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Genotoxicity in child populations exposed to Polycyclic Aromatic Hydrocarbons (PAHs) in the air from Tabasco, Mexico

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Abstract: The economy of the state of Tabasco is based on oil extraction. However, this imposes major effects to the environment and communities. Examples are the Polycyclic Aromatic Hydrocarbons (PAHs) that may be found in the soil, water and sediment of the region. Their volatility makes them available to living beings and results in genotoxic activity. The purpose of this study was to quantify the levels of PAHs in the air at several points in the state, and to analyze their relationship with possible damage to DNA on local inhabitants. Single Cell Gel Electrophoresis Assay (Comet Assay) was applied to peripheral blood lymphocytes of five groups of children between six and 15 years of age. PAH samples were analyzed following US/EPA TO-13-A method. Results indicated the presence in the air of most of the 16 PAHs considered as high priority by EPA, some of which have been reported with carcinogenic activity. Differences ($p<0.05$) were found between PAHs concentration in the gaseous component and in the particulate component of air samples, with the greatest values for the gaseous component. Greatest PAH concentrations were detected in areas with high oil extraction activities. Children groups from high oil activity areas presented genotoxic damage labeled from moderate to high according to DNA migration from nuclei (Tail Length: 14.2 - 42.14 µm and Tail/Head: 0.97 - 2.83 µm) compared with control group (12.25 and 0.63 µm, respectively). The group with greatest cell damage was located in the area with the greatest oil activity. We conclude that the presence of PAHs in the air may represent a health risk to populations that are chronically exposed to them at high oil activity regions.

Keywords: Genotoxicity, polycyclic hydrocarbons, comet assay, single cell gel electrophoresis, PAHs.

Introduction

Oil extraction in southeastern Mexico constitutes 85% of national production. This activity participates in the economic development of the region and country, as well as to environmental impact. Several studies have proved that notable quantities of oil residues and subproducts have been poured into a variety of local ecosystems, affecting biodiversity [4, 21]. It is known that these substances present a risk to human health [9, 20, 23]. Volatile organic compounds have received special attention in recent years, particularly Polycyclic Aromatic Hydrocarbons (PAHs), as these are potential carcinogenic agents [28, 30].

PAHs include compounds that are found naturally in crude oil, creosote, carbon and tar. PAHs, as many as 100, are also formed during the incomplete combustion of carbon, oils, gas, tobacco, gasoline, diesel, and wood. They are produced during highway paving process [1], as well as during burning of forests, agricultural lands and

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waste [2, 14, 18, 24]. The PAHs in the air generated by the combustion are absorbed on particulate matter and liberated to the atmosphere. Some hydrocarbons exposed to the solar light could suffer significative degradations. For example, they could form oxidized derivatives in presence of ozone or form Nitro groups; depending on the chemical composition and the aerosol´s PAHs. The Hydrocarbons route in the organisms forms intermediary groups diols or epoxides, when being united in covalent form to the DNA, which is considerate a way to develop cancer, at the moment of gathering in covalent form to the DNA [5, 14, 29, 13].

The Environmental Protection Agency of the United States of America has recorded 16 PAHs as priority pollutants [8]. This classification considers the affectation level to health and the degree of carcinogenic and mutagenic potential. These compounds include naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo(a)anthracene, benzo(b) fluoranthene, benzo(k) fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(ghi)perylene and dibenzo(ah)anthracene [2, 10, 31].

Epidemiological studies have proved that air pollution originated by incomplete combustion of gasoline; diesel and other fossil fuels may be responsible for an increase in different types of cancer. It has also been proved that air particle extracts containing organic carbon cause alterations in different biological models [14, 22], and that polyaromatic fraction is the main provider of mutagenic effects. It is well documented that PAHs form adducts with several proteins and nucleic acids [13, 24]. For example, benzo(a)pyrene and its metabolites induce genetic damage in germinal and somatic cells, expressing itself as chromosomal aberrations, mutations, adducts formation in DNA, and sister chromatides exchange and micronuclei formation [11, 15, 16, 23]. It is for this reason that it has been employed as a positive control to demonstrate genotoxic sensibility in diverse biomarker tests of early damage. Thus, this compound serves as a reference indicator to predict genotoxicity/carcinogenicity [19, 20, 27].

Evaluations of oil extraction activities impact have taken place for the last 20 years in the state of Tabasco, Mexico. Several studies have reported important PAHs concentrations in the soil, sediment and water of the region [6, 28]. However, there are no available records of the presence of these compounds in the air and/or in air-borne particles. There is also no information on the impact of these compounds on the health of the human population in the region. Thus, the purpose of this study was to evaluate the genotoxic effects in a child population exposed to PAHs in the air, in several sites of the state of Tabasco with different oil activities; there were considerated sampling groups under 15 years**,** for being more susceptible to the changes in the DNA; and because the children are not smokers, the nicotine derivates form adducts with the PAHs and they can cause an indirect damage in the processes of detoxification and repair of the molecular damage.

This study represents one of the first reports of PAH levels in the air in this region, and the first to present evidence of genotoxic damage in human populations of the area. These results will serve as a reference to establish mechanisms to control processes in oil industry, transforming them more efficient, in order to minimize the flow of hydrocarbons to the environment.

Materials and Methods

This study was carried out in the state of Tabasco, Mexico, in the southern Gulf of Mexico. Five sampling points were established in sites with different types of agricultural, urban and oil activities. The following 16 compounds were detected in air samples: acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k) fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(ghi)perylene and dibenzo(ah)anthracene and naphtalene. These compounds are given priority in both the gaseous and particulate components [8]. The total amount of PAHs in each site was estimated as the concentration sum per site of the 16 recorded PAHs over the whole sampling period (November to January). Once the concentration per site was determined, the total concentrations of PAHs were reorganized for two different categories: one group formed with data from sites in the western region of the state where oil activities are intense, and another group formed with data from sites in the eastern region where there is practically no oil extraction activity. EPA/TO-13-A method was used for sampling, detection and quantifying PAHs in the air samples. EMSL/RTP-SOP-MDAD-015 method (Standard operation for the ultrasonic extraction and analysis of residual benzo(a)pyrene from Hi-Vol filters via thin-layer chromatography [8] was used for the extraction of the PAHs from sample filters. Air Samples were taken every 24 h (n = 22), consecutive days with a modified Grasseby High Volume Sampler (Grasseby Inc.) that operated within a calibrated flow range of 0.114 to 0.285 $m^3 min^{-1}$. Each air sample contained a gaseous and a particulate component. Particulate component of the sample was trapped in quartz fiber filters (QFF) and the gaseous component was trapped in 3 inches polyurethane filters (PUF) with a density of 0.020 g m^{-3}. An additional 15% of the filters were blanks. PUF samples were extracted with a hexane-ether mixture (9:1) and QFF samples were extracted with dichloromethane, both in an ultrasonic bath (Branson 3510). The extracted samples were concentrated and then fractionated in columns packed with alumina, silica-gel and anhydrous sodium sulphate. A fraction containing PAHs was obtained by washing columns with 9:1 and 1:1 mixtures of hexane-dichloromethane. Samples were analyzed with gas chromatography in a Hewlett-Packard 5890 system with a flame ionization detector (GC/FID) and a 5% phenyl-methyl-siloxane capillary column (25m x 0.32 millimeters D.I. x 0.52 µm film thickness, Hewlett-Packard 19091B-112). Helium was used as carrier gas with a flow of 2.5 ml

s^{-1}. Temperature ramps were programmed for the column. The volume of the samples that were injected was one microliter [7]. PAHs concentrations that were detected were referred to the volume of the air collected in the sample and corrected considering standard pressure and temperature.

Alkaline modified Single Cell Gel Electrophoresis Assay (Comet Assay) [26] was applied to peripheral blood lymphocytes obtained from four children groups, with ages between six and 15 years old, who lived in regions with different degrees of oil extraction activity, in order to evaluate the possible DNA damage in the inhabitants of the studied regions. For comparative purposes, another group (control group) was formed with 15 children of similar age and conditions, but living in a municipality with no oil activity at all. Lymphocytes were immersed in agarose gel and washed in a cellular lisis solution with detergents and high salt concentrations. Liberated DNA was subjected to electrophoresis under controlled conditions. DNA damage was evaluated as DNA migration; cells with increased frequencies of double chain breakings manifested a greater nuclear DNA migration towards the anode, which was observed under fluorescent microscopy and quantified in microns (Tail Length), as well as in nucleus length units (Tail/Head) in 50 cells per individual. Damaged cells looked like a comet with a markedly fluorescent head, and a tail with a length and fluorescence intensity related to the number of breakings induced by the genotoxic agents [26].

Results and Discussion

In order to have an idea of PAHs distribution in the gaseous and particulate component of the atmosphere of the region, we first presented the sum of all the PAHs lectures obtained during the study period for each air component (Table 1). Total concentration of PAHs in the gaseous component resulted to be more than twice that in particulate component, however this difference turned to be not statistically significant ($p>0.05$).

Table 1: Distribution of total PAHs concentration (ng m^{-3}) in gaseous and particulate components for the total collecting sites. Values at each collecting site were estimated as the sum of 16 PAHs concentrations analyzed throughout the sampling period [n = 22].

PAHs	*Average (s.e.)*	*Median*	*Min value*	*Max value*
Gaseous component	210.16 *(44.6)*	105.68	29.98	915.11
Particulate component	92.24 *(16.8)*	63.27	11.46	347.21
Total [*n* = 22]	302.40 *(50.1)*	242.51	65.30	972.95

Numbers in italic parenthesis represent standard error (s.e.) of data

This is important for human health as these compounds in the gaseous phase, may be transported deeper in the respiratory tract, thus reaching blood/gas diffusion region at alveoli [17]. Distribution of two phases of PAHs in the air is determined by vapor pressure of the compounds, as well as by the atmospheric pressure and temperature. Vapor pressure for organic volatile compounds lies between 10^{-2} and 10^{-9} torr. This and field conditions predominant in this study (day temperature above 40° C) may explain why most PAHs compounds were present in a gaseous phase. PAHs with higher molecular weights remain in the liquid state as a result of their lower evaporation pressures, mostly associated to particles.

Table 2:Distribution of total PAHs concentration (ng m^{-3}) in the gaseous and particulate components of two regions in the state of Tabasco with different oil activities.

	Region with oil extraction activity [n = 12]		*Region without oil extraction activity [n = 10]*		
	Average (S.E.)	*Median (10, 90)*	*Average (S.E.)*	*Median (10, 90)*	*p*
Gaseous component	240.77 *(73.69)*	156.75 (43.8, 469.9)	173.42 *(44.2)*	96.05 (67.2, 392.2)	0.97369
Particulate component	124.72 *(25.22)*	111.83 (48.2, 218.4)	53.26 *(14.3)*	42.62 (12.4, 127.7)	0.03211**
Total	365.5 *(82.7)*	253.55 (98.9, 817.1)	226.69 *(41.5)*	195.37 (92.9, 417.1)	0.22252

Number of samples [n =] are under each region Head. Numbers in italic parenthesis represent standard error *(s.e.)* of data. Numbers in normal parenthesis represent 10 and 90 percentile values. ** ($P<0.05$) for Wilcoxon or Mann-Whitney U test.

In order to investigate the difference in PAHs composition of the air in the regions with different oil extraction activities, sampling sites were distributed in two categories according to oil extraction activities. Table 2 presents the distribution of total PAH concentrations (ng m^{-3}) for gaseous and particulate components of two regions in the state, with and without oil activities. "n" value represents the number of samples collected in each region, and "p" value the probability. Included are the numbers in parenthesis for the standard error (s.e.) of the averages and percentiles 10 and 90 for the medians.

PAHs concentrations recorded for areas with oil activity were greater than those in areas without oil activity, as is shown by the average values of the sampling points in each sub region. In spite of this, it is only the PAH concentration associated with the particulate component in the region without oil activity, that presents significant difference with respect to the region with oil activity ($p<0.05$). PAHs concentration in the gaseous

component was also lower in the region without oil activity, but was not statistically different ($p<0.05$) from the region with oil extraction activity.

High variability is a characteristic of meteorological parameters of these coastal plains, particularly with respect to wind speed and direction, which results in marked variations on pollutants concentration in the air that makes it difficult to establish statistical differences. Table 3 presents a comparison between the concentrations of some individual PAHs detected in the two studied regions. The listed PAHs are important to public health as several institutions have associated them with genotoxic, mutagenic or carcinogenic activities [1, 8, 12]. Their effect on human health depends on the dose, type and route of exposure, as well as on the particular characteristics of the exposed individuals. [30].

Our study shows consistently higher values for all the PAHs in the air from the region with high oil production activity. However, not all differences were significant ($p\geq0.05$). An analysis of these compounds grouped together, presented a significantly greater value for the region with oil extraction activities ($p\leq0.01$), together with the corresponding risk to the health of the population that lives in this area.

Table 3: Health risk PAHs and Pyrogenic PAHs Concentrations (ng m^{-3}) in regions with and without oil extraction activity. Number of samples [n =] are under each region Head.

PAHs with health risk	*Region with oil extraction activity[n = 12]*		*Region without oil extraction activity[n = 10]*		*p*
	Average(s.d.)	*Median**	*Average (s.d.)*	*Median**	
Benzo(a)anthracene†	39.79 *(6.54)*	39.71 (15.96, 66.6)	21.88 *(4.08)*	23.31 (5.01,39.91)	0.0697
Chrysene†	58.81 *(12.75)*	55.0 (11.94, 85.66)	41.79 *(20.1)*	20.57 (3.35, 141.24)	0.0927
Benzo(b)fluoranten††	36.57 *(12.66)*	13.22 (1.20, 88.68)	18.06 *(5.68)*	14.12 (1.07, 43.67)	0.817
Benzo(K)fluoranten††	47.29 *(10.65)*	43.10 (14.38, 89.59)	17.36 *(3.79)*	16.22 (4.29, 35.20)	0.016**
Benzo(a)pyrene†	23.43 *(4.46)*	17.79 (8.82, 43.73)	22.63 *(5.02)*	16.09 (6.97, 46.15)	0.921
Indeno(1,2,3-c,d)pyrene††	56.68 *(22.93)*	28.02 (5.33, 75.32)	41.24 *(7.28)*	36.99 (12.06, 73.95)	0.620
Dibenzo(a,h)anthracene	64.19 *(42.09)*	23.79 (9.98, 40.75)	26.75 *(13.93)*	13.96 (1.04, 92.74)	0.156
Total	46.68 *(7.42)*	31.56 (7.35, 85.67)	27.10 *(3.89)*	17.08 (4.11, 55.17)	0.005**
PAHs with pyrogenic origin					
Phenanthrene(†),††	4.41 (2.07)	1.87 (0.43, 11.32)	5.67 (1.94)	4.51 (0.76, 15.04)	0.176
Fluoranthene†	11.43 (3.19)	7.47 (2.09, 21.52)	8.81 (2.64)	5.08 (3.22, 23.87)	0.488
Pyrene†	6.94 (2.16)	4.98 (2.12, 8.74)	4.79 (1.00)	3.98 (1.57, 10.18)	0.575

Numbers in italic parenthesis represent standard deviation *(s.d.)* of data. Numbers in normal parenthesis represent 10 and 90 percentile values. ** ($P<0.05$) for Wilcoxon or Mann-Whitney U test. † PAHs for hydrocarbon pyrogenic origin. †† wood pyrogenic origin.

PAHs detected most frequently in the air samples were those of the families of polynuclear compounds with three to six rings such as phenanthrene, benzo(a)anthracene, chrysene, benzo(a)pyrene and benzo(b)fluoranthene. These compounds were associated mainly with the gaseous phase of the samples. Lower proportions of high molecular weight PAHs associated with the particulate component of the samples were also detected. Phenanthrene is generally associated with incomplete combustion of fossil fuels [3]. It has been reported that fluoranthene and pyrene may be used as thumb prints for the incomplete combustion of hydrocarbons, and that benzo(a)anthracene, chrysene and benzo(a)pyrene may also be associated at lower concentrations [4]. Those PAHs were present in the air samples of the oil producing region in our study.

Barale and collaborators [2] reported that phenanthrene, indeno(1,2,3-c,d) pyrene, benzo(b) fluoranthene and benzo(k)fluoranthene are PAHs characteristically produced by the burning of wood. These compounds were also detected in the air samples collected in this study from the atmosphere of Tabasco. It is assumed that a fraction of the PAHs collected comes from agricultural burnings that is a frequent agricultural practice in the region.

Genotoxic Damage

DNA damage was evaluated as DNA migration from the nucleus. Individuals from the four analyzed sites presented genotoxic damage labeled from moderate to high (Tail Length: 14.21 to 42.14 µm and Tail/Head: 0.97 to 2.83 µm), compared with the control group (12.25 and 0.63 µm). Tail/head ratios less than 1, where classified as low level DNA damage. Tail/head ratios between 1 and 2 where labeled as medium DNA damage. Tail/head ratios greater than 2, were considered as high DNA damage. Classifying cells in these categories, we could establish the fraction of cells of a subject that are included in each level of DNA damage. After that, proportions of cells in each category were added for all individuals in a sampling site, and the averages of cell fraction in each damage category were graphed for each sampling site (Fig 1).

Thus, the site with greatest genotoxic damage is the one with a greater proportion of individuals with damaged cells than the other groups. As may be seen in figure 1, there is a different level of genotoxic damage in the groups that live in the regions with oil activities. Sites named VH, GM, SM and TL are located in regions with oil extraction activities in increasing order, being VH the lower and TL the higher. It is evident from figure 1, that proportion of cells for the TL site (in red), that are in the high DNA damage category is the highest of all sites. It is also evident from the same figure, that proportion of cells for the UQ site (control group, in green), that are in the high DNA damage category is the lowest of all sites, and most of the cell are located in the low DNA damage category.

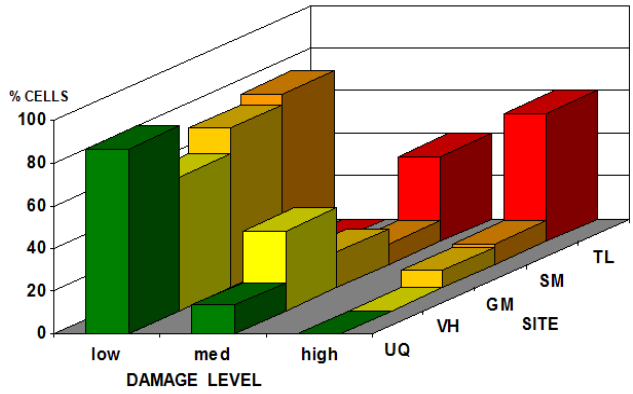

Figure 1: Genotoxic damage to the DNA of the studied populations. Vertical axe represent the fraction of cells per subject with genotoxic damage. Horizontal axe represent 3 levels of genotoxic damage according to values of the ratio Tail/Head. Z axe represent the site of study.

These data shows a relationship between oil extraction activity and genotoxic damage on their inhabitants, suggesting there is a potential risk to the health of these population groups.

Conclusions

This study constitutes one of the few available reports on PAHs in the atmosphere of Tabasco, Mexico, and the first to relate the recorded levels of PAHs with genotoxic activity in the populations of the region. The high variability of the PAHs concentrations found here is associated with the instability of the meteorological parameters. PAHs concentrations varied day to day, indicating diverse origins of the air masses and the contribution of a variety of emission sources.

Low molecular weight PAHs were detected mainly in the gaseous component of the air samples as a result of their physicochemical characteristics (high vapor pressure), whereas the high molecular weight PAHs correspond to low vapor pressures, and were detected mostly in the particulate component of the samples.

The air in the atmosphere of Tabasco contains most of the 16 PAHs that EPA considers as priority pollutants because of their risk to human health. The incomplete combustion of hydrocarbons and incomplete burning of wood seem to be the main sources of PAHs found in the air of the region. Total PAHs concentration recorded was greater in the region with oil extraction activities, in comparison with the regions without these activities.

No limits have been established in Mexico for PAHs concentrations in the air. However, this study shows that the health of the people that live in regions with high oil extraction activities might be at risk, derived from chronically exposure to environmental doses of PAHs. The results obtained from the DNA analyses show different degrees of genotoxic damage in the study groups,

with greater damage in the groups that live in regions with high oil activity, suggesting there is a potential risk to the health of these population groups.

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