

Effects of BTEX Exposure on Hematological and C-Reactive-Protein in Professional and Non Professional Motorcycle Drivers in Cotonou/Benin

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Abstract Benzene, toluene, ethylbenzene and xylene (BTEX) are toxic volatile organic compounds (VOCs) present in polluted ambient air. Air pollution can affect human health through chronic or acute exposure to fumes from vehicles and motorcycles. Our study aims to evaluate the concentration of BTEX in the ambient air and its effects on the biological parameters of motorcycle drivers. We recruited 30 pairs of professional (30) and non-professional (30) motorcycle drivers in Cotonou / Benin. The 3MR3500 organic vapor monitor badges were worn around the necks of these drivers during 8 hours of motorcycle driving. The badges analysis were carried out with gas chromatography flame ionization detector (GC-FID). Biological parameters were measured using the CHEM-7 erba spectrophotometer and the Sysmex XT 4000i spectrophotometer. Our results showed that the means concentrations of BTEX are higher for professional drivers than non-professionals. A statistically significant difference was observed between the two groups with $p < 0.05$. The mean concentrations of BTEX were 77.00 ± 5.45 ; 245.91 ± 18.99 ; 72.53 ± 7.54 professional and 67.25 ± 14.54 $\mu\text{g} / \text{m}^3$ for professional motorcycle drivers and 25.87 ± 6.51 ; 139.75 ± 19.08 ; 64.26 ± 4.99 and 41.60 ± 00.00 $\mu\text{g} / \text{m}^3$ for non-professional motorcycle drivers. Likewise, the biological parameters (hematological and immunological ultra-sensitive C-reactive protein) were also different between the two groups ($p < 0.05$). Professional motorcycle drivers are more exposed and affected to BTEX than non-professionals drivers. This exposure may alter biological parameters.

Keywords: BTEX, professional motorcycle drivers, non-professional motorcycle drivers, sensitive CRP, hematological parameters

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1. Introduction

Ambient air pollution has worsened in recent years and has led to major public health risks [1,2]. The emission of pollutants is linked to various anthropogenic activities such as industrial emissions, storage of petrol and oil, incineration of medical waste and landfills [3]. In addition to these sources, car traffic is also a major source of ambient air pollution, exacerbated by motorcycle taxi

driving in major African cities [4]. Thus, this ambient air pollution is responsible for morbidity and mortality. The World Health Organization (WHO) reported an increase of number of deaths due to air pollution between year 2016 (3 millions) and 2018 (about 7 millions) [5,6]. Some pollutants are associated with increased levels of systemic inflammation markers such as interleukins, Tumor necrosis factor (TNF α), C-Reactive Protein (CRP) and variation of blood cells. CRP is a protein of the innate immune response and rapid expression of inflammation. Its concentration may increase during the inflammatory

response and normalize rapidly after the elimination of the stimulus [7]. This protein is considered as a good marker of the inflammatory response [7,8,9]. In another way, pollutants influence blood cells by inducing several diseases [10,11]. Studies conducted in Benin on ambient air quality have shown that motorcycle taxi drivers have developed several pathologies [12,13,14]. Among the pollutants responsible for these diseases are benzene, toluene, ethylbenzene and xylenes (BTEX), which belong of the family of volatile organic compounds. They are emitted by industries, gasoline, transport etc [3,15]. Generally, different disorders are related to BTEX, ranging from skin and eye irritations, carcinogenic and mutagenic effects, headaches, heart, digestive, kidney, liver and nervous system disorders [16,17]. However, each of these four pollutants has specific health effects [18,19]. Thus, benzene is very toxic and carcinogenic [19,20]. Toluene and xylene can have adverse effects on the cardiovascular, gastrointestinal, hematological, hepatic and renal systems [11,21,22,23]. According to the WHO, in 2013, exposure to ethylbenzene can lead to simultaneous damage to the upper respiratory tract and central nervous system [1,24]. Several studies have shown that the level of benzene in ambient air was above the norm accepted by WHO and has adverse effects on health [12,25,26]. Nevertheless, levels of exposure to BTEX, their effects on blood cells and CRP have been poorly studied. Our work aims to evaluate the concentration of BTEX in the ambient air, its effects on ultra-sensitive protein C-reactive protein (CRP-us) and blood cells in motorcycle taxi drivers called "Zemidjan" compared to the controls in the city of Cotonou/Benin.

2. Methods and Material

Our study was conducted in the city of Cotonou with two groups consisting solely of men, namely professional motorcycle taxi drivers and non-professional motorcyclists as controls. Both groups have at least 5 years of seniority in motorcycle driving. These drivers are matched in pairs of Zemidjan / control having approximately the same age, non-smokers, using same fuels and having same socio-economic conditions. Thirty (30) couples participated in this study, which was approved by the Research Ethics Board of the Institute of Applied Biomedical Sciences (CER-ISBA) of the University of Abomey-Calavi.

2.1. BTEX Sampling and Analysis

A total of 60 badges samples (3M® 3500 organic vapor monitor) worn for 8 hours of driving by the 30 recruited couples were collected. The filters of the badges containing the pollutants were extracted using carbon disulphide (CS₂) and the extracts analyzed out with gas chromatography flame ionization detector (GC-FID).

2.2. Blood Sampling

Five ml (5 ml) of blood were collected after 8 hours of motorcycle driving in dry and EDTA tubes. EDTA tubes were used for Hematological analysis using Sysmex XT 4000i apparatus and dry tubes for CRP-us by CHEM-7 erba spectrophotometers.

2.3. Statistical Analyses

All statistical analyzes were performed using SPSS 20.0 software. Means BTEX concentrations at the professional and control levels were conducted. The t-test was performed to express the significant difference between the two groups. The independence test of the samples were used to compare the biological parameters within the two groups. All results were considered statistically significant values are accepted at $p < 0.05$.

3. Results and Discussion

These results can be divided into two parts such as those for BTEX and biological parameters.

3.1. Results of BTEX

The BTEX badges were worn by thirty professional taxi-motorcycle drivers "Zémidjan" and thirty drivers of non-professional motorcycles. Concerning the ages and socio-demographic statut no statistically significant difference is observed. The Table 1 report the means concentrations of benzene, toluene, ethylbenzene and xylene in both groups. These BTEX values are respectively 77.00 ± 5.45 , 245.91 ± 18.99 , 72.53 ± 7.54 , 67.25 ± 14.54 $\mu\text{g}/\text{m}^3$ for professional drivers and 25.87 ± 6.51 , 139.75 ± 19.08 , 64.26 ± 4.99 , 41.60 ± 00.00 $\mu\text{g}/\text{m}^3$ for non-professionals. There is a statistically significant difference between BTEX concentrations in professional and non-professional drivers. All values of P were $P < 0.05$ and were shown in Table 1.

Table 1. Mean values of BTEX concentrations measured in Professional and Non-Professional motorcycle drivers

Pollutants	Statut	Mean ($\mu\text{g}/\text{m}^3$)	IC95%	P
Benzene	Cas	77.00 ± 5.45	66,30-86,92	0,001
	Control	25.87 ± 6.51	6,85-26,07	
Toluene	Cas	245.91 ± 18.99	186,53-267,77	0,001
	Control	139.75 ± 19.08	45,09-111,78	
Ethylbenzene	Cas	72.53 ± 7.54	38,99-68,65	0,002
	Control	64.26 ± 4.99	4,29-24,28	
Xylene	Cas	67.25 ± 14.54	3,38-24,46	0,000
	Control	41.60 ± 00.00	0,01-23,15	

Cas : Professional motorcycle drivers, Control : Non Professional motorcycle drivers.

Table 2. Mean Concentrations of Hematological Parameters and C-Reactive Protein in Professional and Non-Professional Motorcycle Drivers

Variable	Statut	Mean	95% CI	P-value
Erythrocytes	Cas	3,13±0,96.10 ⁶	2,84-3,49	0,001
	Contrôles	4,99±0,55.10 ⁶	4,79-5,16	
Hémoglobines	Cas	10,52±1,38 g/dl	10,08-11,01	0,001
	Contrôles	15,31±0,94 g/dl	15,0035-15,6198	
Hematocrites	Cas	33,55±4,68 g/dl	32,00-35,12	0,001
	Contrôles	47,06±5,88 g/dl	45,13-49,23	
Plaquettes	Cas	646,40±277,80%	550,68-744,32	0,001
	Contrôles	219,21±44,19%	204,81-234,62	
Reticulocytes	Cas	40,88±12,26 %	36,82-45,29	0,795
	Contrôles	40,12±9,99%	36,84-43,59	
Leucocytes	Cas	7,48±1,94 %	6,78-8,18	0,001
	Contrôles	4,491±0,85%	4,20-4,78	
Neutrophiles	Cas	70,88±15,44%	65,11-76,44	0,001
	Contrôles	57,30±7,13%	54,79-59,73	
Eosinophiles	Cas	2,85±1,09%	2,50-3,24	0,037
	Contrôles	2,36±0,78%	2,09-2,61	
Lymphocytes	Cas	47,64±7,00%	45,16-49,90	0,266
	Contrôles	45,39±8,94%	42,34-48,69	

Hematological and immunological analyzes were performed for all blood samples collected from professional and non-professional drivers. The values of erythrocyte, hemoglobin and hematocrits presented as means are respectively $3.13 \pm 0.96.10^6$, $10.52 \pm 1.38 \text{ g / dl}$ and $33.55 \pm 4.68\%$ in professional drivers and $4.99 \pm 0.55.10^6$, $15.31 \pm 0.94 \text{ g / dl}$, $47.06 \pm 5.88\%$ in non-professionals. Student's t-test showed that these values are statistically significant between the two groups. The Table 2 reports these results as well as those of platelets, leucocytes, neutrophils and eosinophils. A statistically significant difference is observed between the two groups. Parameters such as reticulocytes and lymphocytes do not differ significantly between the two groups. The mean value of the CRP inflammation marker was three times higher in professional drivers compared to non-professionals.

3.2. Discussion

In 2006, AYI-FANOU et al., measured the concentrations of BTEX in Cotonou motorcycle taxi drivers compared to the inhabitants of the village of Sohon. They observed high concentrations of these pollutants in Cotonou compared to those of the village. Our work differs from that achieved in 2006 by the fact that all participants in the study are motorcycle drivers living in the city of Cotonou and chosen in professional / non-professional couples matched by age, type of fuel and socio-economic conditions. As previous studies, our results showed high concentrations of BTEX in the professional motorcycle drivers compared to non-professionals. The means concentrations of benzene, toluene, ethylbenzene and xylene are respectively 77.00 ± 5.45 , 245.91 ± 18.99 , 72.53 ± 7.54 and $67.25 \pm 14.54 \mu\text{g} / \text{m}^3$ for professional motorcycle drivers and 25.87 ± 6.51 , 139.75 ± 19.08 , 64.26 ± 4.99 and $41.60 \pm 00.00 \mu\text{g} / \text{m}^3$ for non-professionals. In all of these studies there is a statistically significant difference between BTEX exposure of professional and non-professional drivers. In

all cases, the values were significant with $p < 0.05$ (Table 1). The means of BTEX concentrations observed during the study exceed the air quality standards recommended by WHO values, the European Union (EU) and Benin for daily exposure [27]. From these results, we can say that all the inhabitants of Cotonou professional or non-professional motorcycle drivers are differently exposed to BTEX but the activity of taxi-motorcycle is more at risk. This can be explained, on one hand, by the duration of exposure to BTEX (the Zemidjans spend more time in traffic than non-professional drivers) and on the other hand, by the quality of the gasoline used in Benin. Indeed, previous work showed that the gasoline used in Cotonou was of poor quality compared to French gasoline [3]. The benzene content is six times higher in Cotonou gasoline than in France and contains almost no ETBE (Ethyl Tert-Butyl Ether). Other studies carried out in Egypt [31], India and South Africa [29,30], have relatively higher values [31] and lower values [29, 30] than Cotonou. Inhaled BTEX can be bio-transformed into non-toxic metabolites eliminated by the organism or toxic effects on some important biomolecules such as hemoglobin, albumin, DNA, proteins involved in inflammation or even the hematopoietic system (bone marrow, lymphoid tissue, production of red blood cells, white blood cells, platelets). Also, we looked for these toxic effects on the blood cells and the C-reactive protein.

Our results showed that the mean number of red blood cells, hematocrit rate and hemoglobin concentration in professional drivers were lower than those of non-professionals. A statistically significant difference is observed between these two groups. In addition, levels of platelets, leukocytes, neutrophils and eosinophils are higher in professional drivers compared to non-professionals. A statistically significant difference between the two groups is also observed. This could be explained by the fact that the presence of BTEX induces a large production of white blood cells involved in the defense of the body. Our results are similar to those of

Rasoul et al., in 2017, which measured hemoglobin and platelets at gas station vendors compared to a control group. They showed that there is a statistically significant difference between the two groups [31]. The work of AYI-FANOUE et al., Found in 2006, a significantly high number of neutrophils among motorcycle taxi drivers professionally exposed to ambient pollution compared to rural inhabitants [25]. Other studies have shown that the exposure of workers in a refinery to benzene induces a significant alteration of their hematological functions [33]. These different studies have shown that BTEX reduces the number of blood cells that could lead to anemia (decrease in hemoglobin concentration). Our results are in contradiction with those of Harati et al., Who showed in 2017 that there is not a significant difference between the hematocrit rate, the hemoglobin concentration and the platelet count in painters car exposed to benzene and the control group. In addition, the same author has shown that there is a statistically significant difference in eosinophil levels between the two study populations [32,39]. Further, studies conducted by Zeinab et al., in 2019, have shown that the platelet rate is higher among taxi drivers than the comparator group [40]. However, the presence of hematoxic metabolites may stimulate the proliferation of white blood cells and platelets. These play an important role in blood clotting. Increasing platelet levels can lead to clots that can clog an artery in the brain, causing long-term strokes and the risk of leukemia. These studies were confirmed by Agabeldour et al., in 2015, who showed that the platelet rate is higher among gas station vendors than the witness. The results from his work show a statistically significant difference between the two study groups [34].

There are several inflammation markers including C-reactive protein. This is one of the important markers of inflammation. Its production is stimulated by interleukins (IL-6), (IL-1) and tumor necrosis factor (TNF) secreted by monocytes and lymphocytes [35]. The mean concentration of CRP measured in the population exposed to BTEX in our study is similar to that of Rioux et al., (2015) and Tunsaringkarn et al., (2013) [38,39]. The increase in the concentration of CRP is correlated with that of ambient air pollutants. Exposure to atmospheric pollutants leads to the production of CRP which can be responsible for several pathologies. According to Ridker et al., (2003), high CRP was associated with an increased risk of diabetes, myocardial infarction, ischemic stroke, and sudden cardiac death [41]. However, there are other diseases such as obesity and cancer that are linked to the increase of this protein [42]. Studies conducted by Lawin et al., (2018) with professional taxi-motorcycle drivers in Cotonou/Benin, showed that their lung function was more affected than that of controls [14]. On the other hand, the studies of Hajat et al., (2015) and Chiu et al., (2016) revealed that the mean concentrations of CRP were lower than those of our study [36,37] but above of the reference value. This decrease in CRP concentration can be explained by the short duration of exposure (24 hours) compared to 5 years in our study.

In conclusion, professional motorcyclists are more exposed to BTEX than non-professionals. Since BTEX are hematotoxic, their effects on exposed subjects are manifested by a decrease in the number of blood cells and

an increase in the production of C-reactive protein. This decrease in blood cells will cause anemia while increasing the concentration of CRP could lead to the development of certain diseases. There is a need to strengthen existing environmental policies to improve air quality and then raise awareness and monitor the health of motorcycle taxi drivers.

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Conflicts of Interest

The authors declare no conflict of interest.

Ethics Approval and Consent to Participate

We received the approval of the ethics and research committee of the "Institut des Sciences Biomédicales Appliquées" (N°46) prior the study and all participants provided written informed consent.

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