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Research Article

Benzene and Lipid Asset

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ABSTRACT

Background: In relation to the medico-social and medico-legal aspects of urban pollution we studied the effects of benzene present in urban pollution on outdoor workers exposed to physical, chemical, and psychosocial stressors. The purpose of this study is to evaluate the possible correlation between the levels of benzene and its urinary metabolites in the blood (Trans muconic acid and S-phenylmecapturic acid) and the parameters of the lipid structure: Total cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), triglycerides and blood sugar.

Materials and methods: From an initial group 1,500 we selected a group of 199 subjects. A blood sample was taken for each worker to assess blood benzene levels and urinalysis to determine the levels of trans, trans-muconic acid and S-phenyl mercapturic acid. We compared the mean and standard deviation of the following lipid parameters: Total Cholesterol, HDL, LDL, Triglycerides and glycemia with benzene and urinary metabolites; we excluded the workers with confounding factors and performed the Pearson's correlation between lipid parameters and urinary metabolites in the total sample and also among age, seniority, sex and BMI; multiple linear regression was performed for the evaluation of the main confounding factors.

Results: We did not find a statistically significant alteration between the values of the lipid structure between the two groups of workers and the parameters of benzene. Triglycerides and HDL are statistically significantly influenced by sex ($p=0.001$) and ($p=0.00$) and BMI ($p=0.00$) and ($p=0.001$) as well as total cholesterol is influenced in a statistically significant way from age ($p=0.003$) and blood glucose from BMI ($p=0.002$) A statistically significant difference was found among the averages of phenylmercapturic S acid values of traffic policeman and police drivers ($p<0.05$), where higher values were among drivers.

Conclusions: The results suggest that occupational exposure to levels of benzene, present in urban pollution, would appear not to influence the values of the lipid parameters in traffic policeman.

Keywords: Lipid structure; pollution, indoor workers; outdoor workers; chemical risk; Benzene; trans-muconic acid; S-phenyl mercapturic acid.

INTRODUCTION

Considering the importance from a medico-social point of view of urban pollution on the general population and its medico-legal effects, we studied the benzene present in urban pollution on outdoor workers, as an indicator of the general population, exposed to physical, chemical, and psychosocial stressors. Benzene is a liquid and colorless organic compound with a characteristic aromatic smell. From the structural point of view it is an aromatic six carbon atoms hydrocarbon, with the formula C_6H_6. It is therefore included in the group of VOCs.

Benzene is present everywhere in the air due to:

- Natural events (forest fires or gas leaks from volcanoes)
- Human and industrial activities that use crude oil and its derivatives as fuels or for the production of lubricants, solvents and adhesives

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• Exhaust gases from motor vehicles, since benzene constitutes about 80% of total emissions into the air where, it degrades, in few days, by reacting with other compounds. Wind and rain, in turn, help dilute and reduce the levels of benzene in the air, as they make it fall and deposit on the ground

Benzene is used in the production of plastics, rubbers, resins, inks, pesticides, adhesives, synthetic fibers (nylon), lubricants, detergents, adhesives, drugs, etc. It is also added as an anti-knock to "green" gasolines to increase the octane number (up to a maximum allowed of 1% by volume).

As regards living environments, contamination by benzene is therefore attributable to the following emission sources:

• Confined spaces: tobacco smoke, glues, paints, furniture waxes, detergents, kitchens, fireplaces.

• External environment: automobile exhaust gases, forest fires, volcanic eruptions, gasoline refueling areas, industrial emissions, oil refineries.

Benzene is rapidly absorbed (in a percentage of 30%-50%) after inhalation. After ingestion, absorption through the gastrointestinal tract is almost 100%, while less than 1% is absorbed through the skin. After absorption, benzene is distributed in body fluids, regardless of the route of intake. The concentrations rapidly decay once the exposure ceases.

Urban pollution is an environmental health problem in many cities around the world not only for the general population, and in particular for urban workers professionally exposed to low doses of air pollutants (fire-fighters, street sweepers, newsagents, taxi drivers and bus drivers, etc.). Motor vehicle exhaust and evaporation loss during the handling, distribution and storage of gasoline are recognized as a major source of aromatic hydrocarbons such as benzene in urban air.

As regards the risks to human health, the toxic effects caused by this organic compound have different characteristics and affect substantially different organs based on the exposure time.

In case of acute exposure, benzene is irritating to the skin and mucous membranes and, like other organic solvents, has an effect on the central nervous system. Depending on the concentration level or the doses, benzene can cause dizziness, light-headedness, headache and vomiting. High concentrations lead to unconsciousness and exposure to very high levels can lead to death.

Longer exposure periods to low doses of benzene have effects on the blood (haematopoietic system). In fact, benzene causes toxicity to the bone marrow (producer of blood cells), causing a reduction in red and white blood cells resulting in anemia. Studies have shown that benzene exposure can cause detrimental effects on female reproductive health and pregnancy outcomes; also some of our previous studies showed hormonal balance changes in both female and male traffic police personnel. Exposed to urban pollutants.

The International Cancer Research Agency (IARC) classifies benzene as a "known human carcinogen" with "sufficient" evidence that it causes acute myeloid leukaemia. As benzene is classified as a genotoxic carcinogen, efforts are being made to reduce occupational exposure of workers and to define long-term effects for chronic exposure to low-dose of benzene for people working in urban environments and for the general population. For these reasons, it is necessary for workers exposed to benzene to identify suitable markers for biological control through the analysis of blood benzene, urinary trans, trans muconic acid (t, t-MA) and S-Phenyl Acid Mercapturic Urinary (S-PMA). The determination of blood benzene at the end of the shift is the most sensitive and specific method as it shows the most recent exposure to benzene [1-20].

Previous studies suggested the possibility that combustion processes can cause effects on the lipid metabolism and on the mechanism of action of some chemical agents present in urban pollution [21].

Although the toxic effects of benzene are known, little is known about the actual damage it causes in other body areas, such as the lipid structure (Total Cholesterol, HDL, LDL, triglycerides and blood sugar).

In our previous studies we suggested the possibility that urban pollution may involve other reactions of the lipid structure [22, 23].

More recently we also suggested the possibility that the cadmium present in urban pollution may cause changes in the lipid structure [24].

Considering the above and that the results of the research conducted so far are uncertain and that no study has been conducted so far on the effects of Benzene present in urban pollution on the lipid structure of employees of the outdoor Municipal Police, we have a suspicion aware that the results could not confirm the 'hypothesis, that benzene, a monocyclic aromatic hydrocarbon, a pollutant present in urban air in very low doses, is responsible for alterations of the lipid structure, in employees of the outdoor municipal police never studied before

The purpose of this study is to estimate the levels of personal exposure to Benzene present in urban pollution and the possible correlation between this exposure and an alteration of the parameters of the lipid structure (Total Cholesterol, HDL, LDL, triglycerides and glycaemia) in a group of outdoor workers in a large Italian city.

MATERIALS AND METHODS

Population

The study was conducted on an initial group of about 1,500 cases of the Municipal Police of a large Italian city, who perform outdoor tasks and are professionally exposed to urban pollutants.

The workers studied are traffic policeman , police drivers or subjects employed in other outdoor tasks, exposed to the urban environment for at least 80% of their working time (7 hours a day for 5 days/week) in a large Italian city with medium-high traffic density. The city where the subjects work was divided in 8 areas considered representative of the air quality. From each of these areas we selected an equal number of workers, randomly sorted.

Workers with the task of traffic policeman were assigned to control vehicular traffic in roads and areas with high and medium traffic density, to monitor and control traffic at intersections, parking lots and restricted traffic areas. The workers with the role of police drivers were assigned to traffic control and specific interventions in the event of road accidents and other activities including driving the car, as a driver or "second on patrol".

The workers with other outdoor duties were assigned to various roles including nucleus for assistance to the marginalized, external

construction workers, external activities in the field of Judicial Police, Environmental Police, etc.

Most of these activities were carried out in outdoor environments and only for the drivers were carried out in the car, for at least 80% of the working time (7 hours a day, 5 days a week). All workers were monitored once during the morning shift (07: 00-14: 00).

For inclusion in the study, each subject was administered a clinical anamnestic questionnaire, in the presence of a physician. The questionnaire included information regarding age, area of residence in the last 5 years, physiological anamnesis, especially focused on exposure to cigarette smoke and the possible presence of immune diseases, past and next medical history and information about non-occupational exposure to benzene.

With regard to exposure to cigarette smoke, we took into account the classification of the World Health Organization (WHO), classifying as smokers all subjects who declare in their medical history that they have smoked at least 100 cigarettes in their life and are currently smokers, or have stopped smoking less than six months before. The subjects thus identical were included in the study, because they accepted to refrain from smoking, according to our indications, for the entire week prior to carrying out the personal sampling.

To avoid the influence of confounding factors, we excluded from the study workers who reported being exposed during non-work activity to solvents, lubricants, detergents, etc. [25]. Drug users and regular alcoholic drinkers (alcohol consumption higher than 2 alcoholic units per day for men, where 1 alcoholic unit corresponds to approximately 12 grams of ethanol) [26]. We also excluded those who reported working in shifts and/or night shifts, practicing competitive sports and having carried out outdoor tasks for less than 1 year [27, 28].

The final sample was therefore made up of 199 workers (78 women and 121 men): 104 assigned to the duties of road operator (65 males and 39 females) and 95 with the role of driver and/or 2nd on patrol (56 males and 39 females).

Biological monitoring (evaluation of blood benzene), the relative urinary metabolites and the dosage of the parameters (Total Cholesterol, HDL, LDL, triglycerides and glycaemia) were carried out on all the subjects in the study; subjects with blood benzene values below the detection limit of the method were excluded from the study.

For the purposes of the statistical evaluation, the following factors were considered: gender, BMI, job title of the workers, age and seniority.

All subjects decided to make their personal information available after being aware that such data would be classified as "sensitive information". They also agreed that these would be treated anonymously and collectively, examined with scientific methods and analyzed for scientific purposes in accordance with the principles of the Declaration of Helsinki.

Blood benzene, metabolites and lipid parameters

The measurement of blood benzene is the most suitable test for evaluating occupational exposure to this agent, since there is a good correlation between the concentrations of Benzene in the air and those present in the blood of exposed subjects [29].

The dosage of blood benzene and the examination of liver parameters were carried out on 199 workers. A 10 mL venous blood sample was taken from each worker. The blood samples were stored in the workplace in the refrigerator at +4°C until they were transferred (inside a special case and at the same temperature) to the laboratory where they were centrifuged and subsequently stored at -20°C until they were tested (within 3 days).

Normal levels of all parameters analyzed were those ordinarily used by the laboratory for clinical analysis: blood glucose 60-110 mg/dL, 120-200 mg/100 ml for total cholesterol, 40-80 mg/100 ml for HDL, 70-180 mg/100 ml for LDL and triglycerides below 150 mg/dl.

The laboratory performed the blood benzene dosage through the extraction method with SPME technique and gas chromatography analysis with mass spectrometry detector with detection limit <150 ng/L. The reference values of blood benzene 0.017-0.72 µg/L are those proposed by the 2017 SIVR [29].

For each worker, the blood sampling for the determination of the benzene values and the urine sampling for the dosage of the metabolites (t, t MA and S-Pma) were performed after 5 continuous working days at the end of the work shift. The urine sample was immediately frozen and kept at -20 °C until analysis. The laboratory performed the analytical determination of t, t MA and S-Pma.

The determination of trans-trans muconic acid was performed using HPLC with UV spectrophoto-metric detector after SPE solid phase extraction [27, 28]. The Limit of Detection (LOD) is 50.0 µg/L. Normal values are between 15-145 µg/g of creatinine for non-smokers [28].

The analysis of S-phenyl mercapturic acid was carried out by extraction with ethyl acetate, esterification and analysis by GC using a mass spectrometry detector [29]. The Limit of Detection (LOD) is 5 µg/L. Values are considered normal if within the range <0.100-0.180 µg/g of creatinine for non-smokers [30-33].

Statistical analysis

The results for the blood benzene values and for those of the lipid parameters (Total Cholesterol, HDL, LDL, triglycerides and glycaemia) were expressed in terms of mean, Standard Deviation (SD) and range (min-max). The T test was used to compare the means in the total sample and after subdivision on the basis of sex, BMI, seniority, age and job.

Pearson's correlation was calculated to verify the level of association between the values of blood benzene and urinary metabolites with other factors that could be influential (age, seniority, sex, BMI), and the parameters of the lipid structure.

Multiple linear regressions was performed, considering the parameters of the lipid structure as a dependent variable and the blood benzene, the metabolites, sex, age, length of service and BMI as independent factors. The results were considered significant when p-values were lower than 0.05. The analysis was conducted using the SPSS Advanced Statistical 21.0 software.

RESULTS

Population

The descriptive characteristics of the sample, made up of 199 workers, are shown in (Table 1).

Table 1: Characteristics of the sample.

Characteristics	Sample 199	Drivers 95	Road workers 104	P
Medium of age ± ds (min-max)	44,20 ± 8,08 (28-64)	45,04 ± 7,74 (28-60)	43,42 ± 8,35 (29-64)	0,080
Medium of working seniority ± ds (min-max)	13,99 ± 8,21 (1-36)	15,98 ± 7,21 (5-34)	12,17 ± 8,68 (1-36)	0,000
Sex	121 M 78 F	56 M 39 F	65 M 39 F	0
Bmi ± ds (min-max)	24,90±3,54 (15,84-34,41)	25,08±3,74 (15,84-34,16)	24,74±3,35 (17,47-34,41)	0,815

Note: *Statistically significant for p<0.05

In the group of driver, we found 3 workers (3.19%) outside the normal range for blood glucose values, 55 (58.51%) for total cholesterol, 1 (1.06%) for HDL, 16 (15.04%) for LDL, 19 (20.21%) for triglycerides. We did not find a statistically significant alteration between lipid trim values and benzene levels.

In the group of road traffic, we found 7 (6.73%) workers outside the normal for blood glucose values, 54 (51.92%) for total cholesterol, 1 (0.96%) for HDL, 7 (6.73%) for LDL, 12 (11.53%) for triglycerides.

There are no statistically significant differences between traffic policeman and police drivers for the parameters of lipid structure, blood benzene and trans-muconic acid, the averages of phenylmercapturic acid values instead are statistically and significantly higher in the group of drivers.

In the total sample 13 workers (6.53%) show blood benzene value above the limit of 720 ng/l proposed by the Italian Society of Reference Values (30), in particular 6 road operators and 7 drivers.

34 (17.08%) workers exceed the maximum limit of 145 µg/g of creatinine for transmuconic acid proposed by S.I.V.R. (30), in particular 19 road operators and 15 drivers. For phenylmercapturic acid 199 subjects (100%) exceed the reference range (0.100-1.89 µg/g of creatinine).

As regards the values of the biological indicators of occupational exposure proposed by the ACGIH, 2 workers (road workers) exceed those for transmuconic acid (500 µg/g of creatinine) and 2 (drivers) for phenylmercapturic acid (25 µg/g of creatinine) [29, 30, 31].

Blood benzene, metabolites and lipid structure parameters

The values of the blood benzene concentrations, its metabolites and the values of the lipid content in the studied population, and in the two subgroups divided by job are shown in (Tables 2 and 3).

A statistically significant difference was found between the mean values of phenylmercapturic S acid of traffic policeman and police drivers (p<0.05), with higher values among police drivers (Table 3).

The statistical regression analysis (Table 4) did not show, in our total sample, a statistically significant correlation between the blood benzene levels and the parameters of the lipid structure.

Table 2: Lipid structure parameters in the two groups.

Parameters	Sample 199	Drivers 95	Road Workers 104	P
Cholesterol tot (mg/dL)	209,74 ± 36,75 (126-317)	213,13 ± 40,791 (126-297)	205,74 ± 32,28 (140-317)	0,134
HDL (mg/dL)	52,11 ± 12,97 (21-91)	52,60 ± 13,144 (30-0,91)	52,48 ± 12,75 (21-81)	0,864
LDL (mg/dL)	134,41 ± 34,25 (56-228)	137,78 ± 37,74 (137,78-37,44)	131,30 ± 29,64 (80-228)	0,139
Triglycerides (mg/dL)	112,32 ± 71,13 (32-416)	111,88 ± 72,407 (32-351)	108,08 ± 68,23 (35-416)	0,735
Blood sugar (mg/dL)	88,37 ± 17,37 (21-220)	88,69 ± 17,60 (63-220)	88,93 ± 16,45 (21-172)	0,95

Note: *Statistically significant for p <0.05

Table 3: Blood benzene and urinary metabolites in the two groups.

Metabolites	Sample 199	Drivers 95	Road workers 104	P
Blood benzene (ng/L)	291,11 ± 303,79 (150-1926)	269,71 ± 302,166 (150-1926)	312,78 ± 274,72 (150-1911)	0,357
Tt muconic acid(µg/g di creatinina)	90,75 ± 82,32 (50-583)	101,76 ± 79,786 (50-490)	100,70 ± 106,60 (50-583)	0,708
Phenyl-mercapturic acid (µg/g di creatinina)	5,65 ± 4,03 (0-50)	6,450 ± 5,60 (5-50)	5,34 ± 1,66 (0,20-15,80)	0,041*

Note: *Statistically significant for p<0.05

Table 4: Correlation in the total sample.

Statisticals	Correlations	LDL Cholesterol	HDL Cholesterol	Triglycerides	TOT Cholesterol	Blood Sugar	AcidS Phenyl Mercapturicides
LDL Cholesterol	Correlazione di Pearson	1	,005	,024	,927**	,070	,096
	Sig. (2-code)		,939	,737	,000	,329	,175
	N	199	199	199	199	199	199
HDL Cholesterol	Correlazione di Pearson	,005	1	-,493**	,168*	-,302**	-,011
	Sig. (2-code)	,939		,000	,018	,000	,877
	N	199	199	199	199	199	199
Triglycerides	Correlazione di Pearson	,024	-,493**	1	,232**	,232**	,061
	Sig. (2-code)	,737	,000		,001	,001	,388
	N	199	199	199	199	199	199
TOT Cholesterol	Correlazione di Pearson	,927**	,168*	,232**	1	,046	,106
	Sig. (2-code)	,000	,018	,001		,518	,135
	N	199	199	199	199	199	199
Blood Sugar	Correlazione di Pearson	,070	-,302**	,232**	,046	1	-,015
	Sig. (2-code)	,329	,000	,001	,518		,828
	N	199	199	199	199	199	199
AcidS Phenyl Mercapturicides	Correlazione di Pearson	,096	-,011	,061	,106	-,015	1
	Sig. (2-code)	,175	,877	,388	,135	,828	
	N	199	199	199	199	199	199

Note: **The correlation is significant at the 0.01 (2-tailed) level.
*The correlation is significant at the 0.05 (2-tailed) level.

DISCUSSIONS

Benzene is among the most studied pollutants in the urban environment due to its known role in some pathologies in various organs and systems. Benzene could act on the lipid metabolism as already suggested in other studies [21]. In fact, it is shown that benzene begins its biotransformation in the liver and that cytochrome P450 catalyzes the addition of a single oxygen atom to the benzene ring forming benzene oxide. Part of this combines with glutathione through the glutathione-s-transferase pathway altering its function, generating oxygen radicals and activating lipid peroxidation. Some pollutants dispersed in the atmosphere seem to act on the lipid structure in a quantitative way, increasing the LDL cholesterol values and causing a decrease in the HDL cholesterol value in exposed subjects in accordance with various studies.

Although some studies showed how the particles from combustion processes are able to generate oxidative damage in the DNA and an increase in blood lipids as a result of the action on the insulin resistance and lipid peroxidation, with the result of a metabolism shifted to lipogenesis, no one has focused on the influence that benzene can have individually on the parameters of the lipid structure [34-36].

Possible mechanisms of action aimed at explaining the alterations induced by benzene on the cardiovascular system are described in the literature: the release into the bloodstream of pro-oxidative and pro-inflammatory mediators capable of altering the LDL cholesterol already present in the blood, and the direct action on the heart and blood vessels of the ultrafine particles translocated into the systemic circulation which would lead to a greater accumulation of LDL in the vessels. Jacob et al., in agreement with other studies showed that it is the effect of chronic exposure to airborne pollutants that causes the serum increase in LDL levels oxidized or not. However, we can hypothesize that benzene; acting on the lipid peroxidation may have a synergistic role on the lipid metabolism, together with the other chemical pollutants present in the atmosphere [37-58].

CONCLUSIONS

As regards the values of phenylmercapturic acid, it emerges, from our study that 100% of the sample is exposed, because the values found are higher than the maximum limit indicated by the SIVR. In particular, these values are higher and statistically significant in the group of drivers, we could hypothesize that since they work in indoor environments, the dispersion and dilution of the concentration of the substance is more difficult than in open

environments (such as for road operators).

As regards the subject of the study, a correlation between benzene and the lipid structure was not found, this may lead to reflect on the advisability of using the parameters of the lipid structure as makers of exposure to benzene; however, we found a statistically significant difference between the averages of the phenylmercapturic acid values in traffic policeman and police drivers ($p<0.05$), with higher values among drivers.

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