plastic bags and stored at –20°C until processed. The PM mass was determined gravimetrically by weighing the filters before and after air sampling and calculating the difference between both weights.

The PM10 concentration was obtained by dividing it by air volume. The reference site was located on the roof of a three-story building, King Fahd Medical Research Center in a residential neighborhood in the northeastern part of Jeddah, west of Makkah, Saudi Arabia. To investigate the impact of petroleum generator on the concentrations PM10 and EOM, air samples were collected from the reference sites using electricity and petroleum generator.

### Analysis of organics from air samples

The organic compounds collected onto or absorbed to the glass fiber filters used in the PM10 air sampler were extracted with acetone in soxhlet apparatus to yield EOM. 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 (Borras and Tortajada-Genaro, 2007). The condensate was weighed and dissolved in appropriate amount of dimethylsulfoxide (DMSO).

All processes were carried out without direct exposure to light. To quantify 16 PAHs in the PM10 samples, the filters were extracted by ultrasonication with tetrahydrofuran and then analyzed using gas chromatography-mass spectrometry (GC-MS). 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 µm ft (Borras and Tortajada-Genaro, 2007).

### Salmonella mutagenicity test (Ames)

The test was performed by the plate-incorporation technique using the *Salmonella typhimirium* strains TA98 which detects various frameshift mutagens and TA100, which detects mutagens that


**Figure 1.** Muzdalifa (A) and Arafat (B) Air sampling sites.

cause base-pair substitutions (Maron and Ames, 1983). The liver metabolic activation fraction (S9) was prepared as described by Ames (Ames et al., 1975). The effect of EOM on mutagenicity was tested with and without the S9 fraction.

Each PM10 organic extract sample was assayed twice in three doses 50, 100, and 250 µg of EOM/plate using triplicates per dose. Revertant colonies were scored after 48 h incubation at 37°C using image pro-plus software (version 6.0.0.260, copyright1993-2006 Media Cybernetics, Inc). The solvent, DMSO (100 µ1), was used alone as a negative control with positive response defined by at least a two-fold increase in revertants over the negative control. Results were expressed as the number of revertants colonies induced by the amount of EOM in cubic meter of air or as revertants per specific EOM dose.

**Single cell gel electrophoresis (comet assay)**

DNA strand breaks were determined by way of the alkaline comet assay (Singh and Stephen, 1997). Incubation of blood samples with the tested PM10 organic extracts, one ml of human peripheral blood from healthy males donor, living in Jeddah, situated in an urban area with limited traffic and industrial influence approximately 105 Km west from Arafat and Muzdalifa (target site) were exposed to 50, 100 and 250 µg of the PM10 organic extracts for 18 h with and without the S9. After treatment, red blood cells were lysed and $1x10^5$ leukocytes were combined with 100 µl of molten low melting agarose were immediately pipetted into comet slide (Trevigen, USA). Slides were then processed and analyzed as described (Elassouli et al., 2007). Benzo[a]pyrene 10 µM, was used as a positive control and 1% DMSO was used as negative control in each electrophoretic run.

Experiments were done in duplicate for each EOM concentration tested. In the single cell gel electrophoresis assay (comet), cells with increased DNA damage display increase migration of DNA from the nucleus toward the anode. The comet forms as broken ends of the negatively charged DNA molecules become free to migrate in the electric field toward the anode. The ability of DNA to migrate is a function of both size of the DNA fragment and the number of broken ends that may be attached to a larger pieces that can migrate shorter distance from the comet head. DNA damage detected by alkaline comet assay was quantified and expressed as tail moment which is the product of tail length and percentage of DNA in the tail.

Fifty cells from slides from each duplicated experiments were examined randomly under fluorescence microscope (Olympus BX-51, Japan). The extent of DNA damage was measured quantitatively using comet analysis system (Loats, USA, high capacity slide analysis system, version 2.3.4, copyright 1997, 2002) based on extended dynamic range imaging (EDRI) technology, The EDRI system is comprised of precision, PCI bus compatible, analog to digital video frame capture electronics, together with a thermoelectrically cooled, computer controlled, instrument grade, solid state, monochrome CCD video camera, and Loats associates, Inc.(LAI) exclusive image capture control and processing software (LAI, 201 East Main St. Westminster, MD 21157 USA) 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. The system automatically generates the values for the comet tail such as its moment, tail length, tail area, percentage DNA in the tail, intensity of fluorescence, tail inertia and other comet tail parameters

**Table 1.** Concentrations of PM10, EOM and Total PAHs.

| | Total PM10(µg/m$^3$) | | PM10 EOM(µg/m$^3$) | | PHAs in the PM10(ng/m$^3$) | |
|---|---|---|---|---|---|---|
| | | | Organic extracts | | | |
| **Site** | **2004** | **2006** | **2004** | **2006** | **2004** | **2006** |
| Arafat | 156 | 160 | 45.6 | 47.2 | 3.36 | 2.22 |
| Muzdalifa | 289 | 600 | 83.2 | 220.0 | 5.17 | 4.76 |
| Reference site | 11.5 | 11.8 | 6.0 | 6.2 | 0.18 | 0.19 |

**Table 2.** Concentrations (ng/m$^3$) of individual PAHs in PM10 organic extracts.

| PHAs | Arafat | | Muzdalifa | | Reference site | |
|---|---|---|---|---|---|---|
| | **2004** | **2006** | **2004** | **2006** | **2004** | **2006** |
| Naphthalene | 0.1 | 1.09 | 0.09 | 0.64 | 0.01 | 0.01 |
| Acenaphthylene | <0.01 | <0.09 | <0.02 | <0.05 | <0.01 | 0.01 |
| Acenaphthene | 0.05 | <0.09 | 0.02 | <0.05 | <0.01 | 0.01 |
| Fluorene | 0.06 | <0.09 | 0.04 | <0.05 | <0.01 | 0.01 |
| Phenanthrene | 0.52 | <0.09 | 0.34 | 0.11 | 0.05 | 0.03 |
| Anthracene | 0.04 | <0.09 | 0.02 | <0.05 | <0.01 | 0.01 |
| Fluoranthene | 0.39 | <0.09 | 0.19 | <0.05 | 0.02 | 0.02 |
| Pyrene | 0.3 | <0.09 | 0.19 | <0.05 | 0.02 | 0.01 |
| Benzo(a)anthracen | 0.17 | <0.09 | 0.09 | <0.05 | 0.01 | <0.01 |
| Chrysene | 0.17 | <0.09 | 0.11 | 0.08 | 0.01 | 0.01 |
| Benzo(b)fluoranthene | 0.18 | <0.09 | 0.13 | 0.13 | 0.01 | <0.01 |
| Benzo(k)fluoranthene | 0.08 | <0.09 | 0.06 | 0.07 | 0.01 | 0.01 |
| Benzo(a)pyrene | 0.24 | <0.09 | 0.15 | 0.25 | 0.01 | 0.01 |
| Dibenz(ah)anthracene | 0.05 | <0.09 | 0.08 | <0.05 | <0.01 | 0.01 |
| Benzo(ghi)perylene | 0.75 | 0.77 | 3.02 | 0.64 | 0.02 | 0.01 |
| Indeno(123cd)pyrene | 0.26 | 0.35 | 0.64 | 0.44 | 0.01 | <0.01 |
| Sum PAHs (ng/m$^3$) | 3.36 | 2.22 | 5.17 | 4.76 | 0.18 | 0.19 |

for each cell examined. Also, it provided the mean and standard deviation for each parameter for all the cells examined.

## RESULTS

### PM10, EOM, and PAH concentrations

The results in Table1 shows the amounts of PM10 and its organic extract in the ambient air collected at Arafat and Muzdalifa during the 2004 and 2006 pilgrimage seasons. The levels of PM10 during 2004 and 2006 in Arafat were 156 and 160 µg/m$^3$ respectively and in Muzdalifa, 289 and 600 µg/m$^3$. The EOM in Aarafat in 2004 and 2006 were 45.6 and 47.2 µg/m$^3$, respectively and in Muzdalifa 83.2 and 220.0 µg/m$^3$. The reference site PM10 levels during 2004 and 2006 were 11.5 and 11.8 µg/m$^3$, respectively and the EOM levels were 6.0 and 6.2 µg/m$^3$ (Table 1). The PM10 and the EOM in the reference site did not vary when air sampler was operated using electricity or petroleum generator.

The big difference in PM10 values between Arafat and Muzdalifa could be in part due to the fact that in Arafat the vehicles stopped during the day time and most of the pilgrims stayed in tent camps, meanwhile in Muzdalifa there is no tents and vehicles move in and out of the area continuously during the night time because of over-crowding, the pilgrims sometimes do not arrive there until late at night.

Chemical analysis of the PM10 organic extract for 16 PAHs are given in Table 2. The total amounts of PAHs in Arafat during the 2004 and 2006 Hajj seasons were 3.36 and 2.22 ng/m$^3$ respectively. In Muzdalifa PAH concentrations were 5.17 and 4.76 ng/m$^3$ in 2004 and 2006 respectively, while the reference site was only 0.18 and 0.19 ng/m$^3$. The major PAHs component was

**Table 3.** Salmonella TA98 revertants (mean values± S.D.) induced in the presence of S9 by different concentrations of PM10 organic extracts collected in Arafat and Muzdalifa (Saudi Arabia) during 2004 and 2006 pilgrimage seasons .

| Site | PM10 organic extract (µg/plate) | Revertants/plate | | Revertants /$m^3$ | |
|---|---|---|---|---|---|
| | | 2004 | 2006 | 2004 | 2006 |
| Arafat | 50 | 72 ± 6 | 70± 5 | | |
| | 100 | 149 ± 10 | 144 ± 8 | | |
| | 250 | 222 ± 18 | 219 ±15 | | |
| | EOM/$m^3$ | | | 61 ± 4 | 66 ± 6 |
| Muzdalifa | 50 | 80 ± 4 | 71 ±5 | | |
| | 100 | 145 ± 8 | 150 ± 10 | | |
| | 250 | 239 ± 17 | 234 ± 15 | | |
| | EOM/$m^3$ | | | 121± 7 | 330 ± 10 |
| Reference site | EOM/$m^3$ | 20 ± 4 | 20 ± 3 | 22 ± 3 | 22 ± 3 |
| DMSO | 100µl | 20 ± 3 | 20 ± 2 | | |
| Spont. Revert. | | 18 ± 4 | 18 ± 3 | | |

benzo(ghi)perylene in both Arafat and Muzdalifa in both pilgrimage seasons. In 2004, the concentration of this compound in Arafat was 22% of the total PAHs and 63% in Muzdalifa. In 2006, the concentrations were 35 and 13% in Arafat and Muzdalifa respectively. In Arafat and in 2004, the second highest PAH compound was phenanthrene (15%) and in 2006 it was indeno(1232-cd)pyrene (16%).

In Muzdalifa, second to benzo(ghi)perylene was indeno(123-cd)pyrene in both 2004 (12%) and in 2006 (9%). The other PAHs detected in Arafat in 2004 were fluoranthene (0.39 ng/$m^3$), pyrene (0.339 ng/$m^3$) and BaP (0.24 ng/$m^3$). In 2006 the concentrations of these compounds were less than 0.09 ng/$m^3$. In Muzdalifa and in 2004 season the concentrations of these compounds were 0.19, 0.19 and 0.15 ng/$m^3$ and in 2006 season their concentrations were ≤0.05, ≤0.05 and 0.25 ng/$m^3$ respectively. The other PAHs were found in <0.10 ng/$m^3$ (Table 2).

**Mutagenicity in the Salmonella test (Ames)**

Bacterial revertants of the salmonella TA98 were expressed as the average number per plate from the triplicate. The spontaneous reversion for TA98 strain was at a frequency that was characteristic of the strain (Maron and Ames, 1983). Table 3 summarizes the mutagenicity results obtained from the EOM collected in Arafat and Muzdalifa. The number of revertants/plate increased with increasing dose at each location during each monitoring year. For example, at Arafat in 2004 the mean numbers of revertants/plate were 72, 149, and 222 at 50, 100, and 250 µg doses.

A similar relationship was seen at Muzdalifa and in other years, indicating a dose-dependent increase in mutagenicity with increasing EOM concentrations. Organic extracts from Arafat and Muzdalifa gave comparable number of revertants at the similar doses which could be due to the similar nature of the constituents of the organic extracts that induced mutation. However, when the mutagenicity was expressed as the sum of net revertants at one cubic meter equivalent concentration of sampled air organic extract, the obtained mean revertants for Muzdalifa was two folds more than Arafat in 2004 and five folds in 2006. In comparison with the spontaneous and reference site the obtained revertants was much lower (Table 3). Chemicals in the organic extract induced mutation only in the presence of S9. Organic extracts from Arafat and Muzdalifa did not show any mutagenicity with Salmonella TA100 strain even in the presence of S9.

**DNA damage (comet assay)**

Similar to the results for the Ames test, these results indicate a dose-response relationship with comet and EOM, as measured by higher percentages of cells with comet and large comet tail moments with increasing EOM. For example 100 and 250 µg/ml caused DNA breaks and induced comet formation in 18 - 22% and in 90-95% of the leukocytes respectively in both pilgrimage seasons compare to the reference site which was only 1-2%. Arafat and Muzdalifa EOMs have profound measurable DNA damaging effects, comet with a tail moment ranged from 2.2 - 4.9, formed with 100 µg /ml and at a dose of 250 µg/ml, the comet tail moment

**Table 4.** Comet rate and comet tail moment induced in human Peripheral blood leukocytes by PM10 organic extracts from three monitoring sites in Saudi Arabia.

| Location | PM10 organic extract (µg/ml blood) | Cells with comet (%) | | Mean tail moment | |
|---|---|---|---|---|---|
| | | 2004 | 2006 | 2004 | 2006 |
| Arafat | 50 | 0 | 0 | 0.01 | 0.01 |
| | 100 | 18±2 | 22±2 | 2.2±1 | 2.5±1 |
| | 250 | 90±4 | 92±5 | 14.5± | 16.2±5 |
| | 47 amount/$m^3$ | | | 0.01 | |
| Muzdalifa | 50 | 0 | 0 | 0.01 | 0.01 |
| | 100 | 18±3 | 22±2 | 2.5±1 | 4.9 ±2 |
| | 250 | 92±4 | 95± 4 | 15.0±2 | 15.4 ±5 |
| | 220 amount/$m^3$ | | | 12.2±2 | |
| Reference site | 250 | 1-2 | 1-2 | 0-0.02 | 0- 0.02 |
| DMSO | 1% | 0.0 | 0.0 | 0.0 | 0.0 |
| Benzo(a)pyrene | 250 (10 µM) | > 95 | > 95 | 29.17 ±3 | 27.9 ±9.6 |

ranged from 14.5-15.5 in both 2004 and 2006 seasons. The comet tail moment in cells exposed to the reference site EOM (250 µg/ml) was 0.0-0.02. The positive control BaP (250 µg/ml of blood) induced comet with tail moment of 29.17. If the EOM concentration per cubic meter of air was taken into account, the comet tail moment induced in Arafat in 2004 was only 0.01, meanwhile in Muzdalifa it was 12.2 (Table 4). Adding S9 to the reaction has no influence on the number of cells with comet or on the comet tail moment.

Figure 2 shows selected photographs for comets formed when blood cells exposed to different concentrations of the EOM. The comet tail moment and the percentage of DNA in the comet tail increased with increasing the EOM does. Figure 2A exhibits cells that are exposed to 1% DMSO (control), which was the solvent for the PM10, cells did not show any DNA degradation, no comet formation and the tail moment was 0.0. Figure 2B shows cells exposed to 100 µg/ml, the comet tail moment and the % of DNA in the tail were 4.9 and 15.58 respectively. Figure 2C shows cells exposed to 250µg/ml, the comet tail moment was 16.22 and the DNA% in the tail was 27.53. Figure 2D shows cells treated with the carcinogen BaP (10 µM), cells exhibited extensive DNA damage forming huge comets with a tail moment of 29.17 and DNA in the tail reached up to 34.93% of the total nuclear DNA.

## DISCUSSION

Epidemiological studies suggest consistently that human are exposed to pollutant mixtures in the ambient air that contain known or suspected carcinogens, such as PAHs, benzene, heterocyclic amine, various aromatic nitroso compounds and many others.

These pollutants are mainly due to the incomplete combustion of fuels (Sasaki et al., 2000). Our investigation has shown that the PM10 concentrations measured in Arafat and Muzdalifa, ranged from 156 to 600 µg/m3, much higher than the concentrations monitored at the reference site (33 µg/$m^3$). Levels at all sites surpassed the world health organization (WHO) 2005 annual limit value of 20 µg/$m^3$ (Krzyzanowski, 2008), and the two pilgrimage sites exceeded the daily WHO limit of 50 µg/$m^3$ and higher than the mean PM10 levels in 20 USA cities and metropolitan areas (Samet et al., 2000). A study in Kuwait at two urban areas and one in a remote desert location showed mean PM10 annual concentrations ranging from 66-93 µg/$m^3$ across the three sites (Brown et al., 2008).

In Spain, PM10 levels reported during episodes were 22.0 µg/$m^3$ at the rural area and 49.5 µg/$m^3$ at the urban site (Rodriguez et al., 2004). In the Metropolitan area of Athens, Greece the daily PM10 concentrations ranged between 32.3 and 60.9 µg/$m^3$ during four year of studies (Grivas et al., 2008). In Kolkata, India, the 24 h average concentrations of PM10 was found in the range 68.2-280.6 µg/$m^3$ for residential area and 62.4-401.2 µg/$m^3$ for industrial area (Karar et al.,2006). The total PAH concentration (ng/$m^3$) in the organic extracts ranged between 2.22-3.36 in Arafat, 4.76-5.17 in Muzdalifa and in the reference site it was 0.18. Comparison of these concentrations with the concentrations in industrial (2.8), urban (5.5) and rural (2.0) area in Flanders, Belgium shows that Muzdalifa has concentration similar to the urban site which was the highest in Flanders.

The major contributor to the PAH in our study was

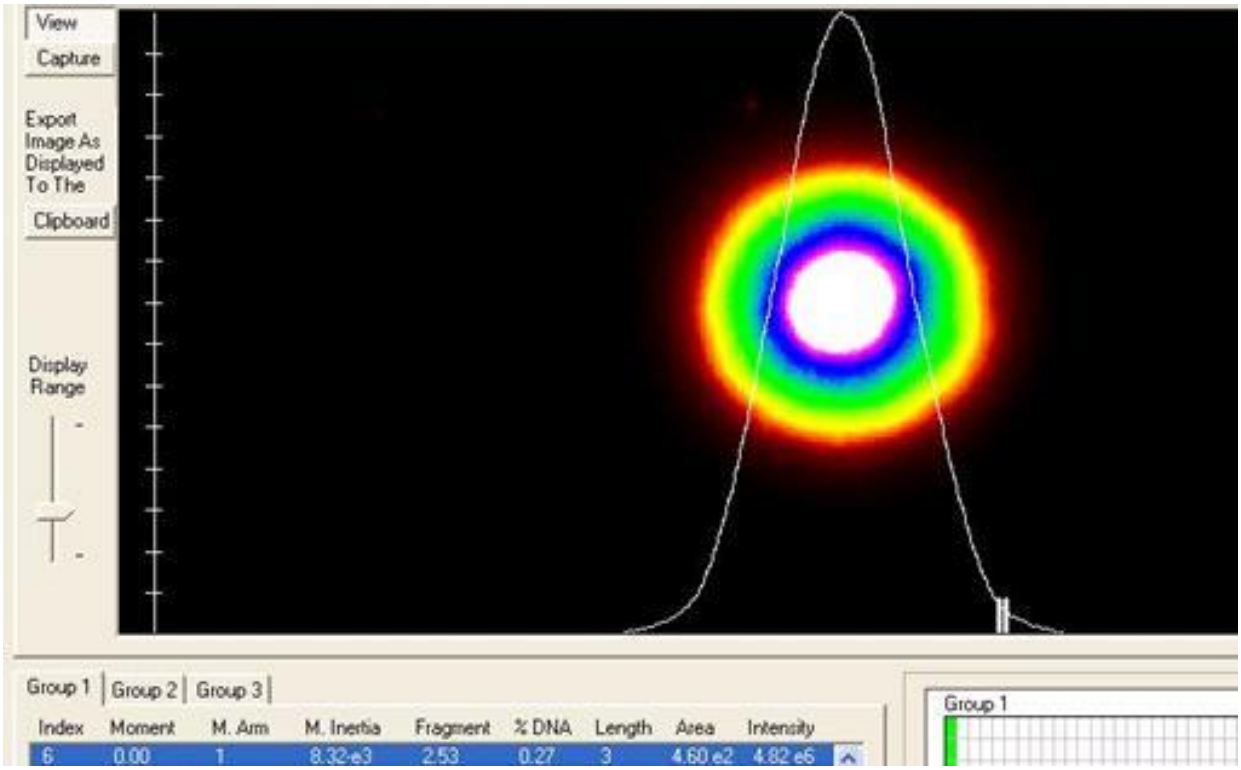


**Figure 2A.** Representative photographs of peripheral blood leukocytes exposed to 1% DMSO (control) and analyzed by comet system. White vertical lines on the x-axes represent the beginning and the end of the comet tail.

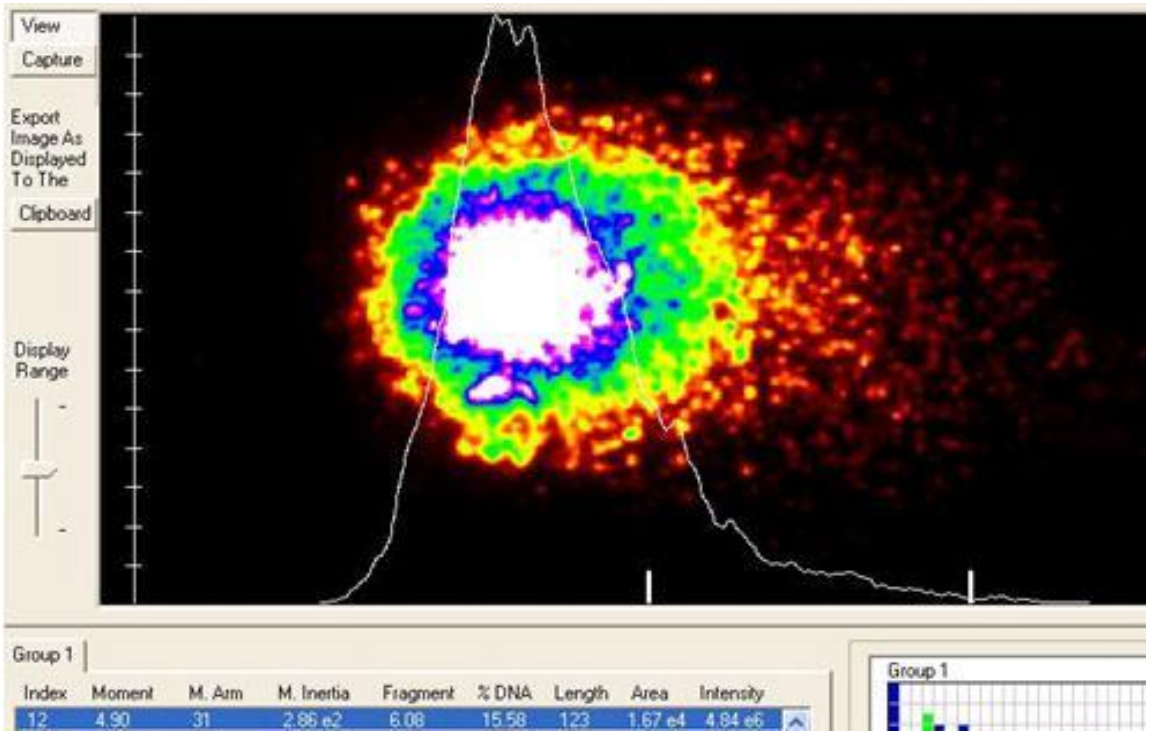


**Figure 2B.** Figure 2 B. Representative photographs of peripheral blood leukocytes exposed to 100 µg EOM and analyzed by comet system. White vertical lines on the x-axes represent the beginning and the end of the comet tail.

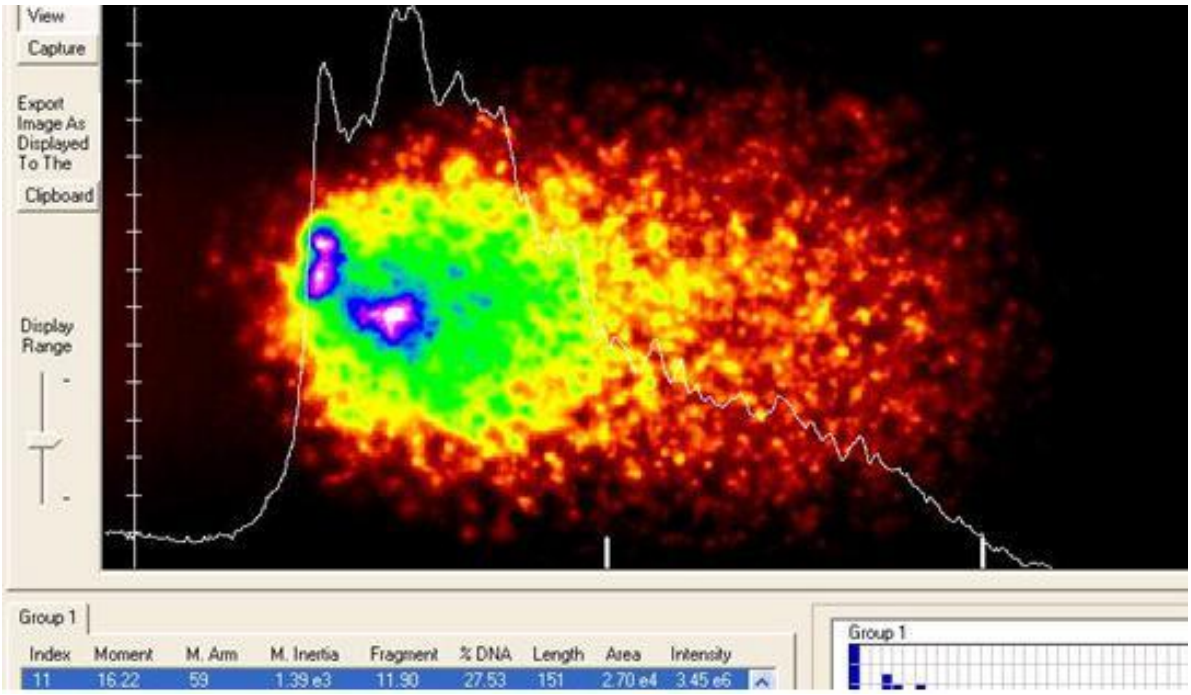


**Figure 2C.** Representative photographs of peripheral blood leukocytes exposed to 250 µg EOM and analyzed by comet system.White vertical lines on the x-axes represent the beginning and the end of the comet tail.

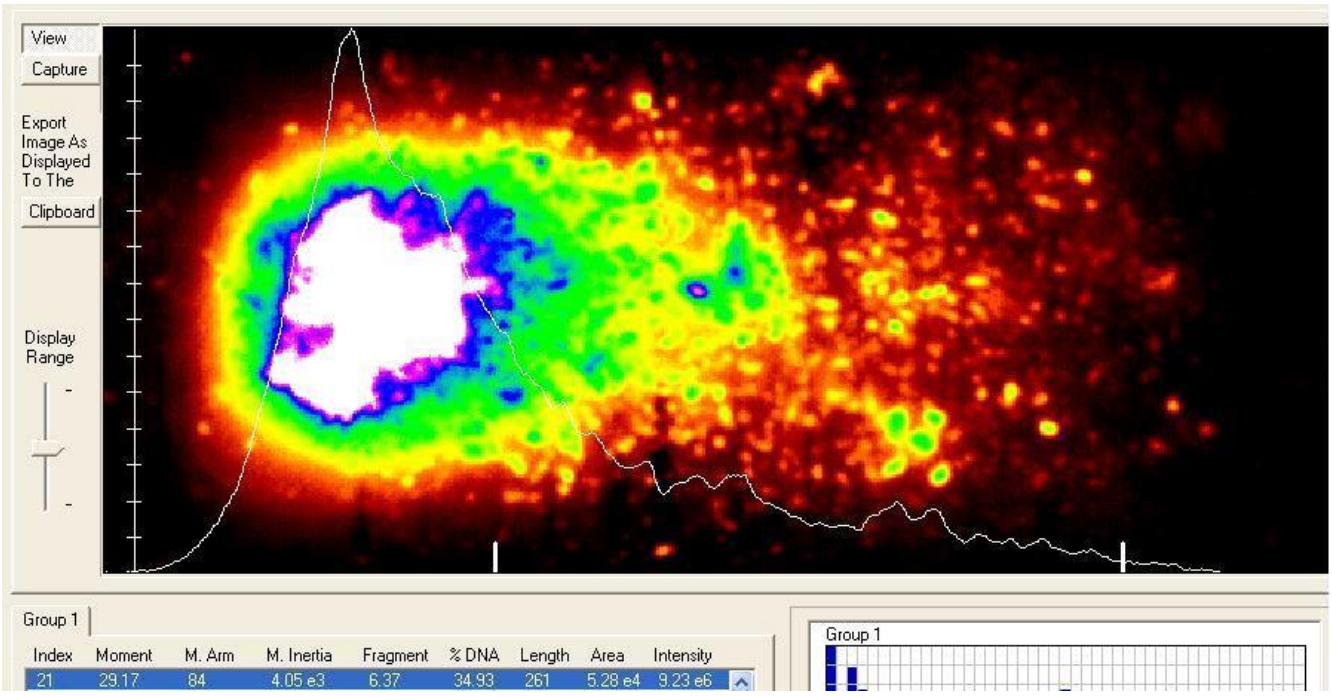


**Figure 2D.** Representative photographs of peripheral blood leukocytes exposed to BaP (10 µM) and analyzed by comet system.White vertical lines on the x-axes represent the beginning and the end of the comet tail.

Benzo(ghi)perylene meanwhile in Flanders it was acenaphtene, possibly due to the difference source of pollutants (Brits et al., 2004). PM10 concentrations and EOM content/$m^3$ of air in Muzdalifa was approximately

2- fold higher than Arafat in 2004 and 4-5 fold higher in 2006. PAHs total concentrations were positively associated with PM10 and EOM levels. This difference in the concentration of PM10 and its composition depends on the traffic intensity, characteristics of pollutants, meteorological condition such as temperature, humidity and wind which vary across different geographic regions and can be influential in the distribution of PM10 (Dominici et al., 2003). The mutagenic and genotoxic potential of the organic extract has been already demonstrated with several short-term tests on prokaryotic and eukaryotic cells (Gabelova et al., 2004). In the present study mutagenicity results in Salmonella have indicated that EOM exhibited a positive response with TA98 but not with TA100 in the presence of S9 only.

This suggested that the mutagens in our samples can induce frame shift but not base substitution mutations. Also, the study suggests that chemicals in the EOM may contain less direct and more indirect-mutagenic substances which need to be activated to the real mutagen by the S9 enzymes. The number of reverse mutation was increased in a concentration-dependent manner and was positively associated with PM10 and EOM levels. Another studies reported that gasoline engine exhibited mutagenicity only in strain TA98, but not in TA100 and in the absence and presence of S9 (Zhang et al., 2007).

Others indicated that PM exhibited mutagenicity in strains TA98 and TA100 (Seagrave et al., 2002). Organic extracts from Arafat and Muzdalifa gave comparable number of revertants at the similar doses which could be due to the similar nature of the constituents of the organic extracts that induced mutation. PM10 organic extracts from Arafat and Muzdalifa were able to induce comet formation in a dose related response without S9 metabolic activation system. Adding S9 to the tests did not exhibit any effect on comet formation or on comet tail moment. Our data are in agreement with those of others who did not find any significant differences in EOM genotoxicity in absence of S9, suggesting that some of the EOM constituents could be a direct mutagen or that the pro-mutagen in the EOM could be activated by blood cells monocytes and macrophage enzymes which shown to express constitutively some cytochrome P-450 enzymes (Baron et al., 1998). Adding S9 to the blood in our study did not enhance the DNA breakage which may be due to the saturation of metabolic pathways needed to activate pro-mutagenic agents by the monocytes enzymes.

In conclusion, this study confirmed the genotoxic properties of organic complex mixtures associated with respirable airborne particles collected in Arafat and Muzdalifa, Saudi Arabia. Air particulate extracts from the two regions showed genotoxic effects in both the Ames test and the comet assay. Although carcinogenic PAHs were present in air samples they could not account for all the genotoxic activity detected. The mutagenicity of airborne particulate is due to at least 500 identified compounds from varying chemical classes. Utilization of Salmonella and comet tests provide us with precise information about the genotoxic potential of environmental chemicals and they can play a central role in continuous biomonitoring of mutagens in the environment to assist in developing a prevention programs to minimize human exposure.

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