

*Full Length Research Paper*

# Hematological changes among Beninese motor-bike taxi drivers exposed to benzene by urban air pollution

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Exposure to high-concentration of benzene in polluted air has been associated with bone marrow deficiency, aplastic anemia and leukemia. However, epidemiological studies have reported conflicting data following human exposure to benzene levels below 1 ppm (that is, 3.2 mg/m<sup>3</sup>). Therefore we investigated the influence of outdoor air benzene on peripheral blood cells among exposed motor-bike-taxi drivers (MBTD) in which specific IgG antibodies against reactive benzene metabolites, such as hydroquinone (HQ) and para-benzoquinone (p-BQ) are identified and quantified for further use as biomarker of exposure. We compared 144 MBTD with 30 unexposed age and sex-matched controls. The mean age  $\pm$  SD (95% CI) were: MBTD 39.5  $\pm$  7.82 (38.2 - 40.7) and village residents 40.3  $\pm$  10.56 (39.1-43.0). Personal benzene exposure was assessed using GABIE diffusive samplers. The levels of specific IgG antibodies to HQ and p-BQ were determined by ELISA. The peripheral blood cells were counted by an automated analyzer. Benzene, Toluene and Xylene levels were much higher in MBTD in comparison to the control group. Benzene exposure levels ranged from 0.012 to 0.550 ppm in MBTD. Their average exposure level per one week was 0.126 $\pm$ 0.206 ppm. Accordingly, MBTD had significantly higher levels of specific IgG antibodies to HQ and p-BQ compared to the controls ( $p < 0.001$ ). WBC, lymphocytes, eosinophils and platelets were significantly decreased in MBTD, whereas RBC and other blood cell numbers remained unchanged. Total WBC, lymphocytes and eosinophils counts were decreased among exposed MBTD compared to unexposed controls. We suggest the use of these blood parameters together with specific IgG antibodies to HQ and p-BQ as biomarkers in biological monitoring of low level benzene exposure. Larger studies are however required to validate this new approach of health survey in workers exposed to benzene.

**Key words:** Benzene, motor-bike-taxi drivers, specific immune responses, peripheral blood cell count.

## INTRODUCTION

Urban air pollution is a major health hazard worldwide. In urban areas, air pollutants mainly originate from incomplete combustion of fossil fuels, e.g. automobile exhaust, residential heating and industrial emissions (Nielsen et al., 1996). The composition of ambient air is complex and depends on the quality of fuel, the type of

engine and engine maintenance (Leong et al., 2002). Vehicle exhaust generally contains polycyclic aromatic hydrocarbons (PAHs), particles and volatile organic compounds (VOC), e.g. carbon monoxide, benzene and also nitrogen oxides. Diesel-powered engines are the major source of particles whereas two-stroke motorbikes and petrol powered cars emitted high level of VOC, including benzene.

Cotonou, the largest town and the economic capital of Benin has more than 1 million inhabitants, about 15% of

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the country's population (INSAE, 2008). Over the past decade the air quality has worsened in the city due to the widespread use of motorbikes and old second-hand cars. According to the data from the National Agency of Land and Transport, there were more than 94,000 motorbikes and more than 350,000 old second-hand cars in the city of Cotonou (Kèlome et al., 2006). Also, gasoline is of poor quality because of illegal import of sub-standard products from the neighboring Nigeria.

In a previous study carried out in Cotonou by our laboratory, we found that motorbike-taxi drivers (MBTD) driving all day were exposed to benzene at a mean level of  $76.0 \mu\text{g}/\text{m}^3$ , while those living in the rural areas were exposed at a mean level of  $3.4 \mu\text{g}/\text{m}^3$  (Ayi-Fanou et al., 2006). It is noteworthy to mention that benzene level in the ambient air in Cotonou is markedly higher than the  $5 \mu\text{g}/\text{m}^3$  recommended by WHO. However, the level of benzene in Cotonou city is much lower than that reported in occupationally exposed workers from other countries (Zhang et al., 1996; Rothman et al., 1996a; Qu et al., 2002; Dimitrova et al., 2005).

Benzene has been classified by the International Agency for Research on Cancer (IARC) as human carcinogen (IARC, 1987). In the body, benzene is metabolized in several steps, starting in the liver by cytochrome P450 2E1, where a variety of ring-opened metabolites and ring-hydroxylated metabolites (e.g. phenols and catechols) are generated (Snyder and Hedli, 1996) (Figure 1).

These circulating phenolic compounds can be transported to other organs, e.g. the bone marrow, where they are further oxidized via reactions mediated by peroxidases into their highly reactive quinones (Subrahmanyam et al., 1991). Quinones such as hydroquinone (HQ) and para benzoquinone (p-BQ) are potent hematotoxic and genotoxic compounds that can be converted by NAD(P)H quinone oxidoreductase (NQO1) back to less toxic hydroxylated metabolites (Smith et al., 2000). Biological monitoring of benzene exposure is based on well-validated urinary metabolites, e.g. trans, trans-muconic acid and S-phenylmercapturic acid (Ducos et al., 1990; Avogbe et al., 2005; Fustinoni et al., 2005; Wiwanitkit et al., 2007).

Exposure to benzene has been associated with adverse health effects, including haematopoietic disorders such as bone marrow deficiency and acute myelogenous leukemia in both rodents and humans (Hayes et al., 2001). However, the mechanisms of benzene-induced hematotoxicity are not totally understood. Numerous studies have reported conflicting results in occupationally exposed workers to benzene levels below 1 ppm (Collins et al., 1997; Lan et al., 2004; Tsai et al., 2004).

There is also evidence suggesting that the immune system can be affected by benzene exposure (Macedo et al., 2007). It has been established that reactive metabolites of benzene, such as HQ, are ultimately responsible for the immunotoxicity associated with benzene

exposure, including suppression of B lymphocytes (Pyatt et al., 1998).

Lange et al. (1973) reported decreased IgG and IgA levels and increased IgM levels in the workers exposed to benzene concentrations of 34 to 49 ppm. In contrast, the levels of IgG and IgM types of antibodies remained unchanged in workers exposed to benzene concentrations of 1.9 to 14.8 ppm (Bogadi-Sare et al., 2000).

At present, only few studies have investigated and characterized hematological changes in humans exposed to benzene levels below 1 ppm (Lan et al., 2004; Tsai et al., 2004). Furthermore, most of the studies have been conducted in different countries, e.g. western Europe and the USA so that there is not much data available on hematological changes in workers (motorbike-taxi drivers) exposed to urban air pollution, such as that occurring in Cotonou (Ayi-Fanou et al., 2006).

Therefore, the aim of this study was to evaluate the hematological changes in MBTD exposed to urban air pollution by benzene in Cotonou, where levels ranging from 0.012 and 0.550 ppm. Moreover, we investigated the influence of benzene on the immune system, showing data on the *in vivo* production levels of specific IgG antibodies against two endogenous metabolites of benzene (HQ and p-BQ). Associations between benzene exposure, consequent immune response and hematological changes in motorbike-taxi drivers working in Cotonou are herein reported.

## MATERIALS AND METHODS

### Subject selection and blood sample collection

The study design and recruitment methods have been described previously (Avogbe et al., 2005; Ayi-Fanou et al., 2006). Briefly, the study group consisted of 144 exposed MBTD working in Cotonou and 30 healthy unexposed subjects living and working in a small village located 80 Km from Cotonou. All participants were males. MBTD with at least 5-years job histories, which had no known disease and were not currently taking medication during the sampling period were recruited.

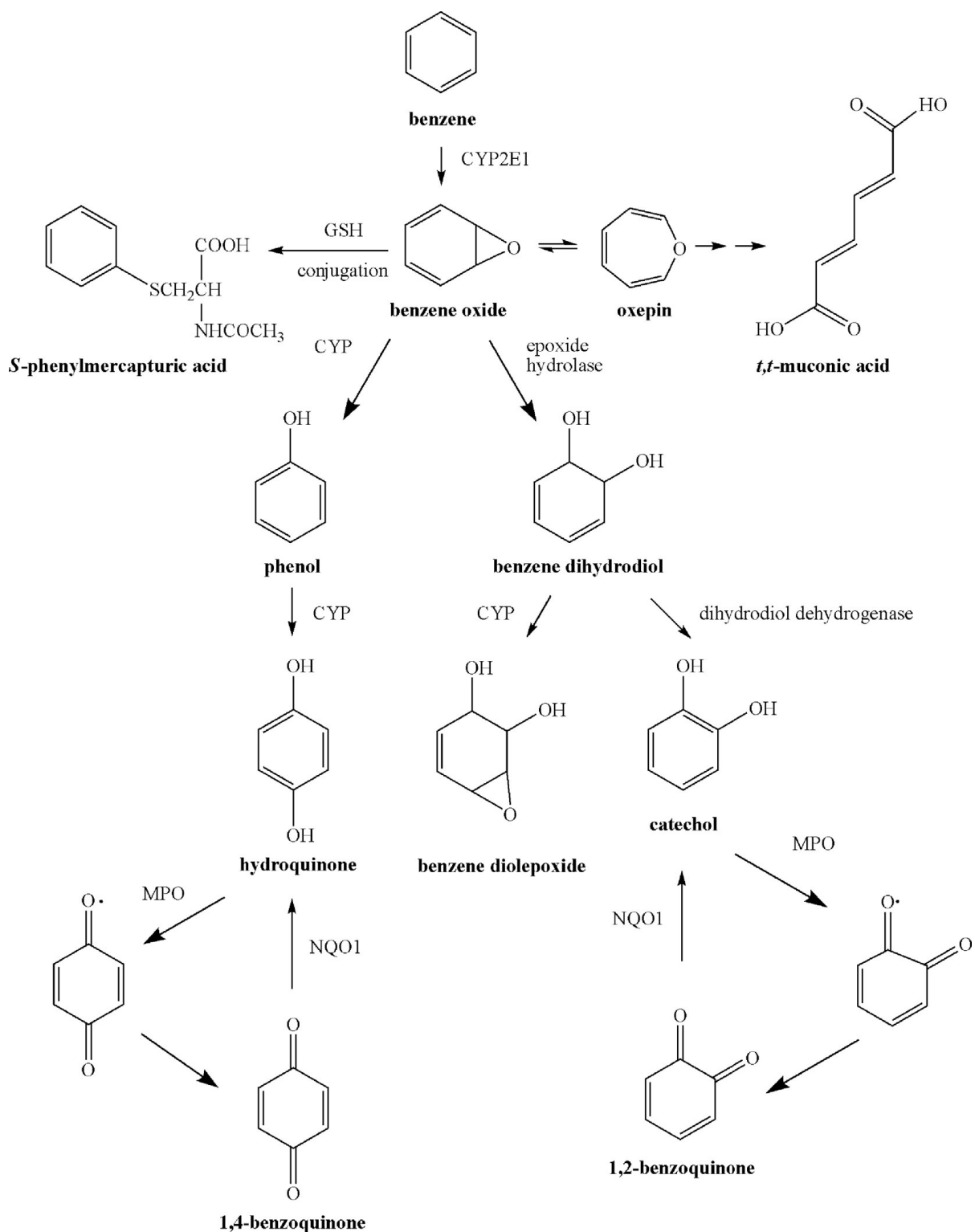
A questionnaire requesting information about the lifestyle and health status of each participant; including tobacco use, an important source of non-occupational benzene exposure, alcohol consumption, eating habits, residence, employment history, previous occupational exposure to other chemicals, height, weight, medication use, existing disease and activities involving exposure to combustion product was completed.

Each participant provided an EDTA blood sample for determination of the levels of the specific antibodies against HQ and p-BQ and blood cell counting. Ambient air measurements were carried out during the working day.

All participants gave their informed consent and the study was approved by the Beninese Environmental Agency (ABE).

### Sampling and analysis of benzene, toluene and xylene

Exposure to airborne benzene, toluene and xylene (BTX) was assessed by using GABIE (Gas Adsorbent Badge for Individual Exposure) diffusive samplers (ARELCO, ARC20001UP, France) containing activated charcoal cartridge. A random selection of



**Figure 1.** Major metabolic pathways of benzene (legend: CYP, cytochrome P450; GSH, glutathione; MPO, myeloperoxidase; NQO1, NAD(P)H:quinone oxidoreductase).

MBTD was performed to obtain individual-level measurements of BTX. Selected MBTD wore GABIE badges while driving all over the city for about sixty-three hours for one-week (that is, 9 h/day).

After sampling, the badges were sealed, preserved at  $-20^{\circ}\text{C}$  and

sent for analysis to the "Centre Commun de Mesure", ULCO, Dunkerque (France). Briefly, BTX were desorbed from the activated charcoal by using 2 ml of benzene-free carbon disulfide (Sigma, France) under agitation for 15 min. The mixture was filtered and 1  $\mu\text{l}$

**Table 1.** Characteristic of the study groups.

Characteristic	Control (n=30)	MBTD (n=144)	P-value
Age, mean years $\pm$ SD	40.3 $\pm$ 10.56	39.5 $\pm$ 7.82	0.607 <sup>a</sup>
<b>Current alcohol use, n (%)</b>			
No	19 (63.3)	86(59.72)	0.702 <sup>b</sup>
Yes	11 (36.7)	58(40.28)	
<b>Current smoking, n (%)</b>			
No	28 (93.3)	133 (93)	0.969 <sup>c</sup>
Yes	2 (6.7)	10 (7)	
Driving hour/day, mean hour $\pm$ SD	NA	6.21 $\pm$ 2.06	
Duration of exposure, mean years $\pm$ SD	NA	11.3 $\pm$ 5.96	
BMI, mean $\pm$ SD; Kg/m <sup>2</sup>	22.35 $\pm$ 1.89	23.54 $\pm$ 3.86	0.08

NA, not applicable. MBTD, motorbike-taxi drivers. <sup>a</sup> Student's t test; <sup>b</sup>  $\chi^2$  test; <sup>c</sup> Fisher's Exact Test.

of the filtrate was analysed on a Gas Chromatograph (CP-3800, Varian USA) coupled to a Mass Spectrometer (1200 TQ, Varian USA) using Factor four VF-5 ms column (0.25 mm internal diameter, 30 m, film thickness 0.25  $\mu$ m). The carrier gas was helium, and the flow rate was set at 1 ml/min. The GC oven was held at 40°C for 5 min and then increased to 310°C at the rate of 5°C/min. The recovery rate of extraction of benzene was 99%.

#### Peripheral blood cell count

Twelve blood parameters derived from a complete blood cell count (CBC) were used in this study. These blood parameters were selected based on data from previous studies on benzene. The parameters were: total white blood cells (WBC) with four WBC subtypes (neutrophils, eosinophils, monocytes, and lymphocytes), total red blood cells (RBC) with five red cell-related measures (hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH)) and platelets.

The samples were analyzed using the same analyzer (Sysmex SE 9000), which was calibrated daily.

#### Determination of specific IgG antibodies against benzene metabolites, HQ and p-BQ

The levels of specific IgG antibodies to HQ and p-BQ were determined as previously described (Dimitrova et al., 2005). Briefly, 100  $\mu$ l of Bovine Serum Albumin 2% (SIGMA) were applied as a carrier to fix the haptens on the flat-bottom microtiter plates. After 2 h of incubation at 37°C, plates were washed 4 times with PBS-Tween 0.05% and 100  $\mu$ l of pure chemicals HQ and pBQ (SIGMA, kindly provided by Prof. Herman Autrup, Aarhus University, Denmark) at 5.10<sup>-5</sup> mg/ml in NaCl 0.9% were added. The plates were incubated for additional 2 h at 37°C followed by a shock-cold fixation at 4°C overnight to allow the formation of covalent bound with BSA. The plates were washed 4 times and 100  $\mu$ l of plasma (1/1000) were added.

After 2 h of incubation at 37°C, plates were washed 4 times and anti-human IgG peroxidase conjugate (1/5000) was added. After 1.5 h incubation at 37°C, the plates were washed again and 100  $\mu$ l substrate solution of ortho-phenyl-enediamine 2.2 mg/ml (o-PDA,

SIGMA) in 0.05 M phosphate-citrate buffer (pH 5.0), containing 0.01% hydrogen peroxide was added. After 30 min of incubation in the dark at room temperature the reaction was stopped with 50  $\mu$ l of 4 M HCl (MERCK), and optical density (OD) was measured at 492 nm using Universal Microplate Reader Bio-tek Instruments ELx800. Each plasma sample was analyzed in triplicate to assure the reproducibility of the data. To check the quality of the results obtained, plasma pools containing a low (from village residents) and high (from MBTD) concentration of specific IgG antibodies were analyzed in each series of assays. The results from within-day and between-day quality controls samples demonstrated high reproducibility (RSD < 10%, data not shown).

#### Statistical analysis

All data were tested for normal distribution using the test of Kolmogorov-Smirnov. Most blood parameters were normally distributed except for eosinophils and WBC, which were skewed right necessitating a logarithmic transformation to obtain an adequate fit with the regression models. Correlation analysis was performed with Spearman rank order correlation. Linear regression analysis was used to estimate the influence of airborne benzene levels on blood cell parameters. We used general linear model (GLM) analysis to evaluate several covariates as potential confounders, including, age, alcohol consumption, BMI, current smoking and length of occupation. A level of P < 0.05 was considered statistically significant. All analyses were conducted using SPSS version 11.0 for Windows software.

## RESULTS

### Demographic characteristics and exposure assessment

The main characteristics of the participating subjects recruited are described in Table 1. There was no statistically significant difference concerning age, body mass index (BMI), current smoking and alcohol consumption between MBTD and the control group. All

**Table 2.** Summary of personal exposure monitoring for one-week.

Parameter	Control <sup>a</sup> (n = 5)		Mean ± SD	MBTD (n = 6)		Mean ± SD	P
	Median	Range		Median	Range		
Benzene (ppm)	0.001	0 - 0.002	0.001 ± 0.001	0.053	0.012-0.550	0.126 ± 0.206	0.0005
Toluene (ppm)	0.001	0 - 0.004	0.001 ± 0.002	0.093	0.026-0.172	0.083 ± 0.057	0.0004
p-Xylene (ppm)	0.001	0 - 0.001	0.001 ± 0.001	0.040	0.01 - 0.151	0.050 ± 0.051	0.008
o-Xylene (ppm)	0.001	0 - 0.001	0.001 ± 0.0001	0.034	0 - 0.091	0.041 ± 0.035	0.002

OD, optical density; MBTD, motorbike- taxi drivers <sup>a</sup>outdoor benzene levels, in previous years as described in Ayi-Fanou et al., 2006.

**Table 3.** Levels of specific IgG antibodies to HQ and p-BQ in study population.

Parameter	Control (n=30)		Mean ± SD	MBTD (n=144)		Mean ± SD	P value
	Median	Range		Median	Range		
Specific IgG antibodies (OD)	0.086	0.07 - 0.10	0.085 ± 0.011	0.241	0.10 - 0.630	0.262 ± 0.117	<0.0001

OD, optical density; MBTD, motorbike- taxi drivers.

participants were of the same social class. Within MBTD, the length of employment was  $11.3 \pm 5.96$  years (mean ± SD).

The results of personal exposure monitoring are presented in Table 2. The levels of BTX over one-week average exposure were much higher in exposed MBTD than in the rural population. MBTD were currently exposed to a wide range of benzene levels, with a median of 0.053 ppm, range: 0.012- 0.550 ppm, as average exposure over one-week.

#### Determination of specific IgG antibodies against HQ and p-BQ

The levels of specific IgG antibodies to HQ and p-BQ are summarized in Table 3. As shown in Table 3, motorbike-taxi drivers had a higher level of specific IgG antibodies to HQ and p-BQ than the rural population ( $p < 0.001$ , Student's t test).

As evidenced by the range, there were large inter-individual variations in the levels of specific IgG antibodies. In addition, MBTD driving more than five hour daily had a significantly higher level of specific IgG antibodies compared to those spending fewer time per day ( $p < 0.001$ , Student's t test, Figure 2).

The benzene concentration in the working atmosphere was significantly correlated with the levels of specific IgG antibodies to HQ and p-BQ ( $r = 0.522$ ;  $p < 0.01$ ). Furthermore, there was a strong correlation between the levels of specific IgG antibodies to HQ and p-BQ and the daily driving hour ( $r = 0.850$ ;  $p < 0.001$ ). In addition, we found a weaker but statistically significant correlation between the levels of specific antibodies to HQ and p-BQ and the duration of exposure i.e., duration of employment ( $r = 0.393$ ;  $p < 0.01$ ). Alcohol consumption had a significant impact on the levels of circulating IgG

antibodies to HQ and p-BQ ( $r = -0.230$ ;  $p < 0.01$ ), whereas other confounding factors, such as age, smoking habit had no effect.

#### Haematological changes

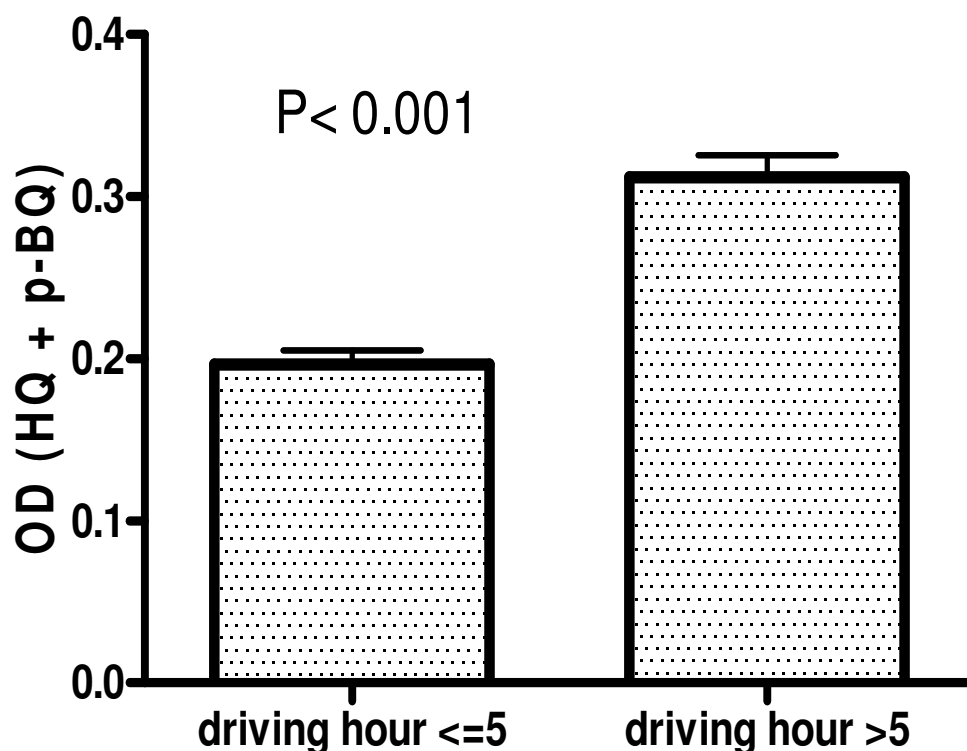
Exposed MBTD had lower total WBC, lymphocytes and eosinophils counts than controls (Table 4). Among WBC subtypes, the most significant difference was found for lymphocytes. Surprisingly, monocytes and neutrophils were significantly elevated in MBTD, even after adjustment for confounding factors. Correlations were significant between specific IgG antibodies to HQ and p-BQ and monocytes ( $r = 0.309$ ;  $p = 0.001$ ) and neutrophils ( $r = 0.277$ ;  $p = 0.001$ ). Red blood cell counts, haemoglobin, hematocrit and MCV were similar between MBTD and controls. The MCHC was significantly elevated in MBTD ( $P = 0.001$ ) and platelets were significantly decreased in MBTD compared to the rural population ( $P = 0.032$ ).

A further analysis of confounding variables with regard to white blood cells suggested that smoking status may have masked the association between benzene and WBC in the unadjusted analyses (not shown).

The analysis showed inverse correlations of specific IgG antibodies to HQ and p-BQ with lymphocytes ( $r = -0.35$ ,  $p < 0.01$ ) and eosinophils ( $r = -0.29$ ,  $p < 0.01$ ). Significant positive correlations were found between specific IgG antibodies levels and neutrophils ( $r = 0.28$ ,  $p < 0.01$ ) and monocytes ( $r = 0.31$ ,  $p < 0.01$ ).

#### DISCUSSION

This study investigated haematological changes in MBTD exposed to benzene at the levels between 0.012 and



**Figure 2.** Mean level of specific IgG antibodies to HQ and p-BQ according to daily driving hours. Legend: OD, optical density; HQ, hydroquinone; p-BQ, p-benzoquinone. The error bars depict the standard errors on the means

**Table 4.** Blood cell counts in relation to benzene exposure in the study population.

Variable*	Control (n=30)	MBTD (n=144)	P- value <sup>a</sup>
RBC ( $\times 10^{10}/L$ )	531 ± 67	516 ± 52	0.126
Hemoglobin (g/dl)	14.26 ± 1.56	14.12 ± 1.43	0.515
Hematocrit (%)	43.89 ± 4.88	44.76 ± 4.32	0.255
MCV (fl)	86.63 ± 7.63	86.93 ± 5.30	0.321
MCH (pg)	28.25 ± 3.36	27.43 ± 2.36	0.030
MCHC	32.54 ± 1.97	35.39 ± 3.22	0.001
WBC ( $\times 10^6/L$ )	5,900 ± 1,213	5,041 ± 1,209	0.001
Lymphocytes ( $\times 10^6/L$ )	3,200 ± 691	1,767 ± 334	< 0.001
Neutrophils ( $\times 10^6/L$ )	2,162 ± 672	3,078 ± 346	< 0.001
Monocytes ( $\times 10^6/L$ )	189.0 ± 89.0	355.8 ± 86.7	< 0.001
Eosinophils ( $\times 10^6/L$ )	351.7 ± 88	126.0 ± 47	< 0.001
Platelets ( $\times 10^9/L$ )	238 ± 66	198 ± 50	0.032

Definition of abbreviations: MBTD, motorbike-taxi drivers; RBC, red blood cell; MCV, mean corpuscular volume; fl, femtoliter; MCH, mean corpuscular hemoglobin; pg, picograms; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cell. \* Means ± standard deviation on raw data, but for WBC and eosinophils, the statistical tests were performed on the log transformed data. <sup>a</sup> P-value was estimated in GLM analyses, adjusting for age, alcohol drinking, BMI, current smoking, recent infection and duration of exposure.

0.550 ppm. The validity of specific IgG antibodies against HQ and p-BQ as an internal biomarker of benzene exposure was also assessed.

We found that exposed MBTD had elevated levels of specific IgG antibodies, as compared to the rural inhabitants. This exposure-dependent increase in the

levels of specific IgG antibodies to HQ and p-BQ is consistent with the findings of an earlier study conducted by Dimitrova et al. (2005). These authors have studied three groups of professionals heavily exposed to benzene (0.05 to 14.73 ppm). They found that percentages of people bearing specific IgG antibody increased with benzene concentration (Dimitrova et al., 2005).

In the present study, we found that the level of specific IgG antibodies to HQ and p-BQ showed stronger association with daily driving hour than with the duration of employment, suggesting that the length of real exposure to benzene is what drives the antibody response.

We found an inter-individual variation in the levels of circulating IgG antibodies to HQ and p-BQ. This variation could be related to differences in benzene metabolism. Furthermore, some of village residents might not declare the domestic use of wood. This may explain increased benzene exposure in some villagers, and hence, at least in part, individual variations seen in the levels of IgG antibodies. Domestic wood burning was previously shown to be an important source of benzene (Gustafson et al., 2007).

At present, the main role of specific IgG antibodies to HQ and p-BQ remains to be determined. Nevertheless, it has been suggested that they could have protective functions, facilitating elimination from the body of reactive metabolites or could be harmful for the immune reactions in the organism (Dimitrova et al., 2005).

Benzene metabolites such as HQ and p-BQ are haptenes, which form antigens in professionally exposed individuals leading to specific antibody generation. The specific phenotype of people for benzene metabolism and the concentration to which they are exposed may induce inter-individual variations. This hypothesis has also been suggested elsewhere (Dimitrova et al., 2005). Exposure to benzene at concentrations, for which no significant toxicity to bone marrow is expected, may lead to first, the haptenes, HQ and p-BQ, forming antigens and second, subsequent antibody production. We do not know if antibody induction also occurs however, with higher benzene concentrations that show toxicity to blood cells progenitors.

In the present study, individuals were all exposed to benzene levels lower than 1 ppm. There was no toxicity at that low concentration of benzene. This makes the difference with the study of Dimitrova et al. (2005). Nevertheless, in both studies the levels of specific antibodies against HQ and p-BQ were about 25 to 30% of that found in exposed individuals. Further studies are therefore needed for fine specificity testing of these antibodies. At present, we hypothesized that the antibody reactivity found in controls may result from cross-reactivity to the carrier protein used to measure antibodies to HQ and p-BQ. These antibodies found in controls might be specific to common recall antigens or simply as a result of minimal exposure to benzene in

controls. Benzene exposure will naturally lead to 3 different types of antibodies: the one type reacts with the haptenes alone, the second type reacts with the carrier protein alone and the 3<sup>rd</sup> reacts with the haptene-carrier conjugates presented to the immune system. Exploring these futures of a haptene-induced immune response with regard to the background responses found in controls, should be targets of subsequent studies. Our results cannot exclude contributions from haptene-like substances in human diet with impact on parameters used in this study. The control participants of the study may equally be exposed to very low levels of benzene deriving from gasoline usage for instance.

We found lower total WBC, lymphocyte, eosinophils and platelet counts in MBTD in comparison to controls. These findings are consistent with many recent studies in which leucopenia has been invariably shown to be the most sensitive effect on blood cells associated with benzene exposure (Qu et al., 2002; Rothman et al., 1996a; Lan et al., 2004; Bogadi-Sare et al., 2000; Rothman et al., 1996b). In one study, however, no decrease was found in WBC number among workers exposed to low levels of benzene (0.14 to 2.08 ppm) (Khuder et al., 1999).

The present data clearly showed reduction of the number of lymphocytes (about 40-55%) with no significant influence of confounding factors. This result is also in agreement with the findings from Lan et al. (2004), that reported decreased absolute lymphocyte counts in shoe workers exposed to < 1 ppm benzene in comparison to unexposed controls (Lan et al., 2004). However, Collins et al. (1997) found no increase in the prevalence of lymphopenia and other measures of hematotoxicity, including MCV and counts of total WBC, RBC, haemoglobin, and platelets which produced similar results among workers with daily 8-hour time-weighted exposures averaging 0.55 ppm. Similar conclusion was reported in a large study on 1,200 employees exposed to a mean average concentration of 0.60 ppm (Tsai et al., 2004).

The mechanism underlying reduction in lymphocytes and eosinophils (reduced by 30-40%) is probably the same, i.e. direct cytotoxicity of benzene metabolites on production of WBC in the bone marrow. Benzene is known as immunotoxic and blood cell carcinogen, inducing anaemia, and blood formula modification (Snyder et al., 1993; Smith et al., 1999).

We found significantly elevated neutrophils counts in exposed MBTD compared to the rural residents. These findings are inconsistent with some previous studies, which reported decreased neutrophil counts as the most sensitive blood parameter in benzene-exposed workers (Qu et al., 2002; Robert et al., 2010). Previous reports suggest that human exposure to benzene concentration of 1 ppm or below does not produce depression of neutrophils and monocytes (Lan et al., 2004; Kang et al., 2005). Increased neutrophil counts have been associated

with cancer (Waugh and Wilson, 2008) and bacterial infections.

In our present study, RBC counts remained unchanged between village inhabitant and exposed MBTD. This result is consistent with many studies, which reported the same at benzene exposure levels ranging from 1.9-14.8 ppm (Bogadi-Sare et al., 2003) and 0.005-5.3 ppm (Kang et al., 2005). However, others reported decreased RBC counts at benzene levels ranging from 0.14-2.08 ppm (Khuder et al., 1999) or found declined RBC in heavily benzene-exposed workers (maximum level of 34 ppm) (Ward et al., 1996).

Considering data from these previous studies, the absence of differences in RBC counts in our study may be due to the low levels of benzene in the ambient air in Cotonou.

Our data corroborated and extended the results of previous studies conducted in people living and working in the city of Cotonou. These studies found an increased in the level of leukaemia (Zinsou et al., 1990), blood neoplasm, neutropeny and lymphopeny (Adjagba, 2000).

In conclusion, exposure to benzene at the levels between 0.012 and 0.550 ppm is associated with decreased WBC, lymphocyte and eosinophil counts in MBTD. These MBTD developed antigens containing HQ and p-BQ haptenes and antibodies of IgG type. The present data allows to conclude that antibodies against p-BQ and HQ are very promising tools and should be further validated for assessment of human exposure to low concentrations of benzene at the working places.

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## REFERENCES

- Adjagba MPD (2000). Study contribution epidemiological cancer in the Republic of Benin. PhD in Medicine at the National University of Benin. No. 868.
- Avogbe PH, Ayi-Fanou L, Autrup H, Loft S, Fayomi B, Sanni A, Vinzents P, and Møller P (2005). Ultrafine particulate matter and high-level benzene urban air pollution in relation to oxidative DNA damage. *Carcinogenesis*, 26: 613-620.
- Ayi Fanou L, Mobio TA, Creppy EE, Fayomi B, Fustoni S, Møller P, Kyrtopoulos S, Georgiades P, Loft S, Sanni A, Skov H, Ovrebø S, Autrup H (2006). Survey of air pollution in Cotonou, Benin - air monitoring and biomarkers. *Sci. Total Environ.*, 358: 85-96.
- Bogadi-Sare A, Zavalic M, Turk R (2003). Utility of a routine medical surveillance program with benzene exposed workers. *Am. J. Ind. Med.*, 44: 467-473.
- Bogadi-Sare A, Zavalic M, Trosic I, Turk R, Kontosic I, Jelcic I (2000). Study of some immunological parameters in workers occupationally exposed to benzene. *Int. Arch. Occup. Environ. Health*, 73: 397-400.
- Collins JJ, Ireland BK, Easterday PA, Nair RS, Braun J (1997). Evaluation of lymphopenia among workers with low-level benzene exposure and the utility of routine data collection. *J. Occup. Environ. Med.*, 39: 232-237.
- Dimitrova ND, Kostadinova RY, Marinova SN, Popov TA, Panev TI (2005). Specific immune responses in workers exposed to benzene. *Int. Immunopharmacol.*, 5: 1554-1559.
- Ducos P, Gaudin R, Robert A, Francin JM, Maire C (1990). Improvement in HPLC analysis of urinary trans-trans-muconic acid, a promising substitute for phenol in the assessment of benzene exposure. *Int. Arch. Occup. Environ. Health*, 62: 529-534.
- Fustinoni S, Buratti M, Campo L, Colombi A, Consonni D, Pesatori AC, Bonzini M, Farmer P, Garte S, Valerio F, Merlo DF, Bertazzi PA (2005). Urinary t,t-muconic acid, S-phenylmercapturic acid and benzene as biomarkers of low benzene exposure. *Chem. Biol. Interact.*, 153-154: 253-256.
- Gustafson P, Barregard L, Strandberg B, Sallsten G (2007). The impact of domestic wood burning on personal, indoor and outdoor levels of 1,3-butadiene, benzene, formaldehyde and acetaldehyde. *J. Environ. Monit.*, 9: 23-32.
- Hayes RB, Songnian Y, Dosemeci M, Linet M (2001). Benzene and lymphohematopoietic malignancies in humans. *Am. J. Ind. Med.*, 40: 117-126.
- INSAE, National Institute of Statistics and Economic Analysis (2008). Dashboard. Economic and Social Council of 4th Quarter, 2002 to 2030, <http://www.insae-bj.org>.
- IARC (1987). Monographs on the Evaluation of Carcinogenic Risks to Humans, Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs, Lyon, France. 1-42: 120-122.
- Kang SK, Lee MY, Kim TK, Lee JO, Ahn YS (2005). Occupational exposure to benzene in South Korea. *Chem. Biol. Interact.*, 153-154: 65-74.
- Kèlomé NC, Lévêque J, Andreux F, Milloux MJ, Oyédé LM (2006). 14C plant isotopic composition ( $\delta^{13}C$ ) evidence for urban CO<sub>2</sub> pollution in the city of Cotonou, Benin (West Africa). *Sci. Total Environ.*, 366: 439-447.
- Khuder SA, Youngdale MC, Bisesi MS, Schaub EA (1999). Assessment of complete blood count variations among workers exposed to low levels of benzene. *J. Occup. Environ. Med.*, 41: 821-826.
- Lan Q, Zhang L, Li G, Vermeulen R, Weinberg RS, Dosemeci M, Rappaport SM, Shen M, Alter BP, Wu Y, Kopp W, Waidyanatha S, Rabkin C, Guo W, Chanock S, Hayes RB, Linet M, Kim S, Yin S, Rothman N, Smith MT (2004). Hematotoxicity in workers exposed to low levels of benzene. *Sci.*, 306: 1774-1776.
- Lange A, Smolik R, Zatoński W, Szymańska J (1973). Serum immunoglobulin levels in workers exposed to benzene, toluene and xylene. *Int. Arch. Arbeitsmed.*, 31: 37-44.
- Leong ST, Muttamara S, Laortanakul P (2002). Influence of benzene emission from motorcycles on Bangkok air quality. *Atmos. Environ.*, 36: 651-661.
- Macedo SM, Vaz SC, Lourenço EL, de Sousa Mda G, Ligeiro-Oliveira AP, Ferreira JM Jr, Almeida SR, de Lima WT, Farsky SH (2007). In vivo hydroquinone exposure impairs allergic lung inflammation in rats. *Toxicol.*, 241: 47-57.
- Nielsen PS, Andreassen A, Farmer B, Overbo S, Autrup H (1996). Biomonitoring of diesel exhaust exposed workers DNA and hemoglobin adducts and urinary 1-hydroxypyrene as markers of exposure. *Toxicol. Lett.*, 86: 27-37.
- Pyatt DW, Stillman WS, Irons RD (1998). Hydroquinone, a reactive metabolite of benzene, inhibits NF- $\kappa$ B in primary human CD4+ T lymphocytes. *Toxicol. Appl. Pharmacol.*, 149: 178-184.
- Qu Q, Shore R, Li G, Jin X, Chen LC, Cohen B, Melikian AA, Eastmond D, Rappaport SM, Yin S, Li H, Waidyanatha S, Li Y, Mu R, Zhang X, Li K (2002). Hematological changes among Chinese workers with a broad range of benzene exposures. *Am. J. Ind. Med.*, 42: 275-285.
- Robert SA, Kerzic PJ, Zhou Y, Chen M, Nicolich MJ, Lavelle K, Armstrong TW, Bird MG, Lin L, Fu H, Irons RD (2010). Peripheral blood effects in benzene-exposed workers. *Chem. Biol. Interact.*, 184: 174-181.
- Rothman N, Li GL, Dosemeci M, Bechtold WE, Marti GE, Wang YZ, Linet M, Xi LQ, Lu W, Smith MT, Titenko-Holland N, Zhang LP, Blot W, Yin SN, Hayes RB (1996a). Hematotoxicity among Chinese workers heavily exposed to benzene. *Am. J. Ind. Med.*, 29: 236-246.



- Rothman N, Smith MT, Hayes RB, Li GL, Irons RD, Dosemeci M, Haas R, Stillman WS, Linet M, Xi LQ, Bechtold WE, Wiemels J, Campleman S, Zhang L, Quintana PJ, Titenko-Holland N, Wang YZ, Lu W, Kolachana P, Meyer KB, Yin S (1996b). An epidemiologic study of early biologic effects of benzene in Chinese workers. *Environ. Health Perspect.*, 104: 1365-1370.
- Smith MT (1999). Benzene, NQO1, and genetic susceptibility to cancer. *Proc. Natl. Acad. Sci. USA*, 96: 7624-7626.
- Smith MT, Zhang L, Jeng M, Wang Y, Guo W, Duramad P, Hubbard AE, Hofstadler G, Holland NT (2000). Hydroquinone, a benzene metabolite, increases the level of aneusomy of chromosomes 7 and 8 in human CD34-positive blood progenitor cells. *Carcinogenesis*, 21: 1485-1490.
- Snyder R, Hedli CC (1996). An overview of benzene metabolism. *Environ. Health Perspect.*, 104: 1165-1171.
- Snyder R, Witz G, Goldstein BD (1993). The toxicology of benzene. *Environ Health Perspect.*, 100: 293-306.
- Subrahmanyam VV, Kolachana P, Smith MT (1991). Hydroxylation of phenol to hydroquinone catalyzed by a human myeloperoxidase-superoxide complex: possible implications in benzene-induced myelotoxicity. *Free Radic. Res. Commun.*, 15: 285-296.
- Tsai SP, Fox EE, Ransdell JD, Wendt JK, Waddell LC, Donnelly RP (2004). A hematology surveillance study of petrochemical workers exposed to benzene. *Regul. Toxicol. Pharmacol.*, 40: 67-73.
- Ward E, Hornung R, Morris J, Rinsky R, Wild D, Halperin W, Guthrie W (1996). Risk of low red or white blood cell count related to estimated benzene exposure in a rubberworker cohort (1940-1975). *Am. J. Ind. Med.*, 29: 247-257.
- Waugh DJ, Wilson C (2008). The interleukin-8 pathway in cancer. *Clin. Cancer Res.*, 14: 6735-6741.
- Wiwanitkit V, Soogarun S, Suwansakri J (2007). A correlative study on red blood cell parameters and urine trans, trans-muconic acid in subjects with occupational benzene exposure. *Toxicol. Pathol.*, 35: 268-269.
- Zhang L, Rothman N, Wang Y, Hayes RB, Bechtold W, Venkatesh P, Yin SN, Wang YZ, Dosemeci M, Li GL, Lu W, Smith MT (1996). Benzene induces aneuploidy of chromosome 9 in the lymphocytes of exposed workers. *Environ. Health Perspect.*, 104: 1325-1329.
- Zinsou CP, Fourn L, Zohoun T (1990). Epidemiological aspects of cancer at the National Centre University Hospital of Cotonou. *Med. Black Afr.*, 37: 230-236.