DNA-Adducts in Subjects Exposed to Urban Air Pollution by Benzene and Polycyclic Aromatic Hydrocarbons (PAHs) in Cotonou, Benin

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Received 6 April 2009; revised 19 July 2009; accepted 21 July 2009

ABSTRACT: Air pollution effect on humans represents a major public health problem. Exposure to genotoxic compounds in the ambient air is evaluated using different biomarkers. In the present study we assessed DNA-adducts levels in apparently healthy people living and working in the city of Cotonou (Benin) in which exposure to air pollutants such as benzene and polycyclic aromatic hydrocarbons (PAHs) mainly benzo(a)pyrene has been evidenced. Rural inhabitants were enrolled as control group. Taxi-motorbike drivers, street food vendors, and gasoline salesmen were recruited in Cotonou whereas suburban residents were recruited in Godomey, 12 km from Cotonou. We found that taxi-motorbike drivers, roadside residents, street vendors, taxi-motor-bike drivers and gasoline sellers had significantly higher levels of DNA-adducts than suburban and village inhabitants ($P < 0.001$; *post hoc*, LSD). Means values were 24.6 ± 6.4, 23.78 ± 6.9, 34.7 ± 9.8, and 37.2 ± 8.1 in the exposed groups *versus* 2.1 ± 0.6 and 3.1 ± 0.8 adducts/10^8 nucleotides, in the two control groups, respectively. We did not find any significant difference within the high exposure groups and inside low exposure subgroups (namely suburban residents and villagers) because the mean individual exposure values to both PAHs and benzene were similar among subjects exposed in the city of Cotonou and those in suburban and village areas. However, there is significant interindividual variations in adducts levels that may reflect variation of genetic susceptibility factors.

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Contract grant sponsors: Denmark Republic (DANIDA Institution), Benin Republic (Ministère de l'environnement, de l'Habitat et de l'Urbanisme, MEHU), and France Republic (IBMC Strasbourg)

Published online 15 December 2009 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/tox.20533

Ranges of adduct level/10^8 nucleotides were: 1–69, 1–76, 3–169, 4–124, 0–9, 0–8 adducts/10^8 for taxi-motorbike drivers, roadside residents, street vendors, gasoline sellers, suburban and village inhabitants, respectively. Our study demonstrated a clear-cut elevated level of DNA adducts in city residents than in none exposed people (or very low exposure levels people) and designate these city residents groups as people at risks for the chronic diseases possibly caused by benzene and PAHs.
Environ Toxicol 26: 93–102, 2011.

Keywords: air pollution; PAHs; benzene; exposed and none exposed subjects; DNA-adducts; ^{32}P postlabelling

INTRODUCTION

Epidemiologic studies published over the past years reported that exposure to ambient air pollution may be associated with increased risk of developing several chronic diseases including cancer in the general population. Human exposure occurs through inhalation of environmental cancer-causing pollutants such as, fossil fuel incomplete combustion products, benzene and PAHs.

Due to an absence of reliable public transportation, more than 80,000 motorbikes circulate in Cotonou (Ayi-Fanou et al., 2006). In the year 2000, the Ministry of Environment, House and Town Planning, estimated the number of all types of vehicles to be about 250,000 in the city of Cotonou, most of them were very often in bad mechanical state.

It has been demonstrated recently that ambient air in Cotonou, the economical capital of Benin, is heavily contaminated with various air pollutants (Ayi-Fanou et al., 2006). The main pollutants were benzene at concentrations of 76.0 ± 26.8 μg/m^3 in Cotonou, compared with 3.4 ± 3.0 μg/m^3 in villages located far from Cotonou. This benzene concentration in the air largely exceeded the 5 μg/m^3 recommended by World Health Organization (WHO IARC 1983) and polycyclic aromatic hydrocarbons (total PAHs) at concentrations of 103 ng/m^3 in Cotonou *versus* 1.6 ng/m^3 in villages. It is well known that prolonged exposure to benzene causes a serious damage to hematopoiesis resulting in aplastic anemia and sometimes in blood cancers (Rinsky et al., 1987).

PAHs are among the major carcinogenic agents which have been associated to an increased risk of respiratory and neoplastic diseases since 1983 (WHO, 1983). PAHs in general and benzene at a lesser extend are capable of forming DNA-adducts following metabolic activation. The capacity of DNA-adducts formation by benzene is evidenced by its covalent binding index of 10 (Arfellini et al., 1985). The DNA-adduct are predominantly formed with guanine or adenine (Nielsen et al., 1996a; Ayi-Fanou et al., 2006). In addition PAHs as well as volatile organic compounds (VOCs), such as benzene are capable of inducing oxidized bases in the DNA through reactive oxygen species production. These oxidized DNA bases are known to be highly mutagenic, (Collins, 1999).

Exposure to those genotoxic compounds is currently assessed using the DNA ATP-γ ^{32}P-postlabeling method of Randerath or personal monitoring of ambient air pollution (Keith and Dirheimer, 1995; Randerath et al., 1999; Ayi-Fanou et al., 2006; Avogbe et al., 2005), both methods qualified for risk assessment. A dose–response relationship between the amounts of bulky DNA-adducts and the levels of PAHs in both occupational conditions and in the general environment have been reported (Schoket et al., 1998). Moreover a study carried out in Copenhagen bus drivers, occupationally exposed to high ambient air pollution by PAHs, revealed significant DNA adducts levels in drivers working in central Copenhagen compared with those driving in the suburbs (Nielsen et al., 1996b).

The objective of the present study was to investigate the relationship between the levels of DNA-adducts as biomarkers of the exposure to PAHs using the ATP-γ ^{32}P postlabeling method in order to evaluate the risk for those exposed to these toxicants in the ambient air. In this purpose four target populations have been selected inside the city of Cotonou, namely taxi-motorbike drivers, roadside subjects, street vendors, gasoline salesmen who were compared with suburban residents and rural inhabitants.

Abbreviations

ABE	Benin Environmental Agency
[γ-^{32}P]ATP	adenosine triphosphate labelled on phosphate γ
B(a)P	benzo(a)pyrene
EDTA	ethylen diamin tetraacetic
DNA	Deoxy ribonucleic acid
GST	glutathion-*S*-transferase
1-(HOP)	1-hydroxypyrene (1-HOP)
LSD	least standard deviation
LsD	least significant difference
PAHs	polycyclic aromatic hydrocarbons
PEI	polyetyleneimine
SB DNA	strand break
TLC	thin layer chromatography
UFP	ultrafine particulate
VOCs	volatil organic compounds
WHO	World Health Organization

MATERIALS AND METHODS

Subjects Recruitment

The study group consisted of 94 healthy consenting volunteers. Except the village participants all others subjects were recruited in the city of Cotonou, e.g., of taxi-motorbike drivers who use to drive daily for at least 8 h in

Cotonou, subjects living near polluted crossroads, such as Marina called "roadside residents," street food vendors, and people selling gasoline for the whole day nearby crossroads with high traffic intensity according to the previously published data (Ayi-Fanou et al., 2006). Some suburb residents of Godomey have been selected as representing an intermediate exposure condition. Each participant completed a consent form and answered questionnaire that provides the following information, age, and sex, history of tobacco use, and health status. Participants were divided into putative two groups, based on the level of exposure following the data obtained by Ayi-Fanou et al. (2006) before the individual exposure evaluation. The low exposure group consisted of suburban subjects and the village residents and the other four were considered as high exposure group. The study was approved by the Benin Environmental Agency (ABE).

Samples Collection

Venous blood samples were collected in Vacutainer EDTA® tubes during a period of 1 week in 2003. For comparison purpose of DNA-adduct levels in the general population, we sampled subjects from the village of Sohon that lies about 80 km far from Cotonou, as a control group. Samples were transported to the laboratory on ice. Upon arrival at the laboratory of Biochemistry and Molecular Biology in Cotonou, lymphocytes were isolated within 3 h using Ficoll-plaque Plus™ procedure (Eurobio) and were stored frozen at -20°C until DNA extraction.

Air Sampling

Air sampling has been performed during at least 6 h per day using a glass fiber filter and a Chromosorb 102 solid sorbent tube. The samples have been analyzed for total PAHs and Benzo(a)pyrene, by high performance liquid chromatography (HPLC), and spectrofluorometric detection (SFD).

Urine Samples

Collection of spot urine samples was performed between 4 and 6 p.m. (i.e., after at least 8 h in the polluted air). Then 1-hydroxypyrene was separated by HPLC and quantified using a Spectrofluorometric detector.

PAHs and (B(a)P Analysis in Ambient Air

Attempts were made to measure 13 PAHs (naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(e)pyrene, perylene, benzo(a) pyrene, dibenz(a,h)anthracene, and benzo(g,h,I)-perylene) following separation by high performance liquid chromatography and fluorescence detection (Jasco 821-FP spectrofluorometer, Japan Spectroscopic). A ChromSpher PAH column (200 mm length, 3 mm internal diameter) from Chrompack was used. Elution was performed at a flow rate of 0–5 mL/min using a gradient of water/acetonitrile from two pumps (Bischoff Chromatography, Germany) according to the following program: 50/50 mixture from 0 to 15 min, then 0/100 for 12 min then 50/50 again for 8 min. One cycle lasting 35 min. Samples were injected with an autosampler (Bischoff, Germany). The detection of PAHs was performed by fluorimetry (82 1-FPJasco spectrofluorometer); for naphthalene, fluorene, and phenanthrene the excitation and emission wavelengths were 280 and 350 nm; for the other compounds, 305 and 430 nm. However conditions for benzo(a)pyrene (BaP) quantification were firstly applied, excitation 263 nm, emission 410 nm. Other conditions were applied for confirmation. Six standard PAH mixtures (Alltech Associates, Deerfield, IL) were used as reference materials. Results are reported as the sum of all PAHs measured (total PAHs concentration).

Hydroxypyrene and Phenol Analysis in Urine

For metabolites measurement in urines the technique included a prior enzymatic hydrolysis of the conjugates with a mixture of glucuronidase and sulfatase from Helix pomatia (Sigma Aldrich); followed by sample purification on octadecyl silica cartridges (Bond-Elut 100 mg, Analytichem, CA). Chromatography was carried out with a model of ET 250/8/4 Nucleosil 10, C18 column (VWR, Chromosorb, France); using two pumps (Bischoff Chromatography, Germany) delivering, either pure acetonitrile (solvent A) or 10% acetonitrile in water containing 0–5 mL glacial acetic acid/L (solvent B) at a flow rate of 1 mL/min to make the elution gradient: from 100 to 20% solvent B in 25 min, from 20 to 0% solvent B in 2 min, isocratic (pure solvent A) elution during 3 min, 100% solvent B in 2 min and re-equilibration of the column for 8 min before a new analysis cycle. Hydroxypyrene was detected by fluorimetry (excitation: 240 nm; emission: 390 nm; 821-FP Jasco spectrofluorometer). The calibration was made with a control urine sample spiked with 10 μg hydroxypyrene/l (Sigma-Aldrich); the detection limit was 0–2 μg/L.

In the same urine samples, phenol was measured by HPLC with UV detection as described previously by Weaver et al. (1996).

Results were standardized to 1 g urinary creatinine.

Enzymes and Chemicals

Proteinase K (EC 3.4.21.64), ribonuclease A (EC 3.1 4.22, micrococcal nuclease (EC 3.1.4.7), spleen alkaline 5′phosphodiesterase (EC 3.1.4.1), and T4 polynucleotide kinase (EC 2.7.1.78) were purchased from Amersham Bioscience and nuclease P1 (EC 3.1.30.1) from Sigma. Ficoll-plaque Plus™, [γ-^{32}P] ATP were also from Amersham Bioscience and phenol (CAS Number 108-95-2), chloroform (CAS Number 67-66-3), isoamyl alcohol (CAS

TABLE I. Some data on individual exposure values to total PAHs and benzene by urinary excretion of phenol and 1-hydroxypyrene as biomarkers, expressed as mean ± SEM

PAHs in Outdoor Air ($\mu g/m^3$)	Benzene in Outdoor Air (mg/m^3)*	Urinary Excretion of Phenol Adjusted to Creatinine (mg/g)	1-Hydroxypyrene Adjusted to Creatinine ($\mu g/g$)
1.6 ± 1.1 $\mu g/m^3$	3.4 ± 3.0 mg/m^3	Village (n = 17) 9.1 ± 1.7[a]	Village (n = 17) 3.07 ± 2.1 (d)
2.5 ± 1.3 $\mu g/m^3$	4.3 ± 2.5 mg/m^3	Suburban (n = 20) 13.5 ± 1.4[b]	Suburban (n = 20) 4.8 ± 2.5 (d)
103 ± 13 $\mu g/m^3$	76.0 ± 26.8 mg/m^3	TMD (n = 13) 155.5 ± 15.6 (c)	TMD (n = 13) 254.3 ± 27.6 (c)
103 ± 13 $\mu g/m^3$	76.0 ± 26.8 mg/m^3	RR (n = 11) 155.5 ± 15.6[c]	RR (n = 11) 254.3±27.6[c]
103 ± 13 $\mu g/m^3$	76.0 ± 26.8 mg/m^3	SV (n = 16) 155.5 ± 15.6[c]	SV (n = 16) 254.3 ± 27.6[c]
103 ± 13 $\mu g/m^3$	76.0 ± 26.8 mg/m^3	GS (n = 17) 155.5 ± 15.6[c]	GS (n = 17) 214.3 ± 27.6[c]

1 ppm = 3.2 mg/m^3
Those bearing the same letter [c] or [d] are not significantly different.
[a]Different from [b] at $P = 0.05$.
[c]Different from [a] and [b] at $P = 0.001$.
[c]Different from [d] at $P = 0.001$.

Number 123-51-3), and the other chemical compounds from Sigma.

DNA Extraction and ^{32}P Postlabeling of DNA Adducts

DNA was extracted from lymphocytes using standard phenol/chloroform/isoamylalcohol extraction method. All DNA samples were stored frozen at −20°C and were shipped on dry ice to *Institut de Biologie Moléculaire et Cellulaire* at Strasbourg (France) for DNA-adducts determination using the ^{32}P-postlabeling method as previously described (Randerath et al., 1985; Reddy and Randerath, 1986; Binkova et al., 1998) with minor modifications. Briefly, 7 μg of DNA was digested by micrococcal nuclease and spleen phosphodiesterase for 4 h at 37°C. Adducts were then enriched by nuclease P1 treatment, afterward a postlabeling reaction was carried out and adduct purification was performed by thin layer chromatography (TLC) on polyetyleneimine (PEI, CAS 26913-06-4) cellulose. The labeled adducts were detected by autoradiography following 24 or 48 h exposure, and quantified with Bio-Imager Bas2000 phosphoranalyser (Fuji, Tokyo, Japan). DNA adduct levels were expressed as mean of DNA adducts per 10^8 nucleotides of two independent assays calculated from the amount of DNA hydrolyzed and the specific radioactivity of the used [γ-^{32}P]-ATP (Phillips and Castegnaro, 1999).

A mixture of adducts from a heavily exposed individual and a less exposed individual (1/2 of the amounts loaded above for single chromatography) was comigrated in identical conditions and quantified similarly. This has been done taking randomly three different couples of individuals. The results are reported in Figure 3.

Statistical Analysis

Values are given as mean ± SEM. of number of DNA-adducts per 10^8 nucleotides. Data were transformed by the natural logarithm before analysis to satisfy the distributional assumptions of conditional normality and homogeneity of variance. Differences between group were analyzed by ANOVA with differences being statistically significant at a P value <5% and a *post hoc* analysis as least significant difference (LSD) at $\alpha < 5\%$. All statistical analyses were performed using SPSS version 11.0 for Windows 2001.

RESULTS

Ninety four subjects were recruited in the present study following the withdrawal of smokers (Tables I and II). The ages and respective numbers of individuals per group are given in Table II. The overall average age is 29 ± 7, with a sexes ratio (M/F) of 0.88 (44/50).

Exposure Assessment

The exposure has been assessed, the following values were found: benzene in the outdoor ambient air was quantified at concentrations of 76.0 ± 26.8 $\mu g/m^3$ in Cotonou, compared with 3.4 ± 3.0 $\mu g/m^3$ in villages located far from Cotonou (Table I). This benzene concentration is a mean value of (n = 9) determinations performed on three consecutive days in three urban polluted areas (101–170 $\mu g/m^3$) where people were recruited. These benzene concentrations largely exceeded the 5 $\mu g/m^3$ recommended by World Health Organization (WHO IARC, 1983).

AS for polycyclic aromatic hydrocarbons (PAHs) they were found to be at concentrations of 103 ± 13 $\mu g/m^3$ in Cotonou *versus* 1.6 ± 1.1 $\mu g/m^3$ in villages and 2.5 ± 1.3 $\mu g/m^3$ in the suburban areas (Table I). The specific values from the polluted areas ranged from 87 ± 16 to 118 ± 11.

Characterization of the airborne concentration of 13 polycyclic aromatic hydrocarbons (PAHs) was performed once per day (6 h) at each location on 3 consecutive days.

Validation of the urinary excretion of 1-hydroxypyrene (hydroxypyrene) as a biological marker of exposure to PAH was also performed once per individual either on day

TABLE II. Information on different groups and levels of DNA-adducts, as determined by ^{32}P-postlabelling method

	Low Exposure Groups		High Exposure Groups			
	Village	Suburban	TMD	RR	SV	GS
Number	17	20	13	11	16	17
Age ± SD	40 ± 12	36 ± 13	36 ± 6	37 ± 9	30 ± 8	32 ± 9
Mean ± SEM of DNA adducts[5]	3.1 ± 0.8	2.1 ± 0.6	24.6 ± 6.4	23.7 ± 6.9	34.7 ± 9.8	37.2 ± 8.1
DNA adduct ranges	0–8	0–9	1–69	1–76	3–169	4–124
P value in comparison of DNA adducts between high and low exposure groups		NS	<0.001	<0.001	<0.001	<0.001

No declared smoker is included in the study. However, some suburban subjects and village residents may have not declare the use of wood fire for meal preparation or smoked fish in their diet.

TMD, Taxi-motor bike drivers; RR, roadside residents; SV, street vendors; GS, gasoline sellers, $\times 10^8$ nucleotides; NS: not significantly different from the "village" value.

1, 2, or 3 since they show-up every day and are exposed all day long, the half life for the urinary excretion of hydroxypyrene being around 18–22 h. The valued were adjusted to creatinine (Table I).

These measurement showed urinary phenol values from 9.1 ± 1.7 mg/g of creatinine for the village, 13.5 ± 1.4 mg/g for the suburban area, and 155.5 ± 15.6 for the more polluted areas in Cotonou (Table I). The difference was highly significant between exposed people in the city of Cotonou ($P = 0.001$) and those in suburban areas and village. These two groups are also different for phenol excretion ($P = 0.05$).

Concerning hydroxypyrene urinary excretion the values ranged from 3.07 ± 2.1 μg/g in village subjects, 4.8 ± 2.5 μg/g and 254.3 ± 27.6 μg/g in heavily exposed individuals (Table I). No difference could be seen between individuals from village and suburban areas while those exposed in the city of Cotonou showed clear-cut difference as compared to village and suburban people ($P = 0.001$).

Benzene has not been measured in the air this time because the air samples have not been stored in appropriate manner, so they were not useable for the purpose of quantifying this volatile compound.

Mean levels of DNA adduct are summarized on Table II. Taxi-motorbike drivers, roadside subjects, street vendors, and gasoline salesmen have significantly higher DNA adduct levels than suburban subjects and rural inhabitants ($P < 0.001$, *post hoc* LSD), Figure 1.

DNA-adduct levels were not significantly different between suburban subjects and village residents ($P > 0.05$, *post hoc* LSD). Furthermore no statistically significant difference was observed in high exposure subgroups, namely taxi-motorbike drivers, crossroad subjects, street vendors, and gasoline salesmen ($P > 0.05$, *post hoc* LSD). Instead a large inter individual variation in DNA-adducts levels was observed. Ranges of adducts per 10^8 nucleotide were for motorbike taxi-drivers, (1–69), roadside subjects, (1–76), street vendors, (3–169), gasoline salesmen, (4–124), suburban inhabitants (0–9), and rural inhabitants, (0–8).

Autoradiographic Maps of the ^{32}P-Postlabeled Bulky DNA Adducts

Typical autoradiographic maps of the ^{32}P-postlabeled bulky DNA adducts from different groups of habitants living in town and village are shown in Figure 2. In all cases where DNA adducts were present, the major spot was quantified by means of the Mac Bas software. In some cases,

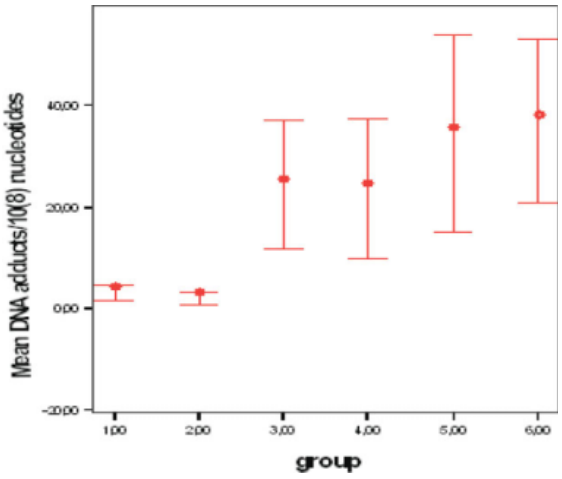

Fig. 1. DNA adducts levels as detected by ^{32}P-postlabeling assay. Anova is statistically significant ($P < 0.001$), and high exposure group is different from the low exposure group ($P < 0.001$, *post hoc* LSD). Data are expressed as mean (point), with SEM (error bar) (1 = village 2 = suburban; 3 = taxi-motor-bike; 4 = roadside; 5 = street vendors; 6 = gasoline sellers). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

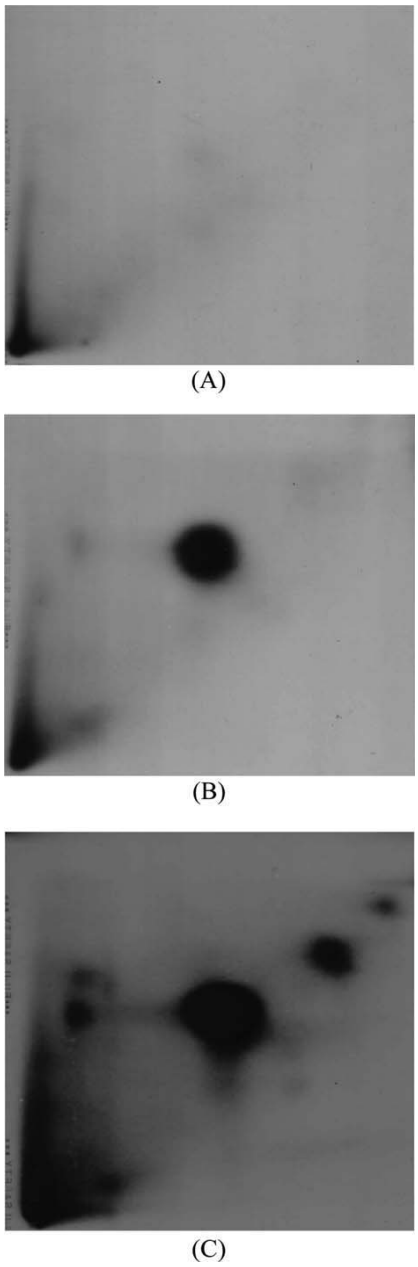

Fig. 2. Typical two-dimensional autoradiographs of ^{32}P-postlabeled DNA-adducts chromatographed on PEI-cellulose TLC plates from circulating white blood lymphocytes. A: No adduct (village resident); B: major adduct ('taxi-motorbike' driver); C: major and additional adducts (street vendor).

additional less intense spots even well resolved on TLC were not taken into account (data not shown). Co migration of DNA-adducts found in exposed groups has shown interestingly that one main spot could be seen indicating a likely identity of adducts that have migrated to this place (data not shown). This is confirmed by the measurement of the distances of migration from the origin in the two dimensions in three different studies (Fig. 3) indicating possible identity.

A mixture of adducts from a heavily exposed individual and a less exposed individual (1/2 of the amounts loaded above for single chromatography) was comigrated in identical conditions and quantified similarly. This has been done taking randomly three different couples of individuals. The results are reported in Figure 3.

Finally, these DNA-adducts have been compared to adducts formed in WBC of rats exposed to a mixture of benzene and PAHs found to be identical for the main spot, (data not shown) indicating that mainly adducts with PAHs are detected herein. Very interestingly some minor adducts spots have migrated similarly to those found in heavily exposed people (Figs. 2 and 3).

DISCUSSION

In the present study we investigated the formation of bulky DNA-adducts as biomarkers of biologically effective dose of PAHs and some volatile compounds mainly benzene in outdoor air in the center of Cotonou in Benin. Individuals enrolled in this study were 94 adults Beninese, some of which were occupationally exposed to varying levels of genotoxic compounds through ambient air pollution, namely, benzene and PAHs. While village residents are exposed to lower levels of the same pollutants, city residents such as taxi-motorbike drivers and roadside residents are exposed to significantly higher levels of such cancer-causing agents in traffic-jammed areas of Cotonou (Ayi-Fanou et al., 2006).

Individual exposure to PAHs and benzene has been determined; it appeared that the values of PHAs exposure (mainly benzo(a)pyrene) in the urban areas are not

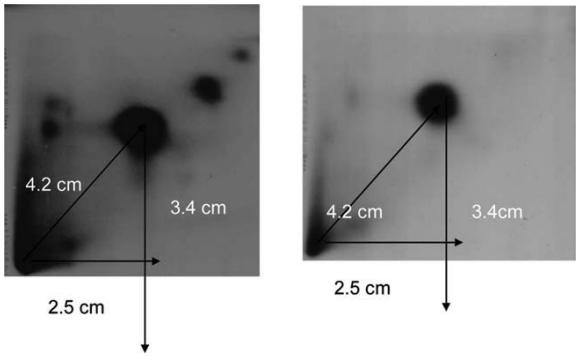

Fig. 3. Super imposition of the spots of the main DNA-adducts and migration distance from the origin.

different. Motorbike taxi-drivers, roadside subjects, street vendors, and gasoline salesmen are apparently equally exposed as indicated by the ambient air concentrations and urinary metabolites. So, it is accordingly normal that the DNA-adducts rates are not different. Our results demonstrated that DNA-adduct levels were significantly higher in city residents i.e., taxi-motorbike drivers, crossroad subjects, street vendors, and gasoline salesmen when compared to rural inhabitants, confirming the significantly higher exposure levels to genotoxic pollutants observed in ambient air in Cotonou. This may be explained by the fact that exposure to pollutants (PAH compounds including B[a]-P) generated from gasoline vapors increased the formation of DNA-adducts in exposed population. These findings are in agreement with those reported by (Ruchirawat et al., 2005) while measuring exposure to PAHs in grilled-meat vendors and clothe vendors in similar areas in Bangkok. Overall, our results are in agreement with a number of studies which reported that populations exposed to environmental air pollution show increased levels of DNA-adducts (Farmer et al., 2003; Singh et al., 2007). However, this is the first study evidencing DNA adduction in an African exposed population as a function of PAH, B[a]-P and benzene outdoor concentrations. Although not yet identified, the major adducts observed may be those generate by PAHs, the major air pollutants found in the ambient air. They could also be similar to the main adducts obtained in bitumen exposed rodents (Micillino et al., 2002; Bottin et al., 2006). Investigations on rat exposed by inhalation (namely whole body and inhalation chamber system) to B[a]-P show that the DNA-adducts found are identical to those found in heavily exposed people (data not shown). The most interesting is that the main spot is absolutely similar while some smaller spots of DNA-adducts migrate more or less to the same distances which are not significantly different following statistical comparison but show some very small differences by superimposition. So, some of the DNA-adducts in WBC of subjects enrolled in the survey may be assigned to B[a]-P in heavily exposed people.

Since DNA damage is believed to be a first step in development of cancer, an increased level of DNA-adducts in city residents may suggest that city residents are of elevated risk for development of chronic lung diseases and cancer related to, benzene and B[a]-P and other PAHs. Similar results have been reported in Thailand, where roadside residents exposed to PAHs (at concentrations about 2.5-fold lower than that found in Cotonou), bear DNA-adducts levels of 0.45 adducts per 10^8 nucleotides, higher than those of rural ones (0.09 adducts/10^8 nucleotides) Ruchirawat et al. (2007). As a matter of fact the levels of DNA adducts in the study of Ruchirawat et al. (2007) are lower than those in our study, probably because of the lower concentrations in the outdoor air in Bangkok and likely because of the improved method of DNA-adducts detection applied in our study.

Likewise, B(a)P-induced DNA-adducts were found significantly higher in policemen working in the streets in Prague compared with nonexposed control (Binkova et al., 2007).

A study carried out in Denmark by (Autrup et al., 1999) found a significantly higher level of carcinogen-DNA adducts in Copenhagen bus drivers than suburban and semi-rural drivers. But levels of DNA-adducts reported in Danish bus drivers (75.4 adducts/10^8 nucleotides) are threefold higher than that we are reporting in taxi-motorbike drivers circulating in Cotonou city (24.6 adducts/10^8 nucleotides).

In this study, we failed to find differences in DNA adducts levels between taxi-motorbike drivers, crossroad subjects, street food vendors and gasoline salesmen, suggesting nonlinearity in adducts formation under high exposure circumstances to PAHs and also to B[a]-P. This absence of dose-response relationship in adducts formation in high exposure circumstances may be explained by several reasons. The main reason is that even though highly exposed, they show comparable urinary metabolites levels in response to similar ambient outdoor air concentrations of B(a)P and benzene. These results are in agreement with those reported by Binkova et al., (1998) in coke oven workers. In contrast, linear dose–response relationship has been found for both 1-HOP and DNA-adduct formation in nonsmoking Royal Thai trafficked policemen as compared with the reference group (Ruchirawat et al., 2002). We acknowledge here that only few people among taxi-motorbike drivers, crossroad subjects, street vendors and gasoline salesmen do smoke. They (all spontaneously declared and those bearing gray teeth induced by tobacco smoking) were discarded from the group of analyzed persons.

So, no declared smoker is included in the study. However some suburban subjects and village residents may have not declared the use of wood fire for meal preparation or smoked fish in their diet. This could have increased their exposure to PAHs and mainly to B[a]-P thus increasing their DNA-adducts levels in such an extent we cannot determine without assessing PAHs in their food intake. This was not the purpose of the present study and should be of concern in further investigations.

Depending on the extent of bioactivation and DNA damage, DNA-adducts can be repaired or lead to apoptosis or eventually to mutation if not repaired. Therefore, genetic polymorphisms in gene involved in detoxication may modulate the amounts of bulky DNA-adducts. Some studies have reported conflicting results regarding the influence of genetic polymorphism, in DNA adducts formation. For example (Garte et al., 2007) found association between glutathione S-transferase (GSTs)-genes inactivation and DNA-adducts formation by PAHs, but other studies did not find any relation (Peluso et al., 2004; Ruchirawat et al., 2007). It

is being suggested that polymorphisms in DNA repair genes involved in nucleotide excision repair may modify aromatic DNA-adduct levels and may be useful biomarkers to identify individuals susceptible to DNA damage following exposure to PAHs (Binkova et al., 2007).

We have previously investigated the level of oxidative DNA damage in mononuclear blood cells (MNBC) by the comet assay, DNA strand breaks (SB), and formamidopyrimidine DNA glycosylase (FPG) sensitive sites in residents from three urban locations in Cotonou, Benin (taxi-motor bike drivers, subjects living near roads with intense traffic and suburban residents) and rural residents. The conclusion reached was that urban air with high levels of benzene and UFP is associated with elevated levels of SB and FPG sites in MNBC, and that NQO1 and GST genes may modulate the effect (Avogbe et al., 2005).

Wood burning particles and volatile wood compound could also be a source of DNA-adducts forming products to which some of the individuals enrolled in this study are maybe additionally exposed rendering the dose–effect relationship difficult to be established in a very accurate way. An illustration of this fact could be found in the study of Avogbe et al. (2005). These authors reported a large gradient in the concentration of ultrafine particulate (UFP) matter, with the highest levels in the center of Cotonou that decrease toward the periphery (Avogbe et al., 2005). UFP are mainly generated by poor combustion of fossil fuels in vehicles and by wood-burning fires. These particles are now receiving an increasing attention worldwide, due to their potential impact on morbidity and mortality (Dochery and Pope, 1994; Pope et al., 2002) with an excess risk of pulmonary and cardiovascular diseases and cancer (Katsouyanni and Pershagen, 1997; Pope et al., 2002). Indeed Each 10 $\mu g/m^3$ elevation in fine particulate air pollution was associated with approximately a 4%, 6%, and 8% increased risk of all-cause, cardiopulmonary, and lung cancer mortality, respectively (Pope et al., 2002).

To draw attention on the ongoing situation of the outdoor pollution in Cotonou some examples were taken from the literature. Robinson and Paxman (1992) have analyzed the role of threshold limit values (TLVs) in national air pollution policy during the 1980s in U.S. These authors focused on 20 carcinogens and 11 substances with nongenotoxic health effects that were regulated by local air toxics programs using TLVs. Data from EPA's National Air Toxics Information Clearinghouse indicate that maximum TLV-based Ambient Air Level guidelines (AALs) frequently exceed minimum TLV-based AALs by a factor of greater than 1000. Cancer potency data from EPA's Integrated Risk Information System suggest significant risks remain at TLV-based AALs. Cancer risks at the median TLV-based AAL exceed 1000 cases per million exposed persons for coke oven emissions (1860) and benzene (2500) for example.

We found that the public health implications of outdoor concentrations of PAHs and benzene are also of concern in industrialized countries such as the USA and Norway or Denmark. A modeled outdoor HAP concentration estimate from the U.S. Environmental Protection Agency's Cumulative Exposure Project was used by Morello-Frosch et al. (2000) to characterize the extent of the air toxics problem in California for the base year of 1990. These air toxics concentration estimates were used with chronic toxicity data to estimate cancer and noncancer hazards for individual HAPs and the risks posed by multiple pollutants. Although hazardous air pollutants are ubiquitous in the environment, potential cancer, and noncancer health hazards posed by ambient exposures are geographically concentrated in three urbanized areas and in a few rural counties. This analysis estimated a median excess individual cancer risk of 2.7E-4 for all air toxics concentrations and 8600 excess lifetime cancer cases, 70% of which were attributable to four pollutants: polycyclic organic matter, 1,3 butadiene, formaldehyde, and benzene.

In conclusion, the data from our study indicate that DNA-adducts levels are correlated with the levels of environmental carcinogenic compounds found in the outdoor air in the center of Cotonou. It seems that at high exposure conditions to benzene and PAHs (mainly B(a)P, DNA-adducts levels are not directly proportional, reflecting either saturation in adducts formation or stimulation of their repair because of chronic exposure and genetic polymorphism. However, the DNA-adducts levels could be correlated with individual exposure values as determined by urinary excretion of hydroxypyrene and phenol.

The highly exposed individuals bearing the higher DNA-adducts levels are being followed in a cohort study to find out those who will develop cancers in the coming times and implement prevention means to protect residents not exposed professionally.

The authors thank Professor Jean Cognet for fruitful discussions, Doctors Latifou Lagnika, Michael Missihoun, and Nicodème Chabi for critical reading of the manuscript.

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