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Research Article

Identification of Hippuric Acid and Phenols in the Urine of Workers with Occupational Exposure

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The current study was conducted on workers in the printing industry in Addis Ababa, the capital and largest city of Ethiopia, which has grown significantly in recent years, providing work for a large number of people whose health motivates us to carry out this work. Because these workers handle substances that appear innocuous but are toxic to human health, such as paint products like VOCs, inhaling these volatile and organic solvents is involuntary. If chronic, it manifests in various symptomatic forms in humans, such as changes in psychomotor function or organ damage, highlighting the need to investigate and evaluate their toxicity depending on the degree of aversion and repercussions experienced by those exposed to this type of solvent. The study was conducted on 40 workers who work in randomly selected locations in Addis Ababa, Ethiopia. For the quantification of total hippuric acid and phenols in urine, two different methods were used. Finally, a survey was carried out to obtain information on the level of knowledge of the toxicity of the chemical substances that use the degree of protection; once the results of the quantitative analyses were obtained and supplemented with the data made by the personal interview, it allowed us to obtain the necessary information to carry out the statistical evaluation and thus to interpret the real substance of the occupational exposure of contamination to which these workers are exposed. The total phenols found averaged 226.61 ± 3.62 mg/L, and hippuric acid is 2.126 ± 0.83 g/L. These values indicate benzene and toluene exposure because they exceed WHO reference values in urine.

1. Introduction

In recent years, the solvent chemical industry has benefited due to the great demand for products that include the manufacture of paints, glues or adhesives, degreasers, and cleaning agents and the production of polymers, plastics, textiles, agricultural, pharmaceutical, etc. Exposure to chemical substances, including solvents such as toluene and benzene, is common since they are used in the manufacture of inks; these exposures are regulated by biosafety standards [1], for example, personal protection implements and the establishment of maximum permissible levels of exposure. Despite this, there is always direct exposure to these solvents due to various factors, including the inadequate use of personal protection equipment or the lack of such implements such as adequate masks, extractor hoods, and fans [2]. This approval respected the technical document of "Protocol of Occupational Medical Examinations and Diagnostic Guides of Obligatory Medical Examinations by Activity" [3], which establishes the procedure for monitoring the health of workers to identify and control occupational risks in the worker, providing evidentiary information to support prevention and control measures in work environments; therefore, it is necessary to carry out periodic monitoring in biological samples of the said workers in order to detect inadequate exposure to these solvents whose maximum limits are regulated based on studies in which they show that they can cause affections to the central nervous system and in the blood, hence the need to evaluate their toxicity, effects, and repercussion on people exposed to this type of solvent [4]. Health and safety issues in tire manufacturing facilities have always been and continue to be of the utmost

importance. Often, the impact of serious accidents can mask illnesses associated with workplace exposures. Because of long latency periods, some diseases are not apparent until after the worker has left work. Many diseases caused by occupational exposures in tire manufacturing plants are never related to the work performed. But diseases like cancer are still prevalent among rubber plant workers [5]. Numerous scientific studies have been conducted with tire factory workers [6]. In some of them, an increase in mortality from bladder, stomach, lung, hematopoietic, or other types of cancer has been identified. This increase is not usually attributed to a specific chemical product, but prolonged exposures to different chemical substances and/or a simultaneous combination of several. Changes often occur in the formulation of the materials used in the manufacture of tires. These changes in the type and quantity of the components constitute an additional difficulty for the detection of the causal agents [12]. Many of the individual components to which workers are exposed are not regulated by government agencies and in many cases have not even been adequately investigated for toxicity or carcinogenicity (22).

According to Ibrahim et al. [7], aromatic hydrocarbons are benzene derivatives with six carbon-hydrogen groups. Plastics, synthetic rubber, paints, pigments, explosives, pesticides, detergents, chemical reagents, scents, and medications include benzene and toluene. They are also solvents in gasoline and physical-chemical analyses. The study examined phenol and hippuric acid levels in urine as biological markers of benzene and toluene exposure and their relationship with hepatic, renal, and hematological alterations: descriptive, cross-sectional, and nonexperimental [8]. Seven employees agreed. End-of-day and end-of-week urine samples were taken. P-Nitroaniline diazotized with alkaline media phenols was detected in urine at 525 nm. Blood samples at the beginning of the work day were used for hematological and biochemical parameters, hepatic transaminases (TGO and TGP), and creatinine. SPSS version 18 reported XSD and percentages. Urine phenol and hippuric acid were normal. Creatinine, transaminases, and hemoglobin were normal. Statistically, phenol levels and TGO were inversely related. All lab personnel use PPE, although most say there is no GPE. A large percentage ignores industrial hygiene and safety.

Cassini et al. (2011) examined the impact of chemical solvents and genotoxic consequences on workers in a Bogotá paint factory using cytogenetic monitoring of micronuclei in lymphocytes and comet detection for DNA damage. Biomarkers of benzene, toluene, and xylene exposure were investigated in the urine of Bogotá workers and a control group. Phenol, hippuric acid, and methyl hippuric acid were measured. Benzene, toluene, and xylene were also monitored. Micronuclei and single-strand DNA breaking was studied in peripheral blood mononuclear cells. Although phenol and methyl hippuric acid concentrations were greater than reference levels, hippuric acid concentrations in urine after exposure were acceptable. One factory had too much benzene. Genetic indicators did not differ between exposed and unexposed workers. The absence of genotoxic effects in the examined cells may be due to low amounts of organic solvents. This effort helps future research and epidemiological surveillance programmes.

Finally, a survey work was carried out to obtain information on the level of knowledge of the toxicity of the chemical substances that use the degree of protection; once the results of the quantitative analyses were obtained and complemented with the data made by the personal interview, it allowed us to obtain the necessary information to carry out the statistical evaluation and in this way to interpret the real substance of the occupational exposure of contamination to which these workers are exposed.

2. Material and Methodology

2.1. Type of Research and Sample Selection. The current research is a descriptive, cross-sectional, and prospective research work, designed according to the orientation applied because it is oriented to experience the indicators of hippuric acid and phenols. The current research investigation sample (population) is made up of 40 urine samples collected from workers who work in the printing industry of Addis Ababa, the capital and largest city of Ethiopia, during the period of November 2021 to December 2021.

2.2. Sample Collection

(i) The collection of samples proceeded to collect the first urine of the morning, in plastic bottles with a wide screw cap

(ii) Each sample was labelled with the date and its corresponding code

(iii) All samples were kept in the refrigerator between 2 and 8°C until analysis

(iv) The analysis was performed within 24 hours of collecting the samples

(v) The analyses were carried out in the laboratory of toxicology and legal chemistry

2.3. Materials. Chemicals used in the study were purchased from Sigma-Aldrich with 98-99% purity, AR Quality, and double-distilled water used with pH 7.01 in the entire study.

2.4. Analysis Methods

(i) Banfi and Marenzi method [9] was used for the quantification of total phenols. Phenols present in urine are determined by quantifying the orange-red complex formed by the reaction of diazotized p-nitroaniline with phenols in an alkaline medium. The coloured complex can be determined spectrophotometrically at 525 nm [10] (Figure 1)

(ii) Weichselbaum and Probstein titration technique [11] was used for the quantification of hippuric acid. Hippuric acid is precipitated by adding sodium chloride in highly acidified urine and refrigerated. The

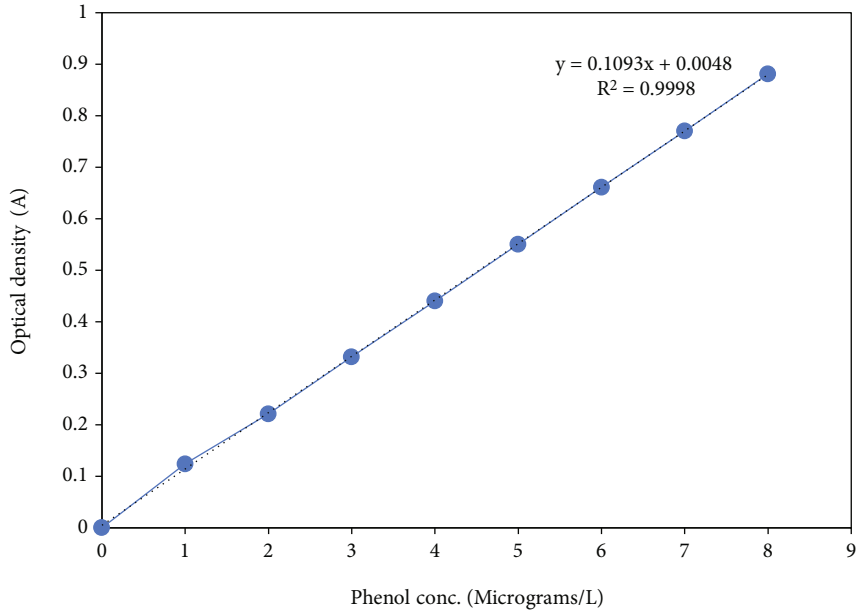


Figure 1: Calibration curve of phenols in a 525 nm spectrophotometer.

Table 1: One-sample t-test statistics.

	No. of samples	Average	Standard deviation	The error of the average
Phenols (mg/L)	40	222.32	51.6112	8.16045

Table 2: Test for a sample.

	Test value = 75				95% confidence interval for the difference	
	t	Degrees of freedom	Sign (two-sided)	Mean difference	Lower	Higher
Phenols (mg/L)	18.057	39	0	147.35	130.85	163.9

precipitated hippuric acid is washed, dissolved with sodium chloride, and titrated with a standardized 0.1 N alkaline solution of sodium hydroxide using phenolphthalein as an indicator [10, 11]

Calculation of results: for the determination of the amount of hippuric acid in each sample by the base acid titration method, the following formula is used:

$$\text{Grams of hippuric acid} = \frac{179 \times mL(NaOH) \times N(NaOH) \times 10}{1000} + \frac{0.123 \times mL \text{ of } urine\ (aliquot) \times 10}{100}, \quad (1)$$

where 179 is the molecular weight of hippuric acid and 0.123 is the correction due to the solubility of hippuric acid.

3. Results and Discussion

3.1. Statistical Results of Phenols. The statistical study of total phenols applies Student's t-test where we want to affirm that there is a significant difference between the mean obtained in the results of the urine samples after having applied the determination of phenols in urine by the Banfi and Marenzi method (that the highest value that could be found is 75 mg/mL, that is, taking this value as the highest found), and then, we found that of the 40 samples the average of phenols was 222.35, with a standard deviation of 51.61 and from the mean of 8.160 (Table 1).

Now, when applying the t-test, we find the statistical value of 18.057 with 39 degrees of freedom with a significance of 0.000 (comparing the Ho with 0.05). That is to say that there is a significant difference between the means found 222.35 mg/L and the maximum value, according to the WHO which is 75 mg/L (Table 2)

3.2. Statistical Results on Hippuric Acid. The statistical study of hippuric acid applies Student's t-test where we want to affirm that there is a significant difference between the mean obtained in the results of the urine samples after having applied the determination of hippuric acid in urine by the Weichselbaum and Probstein method [12]. Assuming that

Table 3: One-sample t-test statistics.

	No. of samples	Average	Standard deviation	Type error of the average
Hippuric acid (g/L)	40	2.04823	0.38231	0.06045

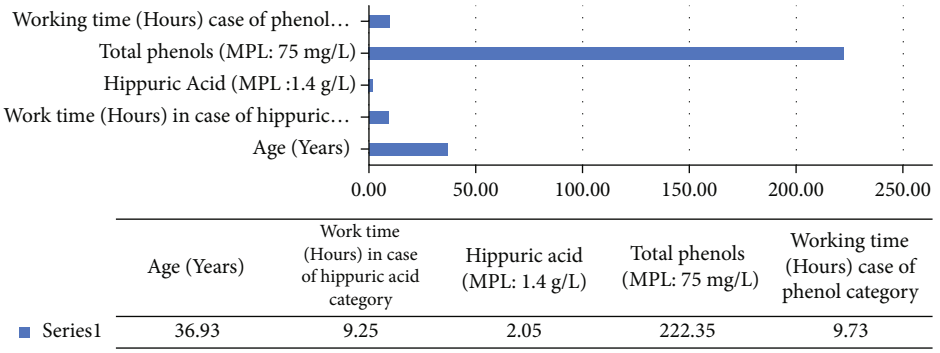


Figure 2: The results (average) of the quantification of total phenols and hippuric acid in the urine of printing workers ($n = 40$; gender: male).

Table 4: Test for a sample.

	Test value = 1.4				95% confidence interval for the difference	
	t	Degrees of freedom	Sign (two-sided)	Mean difference	Lower	Higher
Hippuric acid (g/L)	10.724	39	0	0.64825	0.526	0.771

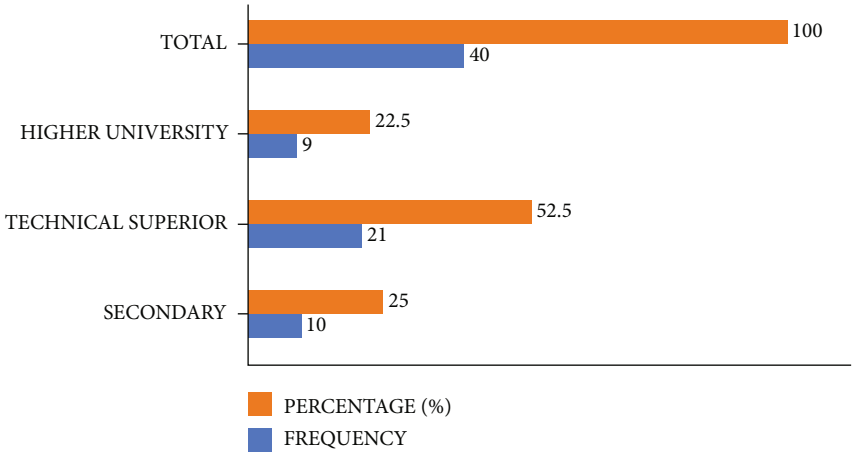


Figure 3: Results according to the level of instruction.

the highest value that could be found is 1.4 g/L, that is, taking this value as the highest found, then we find that of the 40 samples, the average of hippuric acid is 2.04823, with a standard deviation of 0.38231 and E of the mean of 0.06045 (Table 3).

Now, when applying the t-test, it is observed in Figure 2 that we find the statistical value of 10.724 with 39 degrees of freedom with a significance of 0.000 (comparing the Ho with 0.05). That is to say that there is a significant difference between the means found 2.048 g/L and the maximum value, according to the WHO it is 1.4 g/L (Table 4).

3.3. Educational Level Result. It is observed in Figure 3 that of the sample of 40 printing workers, 52.5% have higher technical education, 25% secondary, and 22.5% higher university.

3.4. Result according to Signs and Symptoms. In Figure 4, the sample of the 40 printing workers shows that the highest percentage of symptoms corresponds to those with irritated eyes 20%, then those with nausea 10%, and those with headache 8% and weakness 2%.

3.5. Result according to the Type of Protection Used. It is observed in Figure 5 that in the sample of 40 printing workers, the highest percentage of protection types are

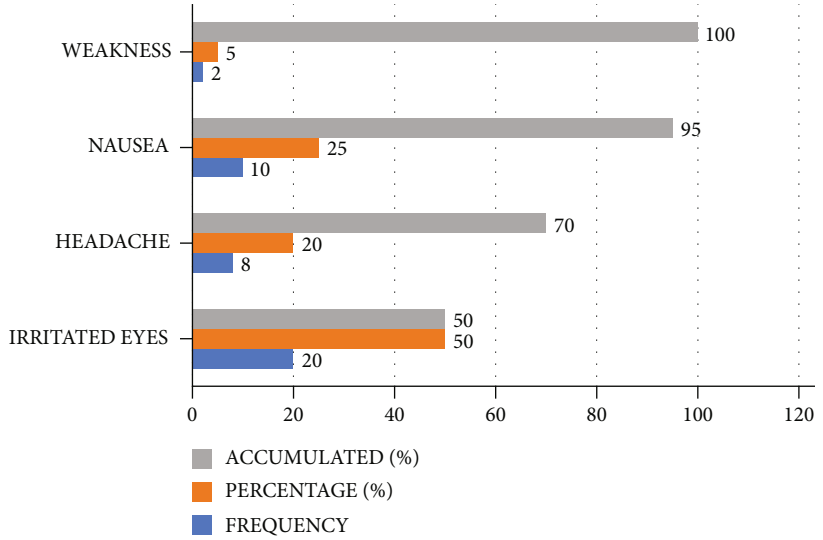


Figure 4: Results according to signs or symptoms.

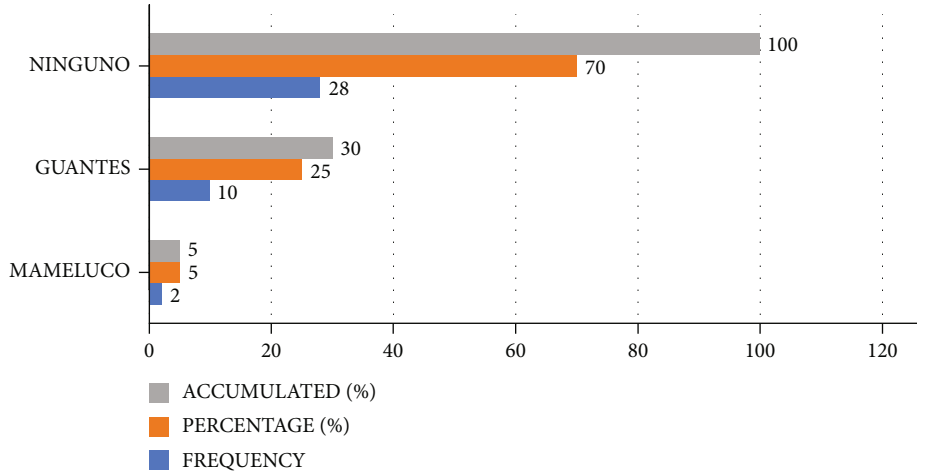
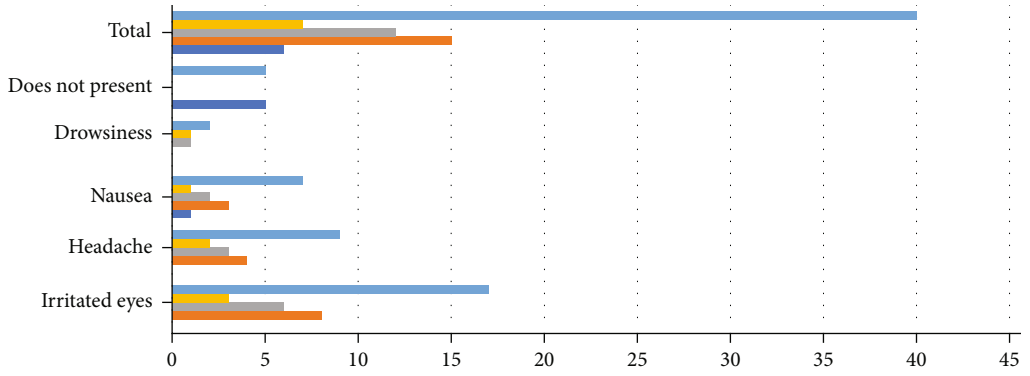


Figure 5: Results according to the type of protection used.



	Irritated eyes	Headache	Nausea	Drowsiness	Does not present	Total
Age groups total	17	9	7	2	5	40
Age groups from 31 to 40 years old	3	2	1	1	0	7
Age groups from 21 to 30 years old	6	3	2	1	0	12
Age groups from 11 to 20 years	8	4	3	0	0	15
Age groups less than 10 years	0	0	1	0	5	6

Figure 6: Result of signs or symptoms found in relation to years of work.

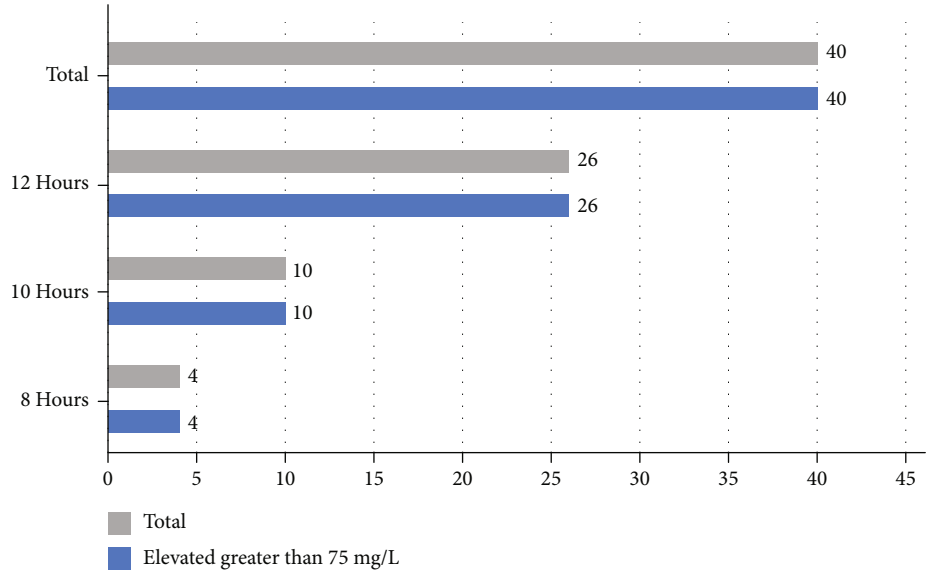


Figure 7: Result of the quantification of total phenols in relation to the working day.

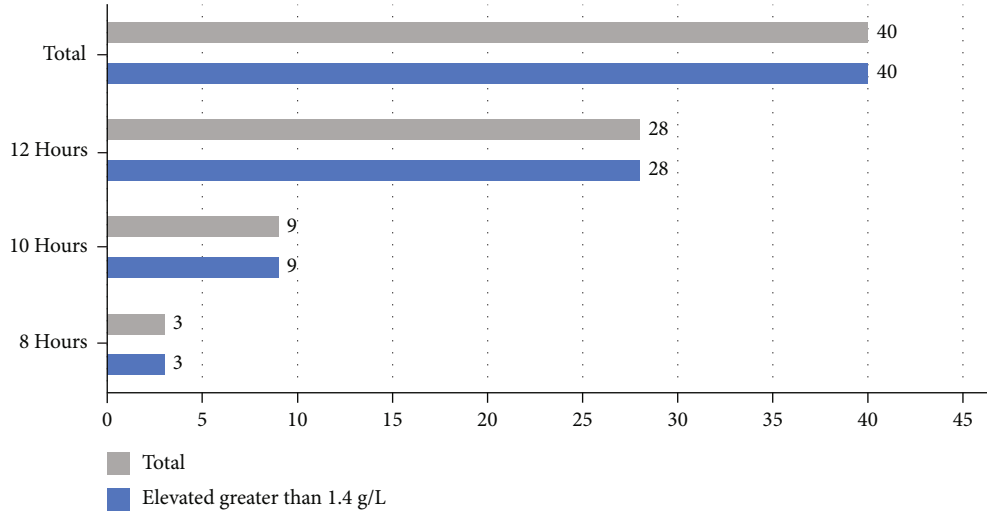


Figure 8: Result of the quantification of hippuric acid in relation to the working day.

gloves 25%, those who use overalls 5%, and without protection 70%, none.

3.6. Result of Signs or Symptoms in relation to Years of Work. It is observed in Figure 6 that the printing workers, according to signs and symptoms in relation to the years working within the range under 10 years of age, present irritated eyes.

3.7. Result of the Quantification of Total Phenols in relation to the Working Day. It is observed in Figure 7 that the sample of the 40 printing workers according to the working day all result in a measurement of total phenols greater than 75 mg/L, which is the limit allowed by the WHO.

3.8. Result of the Quantification of Hippuric Acid in relation to the Working Day. It is observed in Figure 8 that the sample of the 40 printing workers according to the working day all result in a measurement of total phenols greater than 1.4 g/L, which is the limit allowed by the WHO.

4. Discussions

Our study describes the working conditions and risks of exposure to organic solvents, such as benzene and toluene, of workers working in the printing plants in the surrounding area of Addis Ababa. The exposure to solvents in this study and the health effects from exposure to those observed indicate that implementing preventive measures in the printing process is necessary, as other authors have described.

The average result obtained for a concentration of 222.35 mg/L of total phenols in urine far exceeds that presented by Kamal et al. [9] (with 3.3%), indicating that workers who work in printers are more exposed than those who work in paint factories.

In the case of hippuric acid, the average concentration obtained was 2.048 g/L, which was also found high in the study by Kamal et al. [13] (2.09 g/L); Wiwanitkit et al. [14] those do not exceed the normal values that quantify hippuric acid in the printing presses, but in our result, they exceed the values of hippuric acid 2048 g/L. Kamal et al. (2012) observed that the workers present irritated eyes; this is because the solvents they use have volatile solvent compounds which generate this discomfort that is the reason for symptoms that afflict as the presence of confusion, insomnia, irritation at the level of the skin, mucous membrane, and toxic effects in the CNS, liver, and kidney [15]. For those who do not use personal protective equipment, the average time of years working in the area could explain the symptoms indicated [16].

5. Conclusions and Recommendations

5.1. Conclusions. A study was carried out on 40 workers who work in the printing presses, finding the following:

(i) The average concentration of phenols in urine is 222.35 mg/L with extreme figures of 130.8439 and 163.8561 mg/L

(ii) The average concentration of hippuric acid in urine is 2048 g/L with extreme figures of 0.5260 and 0.7705 g/L

(iii) The levels of hippuric acid and phenols in urine exceed the maximum values allowed according to the WHO, which are 1.4 g/L and 75 mg/L, respectively

(iv) 222.35 mg/L of results of total phenols in the urine of printing workers exceeds the maximum limit allowed given by the WHO (75 mg/L)

(v) 2.04823 g/L of hippuric acid results in the urine of printing workers exceeding the maximum allowable limit given by the WHO (1.4 g/L)

5.2. Recommendations. Negotiate with the municipal authorities that they carry out periodic inspections in order to determine if the galleries meet the adequate conditions of personal protection in the work environment. Conduct interviews on proper personal protective equipment such as overalls, gloves, masks, and protective glasses. Evaluate the concentration of benzene and toluene per m^3 of air in the work environment of the people who work in the printing house. It is recommended to carry out further research work on exposure to solvents in the activities that merit it by quantifying their respective biological indicators, but taking into account that if the quantification is carried out in urine, it is carried out in relation to the excretion of creatinine, as this result is more reliable in terms of the clearance of metabolites in urine.

Data Availability

The data underlying the results presented in the study are available within the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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