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Assessment of Occupational Exposure to BTEX in a Petrochemical Plant via Urinary Biomarkers

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Abstract: This work presents the results of the first Serbian monitoring campaign performed to assess the occupational exposure of petrochemical industry workers to benzene (B), toluene (T), ethylbenzene (E), and xylene (X), known collectively as BTEX. The following urinary biomarkers were investigated: phenol, hippuric acid, o-Cresol, p-Cresol, and creatinine. BTEX compounds were collected in 2014 using Casella passive samplers. Multivariate statistical analysis was performed to put in evidence the correlation between the BTEX measured in air and the concentration of urinary biomarkers. While the results indicate an elevated presence of benzene in the air in the working environment studied that surpasses the national and European Occupational Exposure Limits (OEL), the levels of the remaining (TEX) parameters measured were below the OEL. The high relative standard deviations (RSD) for the concentrations of each BTEX compound (68–161 mg m^{-3}) point toward an intensive occupational exposure to BTEX. This was confirmed by relevant urine biomarkers, particularly by the mean values of phenol, which were ten and fourteen times higher than the ones found in the control group (14–12 mg g^{-1} of creatinine). On average, workers are at a higher risk of developing cancer (6.1×10^{-3}), with risk levels exceeding the US EPA limits. Benzene levels should therefore be maintained under tight controls and monitored via proper urinary biomarkers.

Keywords: petrochemical industry; air pollution; BTEX; urine biomarkers; multivariate analysis; carcinogenic risks

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1. Introduction

Oil and petrochemical processing and production complexes present significant challenges for occupational health and industrial safety. Aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (collectively labeled as BTEX) are natural components of the petroleum stream and solvents in various industries [1]. Occupational exposure to these agents may occur during production processes, maintenance of process systems, evaporation, or leaking of poorly maintained underground fuel tanks. The occupational exposure to benzene is regulated in the European Union by occupational exposure limits (OEL), which are 3.25 mg m^{-3} for benzene [2]. According to the same EU legislation, limiting values are 192 mg m^{-3} for toluene, 442 mg m^{-3} for ethylbenzene, and 221 mg m^{-3} for xylene [3,4]. The national Serbian legislation for these compounds is aligned with the European limits [4,5]. Recent studies using urinary biomarkers showed that in occupa-

tional environments, workers are still exposed to significant levels of BTEX, not only in the petrochemical industry, but in the waste management sector as well [6,7].

BTEX compounds pose a risk to human health, as being exposed to them leads to the development of symptoms such as weakness, confusion, and skin irritation and may cause neurological disorders, cancer, hemato-toxicity, and geno-toxicity [8–10]. Benzene, as a class I carcinogenic chemical, is the most toxic. Indeed, long-term exposure to low concentrations of benzene may increase the frequency of cancer and leukemia, and exposure to higher benzene levels may lead to aplastic anemia [11–13]. Monitoring benzene and the other BTEX compounds in occupational settings is therefore a priority in order to further establish their impact on humans and on the environment.

However, economic factors for the plant manufacture also impact the level of benzene, which can be monitored. Indeed, the plant can generate additional profits from pyrolytic benzene, which consecutively result in an increased number of manipulations to condition and store pyrolytic gasoline within plant premises. These treatments would be performed successively in other plants regardless, but as a result, an increased concentration of benzene in pyrolytic petrol, up to 70%, in the working environment can be retrieved. Pyrolytic petrol is used as a solvent, as raw material for aroma extraction (BTX fraction), for setting in high-octane motor gasoline, and for other uses. Therefore, the goal of this study was to assess the impact of a higher level of benzene than normal in a working environment, due to the specific petrochemical activities described above. Occupational medical monitoring is not mandatory for industry in Serbia, with no procedure established.

Urine biomarker analyses are extremely rare and have never been systematically conducted before in Serbia to the best knowledge of the authors. In addition, this analysis is a unique study of highly toxic industrial pollutants in this region. It demonstrates potential impacts on human health and also envisages that these pollutants may impact the wider region and potentially lead to cross-border pollution.

The current study was conducted at a Serbian petrochemical plant for the first time to define specific distribution pathways for volatile organic compounds (VOCs), particularly BTEX, whose presence can be found in urine biomarkers. The results, supported by chemometric analysis, allow establishing and systemizing the relationship between exposure to significant toxic petrochemical parameters and urine biomarkers.

2. Materials and Methods

2.1. Sample Collection and Exposure Assessment

For the first time in 2014, during winter (March) and autumn (October) seasons, a monitoring campaign was conducted at a Serbian petrochemical plant. The monitoring program involved 24 workers and was carried out in two steps. First, the data were collected using personal samplers (with optimal flow 2 L min^{-1}) on ambient BTEX concentrations in occupational settings to evaluate whether these concentrations were in line with national and European OELs. Second, the levels of BTEX urine biomarkers such as phenol, hippuric acid, o- and p-Cresol, and creatinine were monitored. Cancer risk assessment was carried out for workplaces where the benzene concentration in the air exceeded the OEL, calculated as Biological Exposure Index (BEI). BEI guidance values are used for assessing biomonitoring results and represent levels of contaminants most likely to be observed in samples collected from healthy workers exposed to the same extent as workers with inhalation exposure at the target level of contaminants (Threshold Limit Values—TLV).

This monitoring was carried out at a petrochemical plant that is an integral part of an oil and petrochemical complex in the Republic of Serbia. The processing plant uses a variety of raw materials, which contain up to 40% of benzene and up to 80% of other alkyl derivatives. The plant also produces ethylene and by-products, including propylene, pyrolytic oil, and pyrolytic gasoline. The locations investigated were divided into two groups. Each group included 12 workplaces, with four samples taken per location during the sampling period. The groups were classified as low- or high-exposure, based on the probability, strength, and frequency of exposure to BTEX in the working environment and

the associated cancer risk according to national [5] and international laws [14–19]. Descriptions of the workplaces are given in Table 1. A total of 48 samples per group were selected for analysis. Workers' exposure to BTEX concentrations in the occupational environment was assessed using Casella passive–diffusive samplers, optimized at around 2 L min^{-1} flow. The samplers were worn by the workers during their work shift (approximately 8 h) whilst they carried out their regular activities. BTEX compounds were desorbed according to the instructions given by the manufacturer. Urine samples were collected before and after the work shift and stored in disposable polyurethane bottles. Samples were frozen and stored at –20 °C prior to analysis. Biomonitoring and air sampling were carried out on the same day. For the sake of comparison, and urine biomarker values were also investigated for 32 workplaces not exposed to BTEX, also defined in Table 1 as "low" exposure to BTEX (respect to OEL values), which constitute the control group.

Table 1. Major activities of the plant workers who took part in the study.

Workplace	Task (Job Description)	Exposure to BTEX
Production process supervisor	Performing basic diagnostic tests, checking the performance of machines after replacement or repair	High
Steam and turbine compressor operator	Performing basic diagnostic tests, checking the performance of steam and turbine compressors to obtain high-pressure steam for the production process, sealing and repairing the high-pressure pipeline	High
Fraction operator	Monitoring of the fractionation process parameters	High
Field man	Daily maintenance works according to schedules in the field, patrolling the plant sites for dissembling or assembling tasks	High
Warehouse and manipulation engineer	Operating pumps for transporting fluid, measuring levels of raw materials in storage tanks, open pipeline valves, patrolling the warehouse equipment (storage tanks and pipelines)	High
Mechanical engineer	Monitoring equipment and machines at the plant, testing damaged machine parts to determine the level of repair necessary, reparation and replacement	High
Working shift manager	Patrolling the production units and managing the optimal production process during the shift	High
System maintenance and repair engineer	Cutting, threading, grooving, bending, and welding of the high-pressure pipeline and tanks, repairs according to the schedule	High
Board man	Process system control	High
Petrol hydrogenation	Monitoring operation parameters on the section, opening of the valve block at a specific point, required to maintain optimum production	High
Raw materials and product chief	Responsible for receipt and delivery of raw materials and final products	High
Plant supervisor for machines and equipment	Monitoring the cutting, threading, grooving, bending, and welding of the high-pressure pipeline and tank repair	High
Accountant	Dealing with financial issues	Low
Plant manager		Low
Deputy plant manager	Occasional visits to the plant, carrying out tasks according to work systematization	Low
Safety manager	In charge of occupational safety and health for the company, occasional on-site visits	Low
Legal officer	In charge of legal issues in the company	Low

Table 1. *Cont.*

Workplace	Task (Job Description)	Exposure to BTEX
Quality manager	Monitoring implementation of standards and improvement of product quality	Low
Sales manager	In charge of the relationship with customers and ensuring customer satisfaction	Low
Investment and development manager	In charge of development and innovation of the plant and processes	Low
Deputy manager for production	Occasional visits to plant to ensure implementation of standards, planning and monitoring of maintenance tasks	Low
Typist		Low
Helper		Low
Fire safety officer	In charge of fire safety and protection	Low

2.2. Analysis of BTEX and Urine Biomarkers

The analysis of the Casella vials was performed by gas chromatography with a flame ionization detector (GC-FID; Agilent Technologies 6890), using the National Institute for Occupational Safety and Health (NIOSH) method 1501 [20]. BTEX compounds were extracted from the charcoal tubes with 1 mL carbon disulfide (CS2). Internal standard p-cymene (GC + purity, Merck USA) was used at a concentration of 100 μg mL^{-1}. The vials containing CS2 and charcoal were shaken for 30 min and filtered through a SPARTAN 3, 0.45 μm, ∅ 3 mm filter (Schleicher & Schuell). BTEX compounds were quantified by GC-FID using a capillary column (HP—5 5% Phenyl Methyl Siloxane, HP 19091J—413, 30 m × 0.32 mm and 0.25 μm film thickness). Aliquots of 1 μL were taken from the vial and injected into the capillary column. The injector was maintained in split mode at a 2:1 ratio and a temperature of 300 °C. The flow rate of the carrier gas (helium) was 2.6 mL min^{-1}. The injector temperature was 250 °C. The instrumental limits of detection (LOD) for BTEX were 0.5, 0.7, 0.5, and 0.8 μg L^{-1}, respectively.

The urinary biomarkers phenol and cresols [21,22] were used as a measurement of exposure to benzene and toluene and were determined by NIOSH 8305 using a capillary column [19]. The capillary columns employed were glass tubes 7 cm long with 4 mm internal diameter, composed of two sections of activated carbon (100/50 mg) with a 0.5–1 mm granulation (18–35 mesh ASTM) and with plugs of glass wool or polyurethane foam at the ends and between the sections. Prior to analysis, the frozen urine samples were thawed to room temperature, and 0.5 mL urine aliquots were taken. Ten drops of $HClO_4$ were added, shaken, and heated in a water bath for one hour at 95 °C. After the addition of 40 μL of internal calibration standard (thymol solution, 1 mg mL^{-1}), the sample volumes were completed to 10 mL with distilled water in the centrifuge tubes. Sample preparation for GC-FID analysis included pipetting 2 mL diethyl ether into the tube stopper, shaking for one minute, and cooling the tube to 0 °C to allow the phases to separate, then transferring 0.5 mL of the clear ether layer to a culture tube and mixing with a few milligrams of Na_2SO_4. GC-FID analysis was performed in splitless mode. The injector temperature was 290 °C, pressure 15 psi, purge flow 20 mL min^{-1}, one minute purge time, and total flow 26.6 mL min^{-1}. Oven temperature was programmed at 40 °C for one minute, rising at 8 °C min^{-1} to 88 °C and at 45 °C min^{-1} to 290 °C. Prior to measurement, samples were kept at 0 °C to avoid evaporation. The estimated instrumental limit of detection (LOD) was 0.5 μg mL^{-1}.

The urinary biomarker hippuric acid, a measure of toluene exposure, was determined according to NIOSH 8300 [23]: 0.5 mL of diluted urine and 0.5 mL of pyridine were mixed together in a conical centrifuge tube. A total of 0.2 mL of benzene sulfonyl chloride was added and mixed for five seconds on a vibration mixer and kept for 30 min at 20 to 30 °C. The reaction was stopped by the addition of 5 mL ethanol, followed by mixing on

a vibration mixer and centrifuging at 1500 to 2000 RPM (full speed) for five minutes to reduce turbidity. The supernatant was then pipetted and placed in a one-centimeter cuvette in the spectrophotometer (absorbance at λ = 410 nm).

The results were calculated and expressed as the concentration of creatinine in the same urine sample, and to avoid error, the diuresis was not recalculated. The limit of detection of the method was 2.5 g/mL urine for each analyte, and the estimated Standard Deviation was 0.002 g L^{-1}. The urinary biomarker results were corrected for creatinine concentration (mg g^{-1} of creatinine).

2.3. Multivariate Analysis

All statistical data processing was conducted using STATISTICA (Statsoft Inc., Tulsa, OK, USA; version 12.0 for Windows). Principal component analysis and/or factor analysis allowed for identifying the relationships between the ambient BTEX contaminants and the urine biomarkers as their probable indicators. All analyses were performed on a normalized dataset (z-scale transformation) to avoid misclassification due to the broad differences in data dimensionality. Analysis of variance (ANOVA) was done to identify significant spatial and temporal differences ($p < 0.05$). Principal component analysis/factor analysis was conducted to maximize the variation among the variables under each factor, and varimax factors (HFs for low-exposure workers and WFs for high-exposure workers) with eigenvalues > 1 were retained by Kaiser's criteria [24].

2.4. Human Risk Assessment

Human health risk assessment was carried out to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media [14]. Inhalation risk analysis is part of BTEX exposure assessment in the working environment. It has been estimated that for atmospheric benzene concentrations equal to 1 μg m^{-3}, the lifetime risk of chronic leukemia is 4.476×10^{-6} [13], while the probability to develop cancer is in the range of 2.2×10^{-6} to 7.8×10^{-6} [17].

In the current study, cancer risks were calculated for the workers who are highly exposed to benzene using the following equations (1) to (3) [14–18]:

$$E = C \times IRa \times Eda/Bwa \quad (1)$$

$$EL = E \times (D/7) \times (Wk \times 52) \times (YE/YL) \quad (2)$$

$$\text{Cancer risk} = EL\ (\text{mgkg}^{-1}\ \text{day}^{-1}) \times SF\ (\text{mg kg}^{-1}\ \text{day}^{-1}) \quad (3)$$

where E is daily exposure (mg kg^{-1} day^{-1}) and EL is effective lifetime exposure (mg kg^{-1} day^{-1}).

In order to calculate the exposure frequency, standard values were used as given in Table 2 [15–19].

Table 2. Cancer risk assessment parameters.

Parameter	Unit	Value
Pollutant concentration (C)	mg m^{-3}	-
Inhalation rate (IRa)	m^3 h^{-1}	0.83
Exposure duration adult (EDa)	Hour day^{-1}	8
Body weight, adult (BWa)	kg	70
Days per week exposure (D)	Day	5
Weeks of exposure (Wk)	Week	48
Years of exposure (YE)	Years	30
Years in lifetime (YL)	Years	70
Slope factor	mg kg^{-1} day^{-1}	0.029

3. Results

Table 3 reports the various results obtained for the analysis of ambient BTEX samples. The benzene maximum and mean concentration in the air exceeded the OEL levels for the

high-exposure group but not for the low-exposure group, whereas the observed levels of toluene, ethylbenzene, and xylene were lower than the reference OEL values. The mean value for benzene in the analyzed data set was 10.3 mg m^{-3} for the high-exposure group and 0.39 mg m^{-3} for the low-exposure group. Other approximate mean values were 1.35 mg m^{-3} for toluene, 0.23 mg m^{-3} for ethylbenzene, and 0.51 mg m^{-3} for xylene in both profile groups. Compared to national and European OEL values for BTEX, the maximum value for benzene from the high-exposure group was ten times higher than the relevant OEL value. The relative standard deviations (RSD) were calculated between the low- to high-exposure groups as follows—benzene (100–90 mg m^{-3}), toluene (160–139 mg m^{-3}), ethylbenzene (155–153 mg m^{-3}), and xylene (104–67 mg m^{-3})—and confirmed high variation in occupational exposure to BTEX. These results may be explained considering many external factors: (1) seasonal variations, with more days of lower exposition to toxicity, such as during windy autumn and wintertime; (2) worker shifts, as a more intensive technological process is performed during the day; (3) intense exposure of workers in techno-location with an increased risk of chemical hazards; and (4) worker trajectory factors. Future studies could be focused more on these specific factors investigated with multivariate analysis statistical techniques. The use of pyrolytic benzene, without any pre-treatment, removal of aromatic hydrocarbons, and the poor maintenance of the plant, as well as an outdated technology process from the 1960s, are the sources for high levels of benzene in the working environment.

Table 3. Biological statistics and relevant references and monitoring data for the investigated occupational groups.

BTEX from Ambient Air	Exposure Groups		Investigation Data (mg m^{-3})					Relevant Comparison Data
			Minimum	Maximum	Mean	SD	RSD	OEL Values
Benzene	High Low		3.18 0.07	32.11 1.18	10.1 0.39	10.36 0.36	100.11 90.49	3.25
Toluene	High Low		0.06 0.03	7.84 3.92	1.78 0.93	2.87 1.30	160.95 139.56	192
Ethylbenzene	High Low		0.05 0.02	1.56 0.71	0.33 0.14	0.51 0.21	155.61 153.37	442
Xylene	High Low		0.06 0.04	2.02 0.89	0.60 0.42	0.63 0.29	104.37 67.89	221
	Urine biomarkers							Control group mean values / BEI values
Phenol	mg g^{-1} of creatinine	High Low	0.78 0.43	100.9 33.48	14.03 12.63	12.20 25.30	86.74 200.32	2.77 / 250
Hippuric acid	mg g^{-1} of creatinine	High Low	20.67 0.37	143 73.2	70.63 20.43	37.97 18.38	53.97 90.06	2.41 / 1.60
o-Cresol	mg g^{-1} of creatinine	High Low	0.01 0.01	0.79 0.2	0.12 0.02	0.22 0.04	183.33 0.1	n.d. / 0.3
p-Cresol	mg g^{-1} of creatinine	High Low	1.27 0.87	9.55 4.40	3.35 2.44	2.42 1.13	72.24 46.36	5.30 / n.a.
Creatinine	dL^{-1}	High Low	0.90 0.64	2.60 2.66	1.69 1.49	0.50 0.50	29.91 33.70	2.03 / -

Regarding the urine biomarkers, increasing benzene exposure values were accompanied by increased levels of urinary creatinine, with mean values of 1.6 gL^{-1} for low-exposure workers and 1.8 gL^{-1} for high-exposure workers. The comparison of the maximum and mean values of these biomarkers with the Biological Exposure Index (BEI) values for both groups of exposed workers shows that the mean and maximum values of phenol in urine are lower than the BEI values (250 mg g^{-1} of creatinine) [16,25], but considerably

higher than the mean values of the control group with "low exposure" (2.77 mg g^{-1} of creatinine), with maximum values in the high-exposure group going as high as 100.9 mg g^{-1} creatinine. Similar studies, which have carried out biomonitoring of humans exposed to elevated levels of benzene in occupational settings (above 30 ppm), showed a positive correlation with phenol in urine [26–28]. Even at high exposure levels starting at 5 ppm and above, a positive correlation was evidenced between phenol excretion in urine and exposure to benzene [15,26]. In exposed groups, the mean values for phenol in urine, in pre-shift and post-shift urine spots, ranged from 31–126 mg g^{-1} of creatinine. These results point to an interdependence between the level of benzene in the working environment and the urinary biomarker phenol. For cases in which the workers were exposed to benzene concentrations lower than 5 mg m^{-3}, urinary biomarkers such as trans, trans-muconic acid (t,t-MA), s-phenylmercapturic acid (S-PMA), or urinary benzene (BU) are superior to phenol as a biomarker. Some research indicates that t,t-MA shows a positive correlation with food additives (ascorbic acid), while S-PMA is considered a reliable urinary biomarker when the values of benzene are below 1 ppm and BU when monitoring workers' exposure to benzene in the oil industry [11,28–30].

Box and whiskers analyses were performed in order to cover the spread and centers of a data set on the number of input values (96 samples, 48 samples per group of low- and high-exposure workers) and also to increase the significance of performing multivariate analysis with such a base and high level of variation in results. The results are presented as an interquartile range and the mean or average and median (the middle of a data set). Both worker profiles are characterized by a significant dispersion in each of the measured parameters, particularly benzene (Figures 1 and 2). In Figures 1 and 2, the box and whisker plots for the low- and high-exposure groups reflect the large fluctuations observed in the BTEX values. Most of the benzene values fall within the range of the 25% to 75% box values, with maximum values of around 14 mg m^{-3} for most of the samples from the high-exposure group. Toluene showed somewhat higher raw data maximum values for the low-exposure group, and two outliers were observed (Figure 2) at 3.40 and 3.92 mg m^{-3}. Benzene and xylene present similar patterns (ranging from 0 to around 0.5 mg m^{-3}) for the low-exposure group (Figure 2). Several factors may be responsible for the large variations observed during the BTEX analysis. These fluctuations, particularly pronounced for benzene (Figure 1) and toluene (Figure 2), are likely due to their presence in pyrolytic petrol and repeated workers' exposure, which occurred during maintenance interventions in more toxic areas. The data further design the categories of workers at high exposure risk such as steam and turbine compressor operators, raw materials and final product engineers, and field men or worker trajectory factors. This may imply medium to high data oscillations (out-layers).

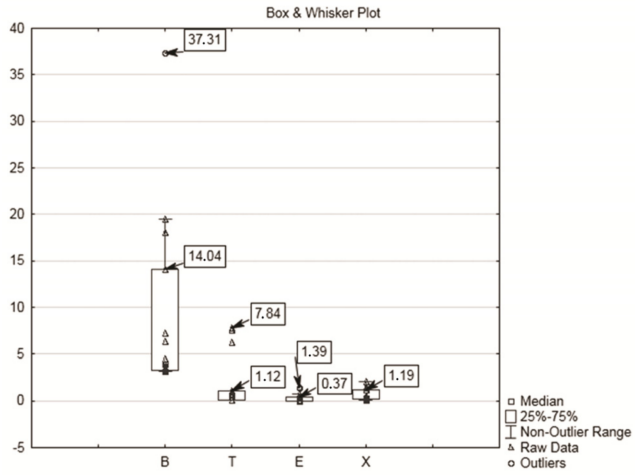

Figure 1. Box and whisker plot with average BTEX values for the high-exposure group.

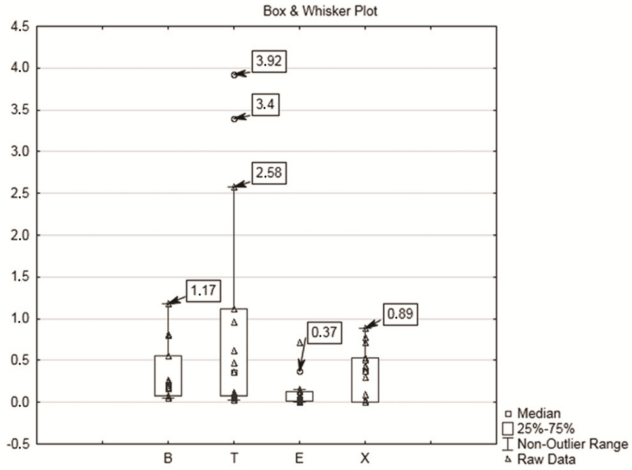

Figure 2. Box and whisker plot with average BTEX values for the low-exposure group.

4. Discussion

Previous data on BTEX exposures in the petrochemical industry were gathered in the case of petrol station workers exposed to gasoline and workers at bus depot stations and petrochemical plants [19,29,30]. Benzene levels were in the range from 4.5 mg m^{-3} to 52 mg m^{-3} with mean values of 14 mg g^{-3} for maintenance tasks in petrochemical plants, i.e., cleaning tanks and pipe maintenance. In extreme cases, such as for a petrochemical refinery, benzene values were as high as 160 mg m^{-3}, but typical values are in the range of 3.5–77.9 mg m^{-3} [31,32]. The available data on mean exposures to TEX were as follows: toluene ranged between 0.14 and 2.2 mg m^{-3}, xylene ranged between 0.59 and 1.1 mg m^{-3}, and mean exposure to ethylbenzene was 0.26 mg m^{-3}. These TEX levels in occupational settings in petrochemical workplaces were below OEL [33–35], such as the TEX exposure data obtained in this study.

The biomarker used for toluene was hippuric acid, and for both the high- and low-exposure groups, its levels exceeded the control group by 30 and 10 times, respectively, and also exceeded the BEI (Table 3). Levels of hippuric acid in urine are also affected by drugs, food, additives, and the consumption of benzoate-containing soft drinks, whose metabolism product is hippuric acid. Co-exposure with toluene may also alter benzene metabolism, since both benzene and toluene are metabolized by the same isoenzymes of cytochrome P450 (CYP) and especially CYP2E1 [36–39]. However, hippuric acid, followed by o-cresol, is the leading biomarker for toluene exposure assessment at 50 mg m^{-3}, with un-metabolized toluene and benzylmercapturic acid in urine being used when the air toluene levels are at 2 mg m^{-3} [36–38]. A study from Lovreglio et al., 2010 [34], evaluated the validity of potential biomarkers for their use in biological monitoring for exposure to low concentrations of benzene and toluene. It suggested the use of urinary benzene and urinary toluene and S-benzyl-mercapturic acid (SBMA), taking into account the metabolism and co-exposure of those two solvents.

The mean values of p-Cresol are lower than the control group for both exposure groups. The higher levels of p-Cresol compared o-Cresol are explained by the fact that p-Cresol is physiologically excreted in large amounts as a degradation product of tyrosine, and o-Cresol is specifically the best biomarker to follow in order to protect workers from potential effects of toluene exposure. Only 1% of inhaled toluene is excreted to o-Cresol [22,39]. The differences between cresol and hippuric acid (both are toluene metabolites) may be attributed to the different excretion times. Cresol is excreted 1 h after exposure, while the highest hippuric acid concentration is typically observed four to six hours after exposure [40,41]. O-Cresol showed low values compared to the BEI value of 0.3 mg g^{-1} of creatinine, which are in agreement with the low levels of airborne toluene.

Unlike in many developing countries, where exposure to benzene levels in excess of 3.25 mg m^{-3} are tightly controlled, the BTEX exposure assessment in this work showed that

benzene levels are still above the OEL. Increased monitoring and biomonitoring of benzene and the selection of appropriate biomarkers must therefore be a priority to ensure a proper assessment of risk exposition levels and, subsequently, with proper safety management procedures, to contain BTEX exposures below the OEL. Urinary metabolites are favored due to the ease of sample collection in comparison to blood metabolites, which require invasive sample collection procedures. This study showed high exposure of workers to benzene, using phenol and hippuric acid as biomarkers. The validity of these markers has been demonstrated for levels in the air exceeding 10 mg m^{-3}, which is the case in this study. However, to achieve better exposure assessments, the selection of appropriate urinary metabolites must consider other parameters such as metabolism mechanisms, time of sample collection, and other confounding factors [22,27,40–43]. For example, smoking cigarette behavior has been confirmed to be a strong confounding factor for the urinary excretion of benzene metabolites at low levels and must therefore also be taken into consideration and evaluated in urinary biomonitoring [40,41].

4.1. Cancer Risk Assessment

The potential risk of 1×10^{-5} = 1 in 100,000; 1×10^{-4} = 1 in 10,000; and 1×10^{-3} = 1 in 1000 is based on the probability of developing cancer in a population sample. According to the American Environmental Protection Agency (USEPA) [14], a cancer risk above 1×10^{-6} is unfavorable, as it significantly increases carcinogenic potential in humans. Table 4 shows the cancer risk assessment for workers exposed to benzene concentrations that exceed the OEL; all employees studied exceed the critical guideline value.

Table 4. Cancer risk assessment for the high-exposure group.

Workplace	Benzene Concentration (mg m^{-3})	E (mg kg^{-1} day^{-1})	EL (mg kg^{-1} day^{-1})	Cancer Risk
Steam and turbine compressor operator	37.31	3.303	0.778	2.26×10^{-2}
Raw materials and final product engineer	19.44	1.721	0.405	1.18×10^{-2}
Field man	18.06	1.599	0.377	1.09×10^{-2}
Mechanical engineer	14.04	1.243	0.293	8.49×10^{-3}
Warehouse operator	7.23	0.640	0.151	4.37×10^{-3}
System maintenance and repair workers	6.35	0.562	0.132	3.84×10^{-3}
Supervisor for machines and equipment	4.46	0.395	0.093	2.69×10^{-3}
Production process supervisor	3.96	0.350	0.083	2.39×10^{-3}
Shift manager	3.61	0.319	0.075	2.18×10^{-3}
Fractionator operator	3.37	0.295	0.069	2.04×10^{-3}
Boardman	3.33	0.286	0.067	2.01×10^{-3}
Petrol hydrogenation operator	3.30	0.281	0.066	2.00×10^{-3}

The cancer risk estimation results for the petrochemical plant employees in Serbia show that, on average, these employees are at high risk, with a potential 6 in 1000 chance of developing cancer (6.10×10^{-3}). Of particular concern are workers who are exposed to benzene concentrations above 18 mg g^{-3}. The OEL level is exceeded ten times (Table 3) regarding the maximum benzene for the high-exposure group, resulting in a 1 in 100 chance of developing cancer over their lifetimes. The average cancer risk estimations of this study are higher than the risks estimated for occupational exposure in the petroleum industry, with, for instance, gasoline pump attendants and bus depot workers having reported risks of 1.75×10^{-4} and 1.33×10^{-3}, respectively [19,42–46]. In a Bulgarian refinery, where benzene levels were above 3 mg m^{-3}, the lifetime cancer risk was estimated at 44 in 1000 and 28 in 1000, which are much higher than the data obtained in this study [19]. The higher cancer rate may be explained by the high content of benzene in product streams and by the inappropriate use of protective equipment. Additionally, the high level of benzene in this study may result from smoking habits, which is confirmed in this study, in which 67.65% of workers, of which 83.56% are male, are smokers. These facts could apply to phenol concentration in urine in the male group. However, the role of smoking behavior in increasing the levels of BTEX compounds in urine needs to be confirmed with further analysis [5,6]. A recent medical study in Serbia showed that exposure to petrol

and petroleum derivates in a refinery (part of the same petroleum complex as the plant studied herein) was associated with genotoxic effects on the lymphocytes in the exposed cohort [4]. In a similar medical study in Hungary, in which workers were exposed to maximal mean benzene concentrations of 43.8 mg.m^{-3}, increased values of most of the investigated geno-toxicological biomarkers were observed in the exposed subjects [9].

One of the possible explanations for the relatively low values of phenols (compared to BEI values) in the urine of workers in relation to the high values of benzene in the environment may be linked to alkylbenzenes present in that same environment, which compete with benzene [15]. It can be assumed that the metabolism of benzene (oxidation process) to phenol takes place with more difficulty (or more slowly) in the presence of alkylbenzene, which is a mitigating circumstance from the aspect of the health of those exposed. Also interesting are the findings of o-cresol, which occurs in a small number of subjects and is most likely linked to short exposures to high concentrations of toluene in the environment. Further research is needed to confirm this assumption.

4.2. Multivariate Analyses of BTEX and Urine Biomarkers

The complex nature of the correlation between BTEX and urine biomarkers was analyzed by multivariate analysis and more precisely by principal component analysis (PCA)/factor analysis (FA) performed on the normalized data. Standardized component loadings (normalized data) were applied to maximize the variation among the variables under each factor, and those varimax factors (VFs) with eigenvalues > 1 were retained (Kaiser's criteria) [24]. The results are shown in Table 5 and Figure 3. Varimax factors (HFs and WFs) of the entire data set (Table 5) revealed four HFs for the low-exposure group (HF1, HF2, HF3, and HF4) and three WFs (WF1, WF2, and WF3) for the high-exposure group, with eigenvalues > 1, highlighting the most important variables. Special focus was given to the values with loading > 0.70, which were considered significant (strong, Table 5). For the investigated workplaces, HFs explained 91% and WFs 78 % of the total variance in the data set. The first and most important component, HF1 of the low-exposure group, accounting for 41.9% of the total variance, was significantly positively correlated with the BTEX parameters and showed a strong and expected association, implicating the same or similar sources of petrochemical industry emissions, dominantly pyrolytic petrol, the same as for the WF1 factors from the high-exposure group.

Table 5. Loadings of experimental variables (9) on significant principal components for the low- and high-exposure groups' data sets based on 45 samples per group.

Variable	Low-Exposure Group				High-Exposure Group		
	HF1	**HF2**	**HF3**	**HF4**	**WF1**	**WF2**	**WF3**
B	**0.891**	0.039	−0.142	−0.242	**0.918**	−0.143	−0.047
T	**0.991**	0.072	0.008	−0.036	**0.988**	0.066	0.067
E	**0.927**	0.091	0.102	0.151	**0.892**	0.298	0.059
X	**0.951**	0.131	0.003	0.140	**0.943**	0.118	−0.049
C	0.034	0.058	−0.129	**0.981**	−0.230	−0.159	**−0.845**
H	0.093	0.247	**−0.801**	−0.090	0.103	**0.844**	0.191
P	−0.124	**−0.981**	0.070	−0.006	0.011	**−0.927**	0.178
oC	−0.085	**−0.972**	0.102	−0.060	−0.336	−0.364	0.403
pC	−0.094	−0.069	**−0.851**	0.277	−0.482	−0.291	0.486
Eigenvalue	3.77	2.02	1.39	1.00	4.14	1.74	1.17
% Total variance	41.9	22.5	15.39	11.1	45.9	19.3	13.0
Cumulative % variance	41.9	64.3	79.73	90.9	45.9	65.3	78.2

Note: Bold values indicate strong loadings. Symbols as follows: B—Benzene, T—Toluene, E—Ethylbenzene, X—Xylene, C—Creatinine, H—Hippuric acid, P—Phenol, oC—o-Cresol, pC—p-Cresol.

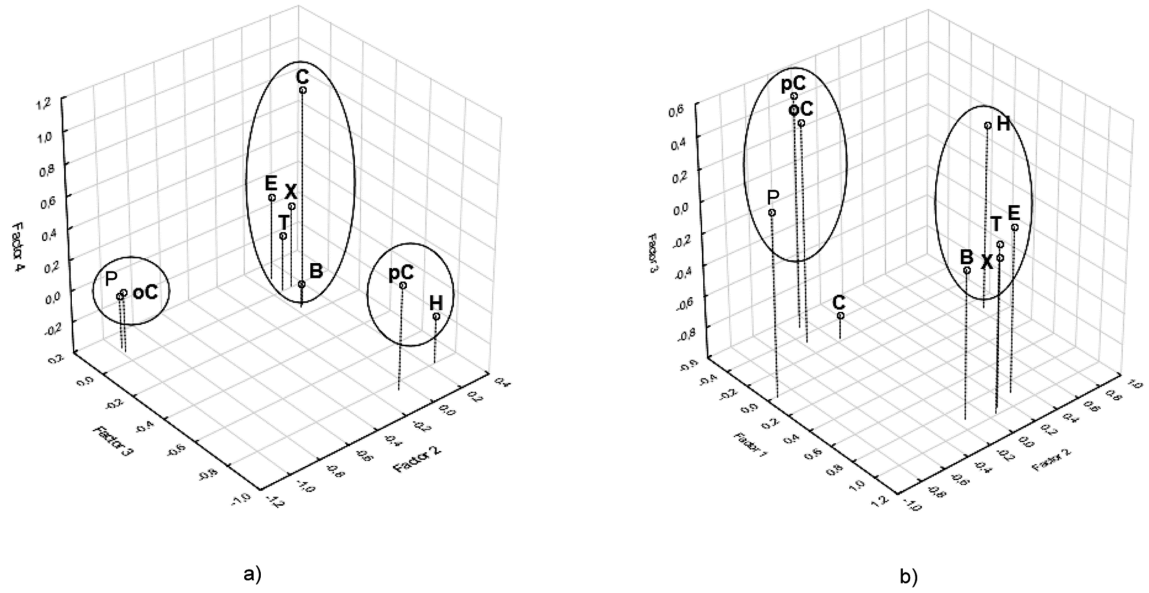

Figure 3. Three-dimensional factor plot (3D) of BTEX parameters and urine biomarkers assembled in three groups for the (**a**) low-exposure group and (**b**) high-exposure group.

The second component (HF2), accounting for 22.5% of the total variance and negative significant loading of phenols and o-Cresol (Table 5), indicates the same sources for those biomarkers and simultaneous exposure to benzene and toluene in the working environment, since in the absence of co-exposures, levels of urinary metabolites increase with increasing exposure to parent aromatic hydrocarbon [35].

The third group (HF3) of urinary hippuric acid (H) with loading of −0.80 and dominant p-Cresol with loading of −0.85, shows the influence of occupational exposure to toluene [47]. The high loadings of creatinine in group HF4 (0.98, Table 5) imply that its presence is largely due to different sources other than those of BTEX. Those parameters are also related to individual-specific factors such as food intake, drugs, and smoking habits [39,40].

For WF2, the significant but different sources of hippuric acid (0.84) and phenols (−0.92) are shown in Table 5. The creatinine loading in WF3 can be explained in the same way as the HF4 sources.

In order to perceive the wider dependence of BTEX and urinary biomarker parameters, the group of varimax factors (HFs and WFs) of the entire data set (Table 5) was compared with the three factor components, as illustrated by the three-dimensional scatter plot (3D, Figure 3) with somewhat different results than those reported in Table 5. The analyses were performed on a normalized dataset (z-scale transformation) to avoid misclassification due to the wide differences in data dimensionality. However, due to complex air-to-biosystem transfer phenomena, some deviations were expected and effectively found. The three groups of parameters have different cluster arrangements in relation to BTEX with creatinine for the low-exposure group (Figure 3a) and BTEX with hippuric acid for the high-exposure group (Figure 3b). The BTEX and creatinine cluster in the first major group (Figure 3a) indicates their similar distribution patterns and sources and may be explained by the mechanism of urinary creatinine, which was found to increase the excretion of most BTEX, suggesting a similar mechanism of excretion for these chemicals [35]. Hippuric acid is also clustered with BTEX. This is expected due to the higher concentration of benzene and toluene in the outdoor working environment and its co-exposure and implication on this biomarker.

The different outcomes issued from PCA/FA analysis and gathered in Table 5 and Figure 3a,b may be linked to some differences in chemo-metric analysis of parameters, e.g., for the high-exposure group, hippuric acid and phenol (WF2, Table 5) showing

different complexing. Compared to data from the 3D plot, Figure 3b, hippuric acid is complexed with BTEX, and another grouping of phenol with o- and p-Cresol was observed, representing a new data set, which differs from Table 5 (WF2). It is possible that during that interval of time, some even higher maximum concentrations of the BTEX were emitted during the day, and as such, they could serve as a warning signal. Nonetheless, the whole monitoring should be repeated (which is also performed in practice) in the same way in another season (summer and spring measurement).

The above-mentioned parametric analysis and comparative estimations confirm the significance of air and biological monitoring and the importance of following the variations in the concentrations of priority substances, particularly BTEX, in ambient working environments and specific urinary metabolites. A comprehensive monitoring plan, scheduling various system activities at different time intervals, will provide better air monitoring and assessment of occupational exposure. The results obtained in this study and similar other studies may contribute to better national health and safety strategies and to increase occupational safety where pollutants like BTEX are present.

The values of benzene above the threshold level for most workers' duties indicate that effective protective measures and strategies should be taken in terms of using appropriate protective equipment among employees such as functional chemical masks or VOCs absorbent masks [7]. Unfortunately, in these places, so far only a few workers are wearing gloves and in some cases protective glasses. Greater control is also needed when handling raw materials and preventing them from leaking and evaporating, and it would be beneficial for workers' health follow-up to wear passive dosimeter badges attached to their collar to measure and monitor benzene levels.

For working places where elevated concentrations of phenol in the urine were found, workers should be referred for additional medical examinations and removed from their jobs for a certain amount of time. It would also be necessary to organize the workers' activities and to elaborate the shift schedule taking into account this study.

For the petrochemical industry, it is necessary to perform scheduled and more detailed monitoring, to establish safety strategies, in order to protect the health of workers and protect the environment [8,48].

5. Conclusions

Monitoring of BTEX in a petrochemical plant in Serbia showed levels of benzene in the air exceeding the OEL. Benzene's urinary metabolite phenol was also found to have higher values compared to the non-occupational exposure group and to the biological exposure level. However, hippuric acid is present at high levels, and its concentration in urine could be the result of lifestyle (drugs, food, additives, or consumption of benzoate-containing soft drinks) and habits. Toluene, ethylbenzene, and xylene levels in the air were lower than the OEL with correspondingly low levels of urinary metabolites. This is the first biomonitoring study that assessed the occupational exposure to BTEX using urinary biomarkers in Serbia in a petrochemical plant.

Petrochemical facilities represent a significant source of BTEX emissions and exposure for employees. Benzene levels in occupational settings in petrochemical plants need tight controls with extensive air monitoring to ensure that exposure is below occupational limits. Biomonitoring data and selection of appropriate urinary biomarkers can be used to ensure compliance with industrial hygiene guidelines. Those guidelines should be updated and be more rigorous, especially regarding mutagens and carcinogens in the working environment, including benzene. For workplaces with higher levels of benzene health monitoring, the frequency of controls should be increased. More effective protective strategies are required to minimize exposure and related occupational hazards, and on-site ventilation systems should be improved. The health risk assessment conducted indicates that the studied employees are at carcinogenic risk, with cancer risk values exceeding the limits defined by the USEPA. This study has implications for workplaces where benzene is present in the working environment, where workers are exposed to BTEX on a daily basis, and where no

studies have been undertaken to analyze the effects of BTEX on their health, e.g., petrol station workers, taxi drivers, and policemen.

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Institutional Review Board Statement: Ethical review and approval were waived for this study, due to human biological samples are collected and analyzed according to the protocol within regular and mandatory medical examination for workers exposed to BTEX and all subjects were previously informed and agreed to use the results of the analysis for scientific research purposes. Study and protocol were conducted according to the guidelines of the Declaration of Helsinki.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due national database on occupational diseases is not available online.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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