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Determination of Benzene and its Metabolite Phenol in the Urine Samples of Gas-Station Workers in the Greater Amman Municipality

***Mahmoud A. Alawi* ***

ABSTRACT

Thirty one urine samples were gathered from workers in twenty automobile gas- stations in the area of Greater Amman Municipality. The samples were analyzed for the contents of the aromatic benzene and its main metabolite phenol using High Performance Liquid Chromatography (HPLC) with the UV-detector after steam distillation and Liquid-liquid extraction. The benzene concentration in the urine samples ranged from 0.80 to 9.44 mg/L and for phenol between 0.16 and 20.77 mg/L. A clear correlation was found between the concentrations of phenol and benzene in the urine samples. Workers whom urinary benzene concentration was 6.5 mg/L or higher were found after six years that they get the Leukemia.

Keywords: Benzene, Phenol, Gas- Station Workers, Urine, Amman.

1. INTRODUCTION

Individuals employed in industries that manufactured or use benzene may be exposed to the highest levels of benzene (Agency for Toxic Substances, 1991). Benzene is found in emissions from coal and oil, motor vehicle exhaust, and evaporation from gasoline service stations and in industrial solvents. These sources contribute to elevate levels of benzene in the ambient air, which may subsequently be breathed by the public (Agency for Toxic Substances, 1991). Tobacco smoke contains benzene and account for approximately 50% of the public's exposure to benzene (Agency for Toxic Substances, 1991). Individuals may also be exposed to benzene by consuming contaminated water (Agency for Toxic substances, 1991), which contains more than 5 µg/L according to the Canadian guidelines for drinking water (Kindzierski, 1998).

Measurement of benzene in an individual's breath or blood (Angerer, 1991) or the measurement of some metabolites in the urine (phenol) can be used to estimate the levels of exposure (Agency for Toxic Substances, 1991). But it is pointed out that most of these methods do not fully reflect benzene exposure at very low pollution level as in the general environment (Kouniali, 2003 and Melikian, 2002). In the last decade, many publications have shown that the urinary unchanged benzene is well correlated with environmental exposure to benzene (Ikeda, 1999) and could be used for biological monitoring.

Most people are exposed to small amounts of benzene on a daily basis. One can be exposed to benzene in the outdoor environment, workplace, and at home. Exposure of the general population to benzene is mainly through breathing contaminated air. The major source of benzene exposure is automobile service stations.

Exposure to benzene liquid and vapor may irritate the skin, eyes, and upper respiratory tract. Redness and blisters may result from dermal exposure to benzene (Sittig, 1985).

Tests involving acute exposure of animals, such as the LC_{50} and LD_{50} tests in rats, mice, rabbits, and guinea pigs, have shown that benzene has low acute toxicity from inhalation, moderate acute toxicity from ingestion, and low or moderate acute toxicity from dermal exposure (U.S. Department of Health,1993).

Chronic (long-term) inhalation of benzene causes disorder in the human blood. Benzene specifically affects bone marrow (the tissues that produce blood cells). Aplastic anemia (a risk factor for developing nonlymphocytic leukemia), excessive bleeding, and damage to the immune system (by changes in blood levels of antibodies and less of white blood cells) may develop (Agency for Toxic Substances, 1991).

* Department of Chemistry, Faculty of Science, University of Jordan, Amman, Jordan. Received on 3/4/2008 and Accepted for Publication on 24/6/2008.

Increased incidence of leukemia (cancer of the tissues that form white blood cells) has been observed in human occupationally exposed to benzene (U.S. Environmental Protection Agency, 1993). Environmental Protection Agency (EPA) has classified benzene as a Group A, known human carcinogen (U.S. Environmental Protection Agency, 1993). EPA estimates that, if an individual were to breathe air containing benzene at 0.1 µg/m^3 over his or her entire lifetime, that person would have theoretically no more than a one-to-million increased chance of developing cancer as a direct result of breathing air containing benzene. Similarly, EPA estimates that breathing air containing 1.0 µg/m^3 would result in not greater than a one-to-hundred thousand increased chance of developing cancer, and air containing 10.0µg/m^3 would result in not greater than a one-to-ten thousand increased chance of developing cancer (U.S. Environmental Protection Agency, 1993).

In this study the levels of benzene and its metabolite phenol were monitored in thirty one urine samples taken from workers in twenty automobile Gas-stations in the area of Greater Amman municipality, who inhale benzene with the car fuel fumes.

2. GENERAL EXPERIMENTAL

Reagents

Analytical grade benzene, phenol, p-cresol (internal standard), ethyl acetate, sodium hydroxide, hydrochloric acid, acetic acid, sodium chloride and anhydrous sodium sulfate were purchased from Merck (Germany). Methanol (HPLC-quality) used for the mobile phase was purchased from GCC (UK).

HPLC Apparatus and Conditions

An isocratic HPLC-apparatus was used which was consisted of a Beckmann 114M solvent delivery pump connected to a Beckmann injector with a 20µl loop. A Varian Prostar UV detector 325 Module was used for detecting the concentration of benzene and phenol.

For the measurement of benzene a Reversed Phase RP-C8 column (250 x 4.6 mm, 5 µm) was used. The optimized eluent was composed of methanol/ water/ acetic acid (34:65: 1 v/v/v %) pumped at an optimized flow rate of 1.0 ml/min. The UV-detector was used at a wavelength of 280 nm and at a sensitivity range of 8. The attenuation of the used integrator was 0.0005.

For the measurement of phenol a RP-C18 column (250 x 4.6 mm, 5 µm) was used. The eluent was the same as that used for the measurement of benzene delivered at an optimized flow rate of 1.5 ml/min. The UV-detector was used at a wavelength of 218 nm and at a sensitivity range of 8. The attenuation of the used integrator was 0.0005.

Sampling and Sample Extraction

Thirty one urine samples were gathered from workers in twenty car service stations in the Greater Amman Municipality. The samples were gathered from the volunteers at the end of working day (after at least 8 hours working time) in glass containers, kept closed in a refrigerator and were analyzed in the same day.

The samples were extracted for benzene analysis as follows: 10.0 ml of the urine sample was placed in a 500-ml round bottom flask, then the sample was steam distilled. The distillate which was collected for 30 minutes in 250-ml round bottom flask, was transferred to a 250-ml separatory funnel. 2 ml ethyl acetate which contains 5 ppm p-cresol was added and the mixture was extracted for 5 minutes. The organic layer was dried over 1g anhydrous sodium sulfate, then transferred to a 2 ml vial. From this solution 20 µl were injected onto the HPLC column under the above mentioned conditions.

The samples were extracted for phenol analysis as follows: 10.0 ml of the urine sample were placed in a 500-ml round bottom flask, then it was steam-distilled. The distillate which was collected for half-hour in a 250-ml round bottom flask was transferred to a 400-ml separatory funnel and made alkaline with few drops of 2M NaOH. Five grams of highly pure sodium chloride were added and the solution was shaken for few minutes, and then 10ml ethyl acetate were added and the mixture was shaken for 5 minutes. The organic layer was discarded. Then the aqueous layer was made acidic by adding few drops of 1M HCl, 2 ml ethyl acetate containing 5 ppm p-cresol was added and the mixture was shaken for 5 minutes. The aqueous layer was discarded. The organic layer was dried over 1g anhydrous sodium sulfate, and t transferred to 2 ml vial. From this solution 20 µl were injected onto the HPLC column under the above mentioned conditions.

Minimum Limit of Detection

From the noise of the base line, the minimum limit of detection was calculated as signal-to-noise ratio of 3:1 and found to be 0.05 µg/ml for benzene and 0.05 µg/ml for phenol in urine when using 10 ml sample.

Calibration Curve, Linearity and Recoveries

For benzene, the relationship between concentration and peak area ratio between benzene and p-cresol (Internal standard) was linear for the range between 0.2 -1.8 µg/ml. The regression equation was Y = 0.1511X + 0.055 with a correlation coefficient of 0.9979. For phenol, the relationship between concentration and peak area ratio between phenol and p-cresol was linear for the range between 0.1 - 5.9 µg/ml. The regression equation was Y= 0.7372X + 0.1105 with a correlation coefficient of 0.9970.

The percent recoveries from spiked urine samples in the range of 1-10 µg/ml were found between 91.7 and 103.6% for benzene and between 83 and 107% for phenol.

3. RESULTS AND DISCUSSION

The results of the analysis of the urinary benzene and phenol are shown in table (1). Also table (1) contains information taken from the questionnaires filled by the volunteers like smoker or non-smoker and about health problems lime leukemia, headache or dizziness. Figure (1) shows the correlation between the concentrations of benzene and phenol in the same sample. This relationship has a regression equation of Y= 1.4735 X + 0.00 with a regression coefficient of 0.8677. This means that benzene is the source of phenol in the urine and the concentration of phenol is almost directly proportional to the concentration of benzene in the urine.

Benzene is carcinogen which leads to leukemia and is strictly regulated by the Occupational Exposure Limits (1). On the other hand, benzene is widely used as organic solvent, synthesis of materials and is contained in petrochemicals like automobile gasoline and fuel. Therefore, benzene contamination exists everywhere.

Table 1.

Average Concentrations (n=3) of Benzene and Phenol in the Urine Samples.

Donor Number	Smoker/ Non	Health Problems?	Benzene [mg/L]	Phenol [mg/L]
1	Non	H/D*	1.49±0.08	0.92± 0.06
2	Smoker	-	4.91±0.13	5.58±0.14
3	Non	H/D	2.10±0.10	1.51±0.08
4	Non	H/D	1.71±0.09	0.98±0.06
5	Non	H/D	1.06±0.07	0.72±0.05
6	Non	H/D	1.49±0.08	0.83±0.06
7	Non	-	0.72±0.05	0.61±0.05
8	Non	H/D	1.70±0.09	0.96±0.06
9	Non	H/D	2.00±0.10	1.43±0.07
10	Non	H/D	1.82±0.10	1.14±0.06
11	Non	H/D	1.75±0.09	1.03±0.05
12	Non	-	0.51±0.05	0.52±0.03
13	Non	H/D	2.40±0.11	2.11±0.08
14	Non	H/D	2.24±0.10	1.58±0.07
15	Smoker	Leukemia**	6.51±0.15	13.44±0.15
16	Non	-	0.28±0.04	0.18±0.01
17	Non	-	0.58±0.05	0.52±0.02
18	Smoker	H/D	4.62±0.12	4.27±0.12
19	Non	H/D	2.36±0.11	1.70±0.06
20	Smoker	H/D	4.02± 0.12	3.01±0.12
21	Non	-	0.67±0.06	0.53±0.03
22	Non	H/D	1.45±0.07	0.79±0.04
23	Non	-	0.71±0.06	0.61±0.03
24	Non	-	0.80±0.06	0.64±0.03
25	Non	H/D	1.95±0.10	1.43±0.05
26	Smoker	Leukemia	9.44±0.25	20.77±0.30
27	Smoker	H/D	4.11±0.12	3.15±0.10

28	Non	-	0.17±0.02	0.16±0.02
29	Smoker	H/D	4.97±0.12	10.33±0.13
30	Smoker	H/D	4.04±0.11	3.12±0.12
31	Smoker	H/D	3.38±0.10	2.15±0.10

** H/D means Headache and dizziness*

***Information after 6 years from sampling date*

Moreover, it is known that tobacco smokers are also exposed to benzene (Smith, 1997).Therefore, it is important to assess the human health risk caused by exposure to benzene through direct monitoring of benzene in the urine.

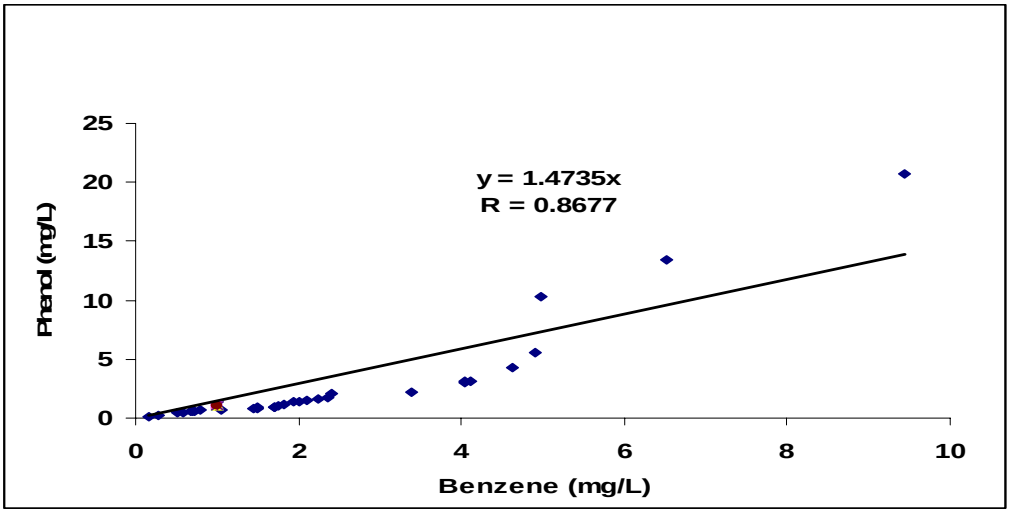

Fig.1. Correlation between Benzene and Phenol Concentrations in Urine

There is very little work about the analysis of benzene in the urine. Sun et. al (S. Suna, 2005) have analyzed benzene in the urine of smokers (range 0.08 – 0.42 µg/L) and non-smokers (0.02 – 0.17 µg/L). But this is the first time that benzene is analyzed in the urine of car-service station workers. Any how, the maximum allowable concentration for benzene is zero (Nell) (Seeger, 1988), because benzene is carcinogen. Therefore any amount could be found in the urine means a high risk for the human health. The benzene concentrations which we found (range 0.8 – 9.44 mg/L urine) is 10-22 fold higher than that found in the urine of smokers (S. Suna, 2005) and is high enough to predict that the Automobile Gas-station workers are exposed to high amounts of benzene. We found after six years from the sampling date that the two donors with the numbers 15 and 26, whom their urinary levels of benzene and phenol is extremely high, became Leukemia cases and most of the smoker donors among the car-service workers complains from headache and/or dizziness as shown in table (1).

Further studies on the relationship between the urinary benzene and environmental benzene exposure in the general population and especially the workers in the care service stations are necessary.

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تقدير تراكيز البنزين وناتج أيضة الفينول في عينات بول من عمال محطات البنزين من منطقة عمان الكبرى

محمود علاوي *

ملخـص

جمعت واحد وثلاثون عينة بول من عمال محطات البنزين في عشرين محطة بنزين في منطقة عمان الكبرى. حللت العينات على تراكيز البنزين الحلقي وعلى أهم نواتج أيضة ألا وهي مادة الفينول، باستخدام جهاز الكروماتوغرافيا السائلة ذات الأداء العالي وباستخدام المكشاف الفوق بنفسجي وذلك بعد الاستخلاص بطريقة التقطير البخاري والاستخلاص بالمذيبات. كانت تراكيز البنزين تتراوح بين 0.8-9.44 ملغرام/لتر وتراكيز الفينول بين 0.16–20.7 ملغرام/لتر من البول. وجدت علاقة واضحة ومباشرة بين تراكيز الفينول وتراكيز البنزين في البول. بعد ستّ سنوات من جمع العينات وتحليلها وجد أن المتبرعين الذين كانت عيناتهم تحتوي على تراكيز بنزين تساوي أو أعلى من 6.5 ملغرام/لتر وعددهم اثنان قد اصيبوا بسرطان الدم (اللوكيميا).

الكلمـات الدالـة: البنزين، مادة الفينول، عمال محطات البنزين، البول، عمّان.

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