Saudi Journal of Biological Sciences 30 (2023) 103538

HOSTED BY

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Evaluation of environmental and biological monitoring methods for toluene exposure assessment in paint industry

Mansour A. Balkhyour [a], Radhouane Chakroun [a,*], Faycal Faidi [b,c]

[a] Department of Environmental Sciences, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[b] Al-Qunfudah Center for Scientific Research(QCSR), University College of Al-Qunfudah, Umm Al-Qura University, 21912 Al-Qunfudah, Saudi Arabia
[c] Biology and Occupational Toxicology Laboratories Department, Tunisian Occupational Safety and Health Institute 5, Rue Mustapha Khaznadar, 1007 Tunis, Tunisia

ARTICLE INFO

Article history:
Received 21 June 2022
Revised 9 November 2022
Accepted 7 December 2022
Available online 13 December 2022

Keywords:
Biomonitoring, blood
Hippuric acid
Occupational exposure
ortho-Cresol
Paint
Toluene
Urine

ABSTRACT

The aim of this study was to assess the exposure to Toluene in paint industry and to evaluate the environmental and biological monitoring techniques for the assessment of occupational exposure to this aromatic hydrocarbon. In this study, personal active and passive air sampling for toluene measurements, blood and urine sampling respectively for B-Tol and HA or U-Tol analyses for eight workers from two paint and thinner production factories were collected during four successive working days. Correlations were analyzed between biological indicators and environmental toluene exposure levels.

The concentration of Toluene measured in air samples ranged from 0.2 to 414.0 ppm (mean = 59.8 p pm), with high variability of atmospheric levels between activities and between days. No significant difference was found between airborne toluene concentrations measured by the two sampling methods. The correlation between air concentrations sampled by the diffusive sampling method and the biomarkers was the best for HA ($r = 0.902$, $p < 0.01$), followed by B-Tol ($r = 0.820$; $p < 0.01$), o-Cr ($r = 0.691$; $p < 0.01$) and U-Tol ($r = 0.607$; $p < 0.05$). The correlation was better between air concentrations and urinary metabolites HA and o-Cr for exposure levels higher than 50 ppm ($r = 0.931$; $p < 0.01$), and lower than 300 ppm ($r = 0.827$; $p < 0.01$), respectively.

According to our results, workers in the studied industries are highly exposed to Toluene. Given the high correlation found between toluene concentrations in samples taken on dosimeters and those actively sampled on charcoal tubes, it may be assumed that both sampling methods are valuable. Despite the influencing factors, HA was found to be a reliable biological indicator for the monitoring of occupational exposure to toluene for high exposure levels. However, B-Tol seems to be an interesting alternative, since it is more specific and showed the best correlations with airborne toluene levels.

1. Introduction

Toluene is an aromatic hydrocarbon widely used in industry either as organic solvent or as a starting product for chemical synthesis. It is also an important constituent in petroleum products, such as gasoline to which it is added to improve octane ratings. People working with gasoline, paint, lacquer, or dyes are the most directly concerned with occupational exposure to Toluene. The main effects of exposure to toluene are related to central nervous system dysfunctions (ATSDR, 2017). Neurobehavioral effects have been observed among workers exposed to toluene with regard to controls (Foo et al., 1990). Moreover, workers exposed to low toluene concentrations have been reported to experience neurological impairments (Shih et al., 2011). Additionally, color vision impairments have been reported among rotogravure printing shop workers (Chung et al., 2004). Chronic exposure to inhaled toluene may also lead to irreversible kidney damage (ATSDR, 2017). Moreover, several toluene exposure occupational studies reported reproductive effects including spontaneous abortion, congenital malformation and fertility reduction (Bukowski, 2001). Furthermore, the mutagenic risk through chromatid exchanges has been shown to be higher in rotogravure printing plant workers compared to a control group (Hammer, 2002). Therefore, a precise

* Corresponding author.
E-mail addresses: mbalkhyour@kau.edu.sa (M.A. Balkhyour), rshagroon@kau.edu.sa (R. Chakroun), fafaidi@uqu.edu.sa (F. Faidi).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2022.103538
1319-562X/

Table 1
Time weighted average occupational exposure limits (TWA-OELs).

	OSHA-PEL (ppm)	ACGIH-TLV (ppm)	NIOSH-REL (ppm)
TWA-OELs	10	20	100

OSHA-PEL: Occupational Safety and Health Administration - Permissible Exposure Limit.
ACGIH-TLV: American Conference of Governmental Industrial Hygienists - Threshold Limit Value.
NIOSH-REL: The National Institute for Occupational Safety and Health - Recommended Exposure Limit.

toluene exposure assessment may be helpful for the protection of workers and the prevention of occupational diseases. Exposure assessment can be done through air monitoring techniques using passive (PS) or active sampling (AS) methods. Most passive samplers operate on the principle of diffusion. Active sampling on the other hand, involves the use of an air sampling pump in order to actively pull the air through a collection device (coconut charcoal sorbent tubes). PS method is easy to undertake, and is well accepted by workers. It also allows sampling during long periods, which permits easier determination of the time-weighted average concentration. However, the sampling rates are provided by constructors and depend on environmental conditions, air speed and temperature in particular. The advantage of AS is that the sampling flow rate and duration (which may be shorter) can be set by the operator.

Biological monitoring is also considered as a helpful and powerful tool for the assessment of exposure to chemicals. Measurements of biological indicators allow the estimation of the body burden of contaminants and evaluate exposure from all routes of entry. However, chemical pollutants may have several biological indicators of exposure. Because of their low half-life, urinary metabolites are most commonly used to evaluate occupational exposure to volatile organic compounds. Urinary metabolite concentration at the end of the shift may reflect the level of exposure of the day. Hippuric acid has been for a long time used for the assessment of exposure to Toluene. However, the pollutant measurement itself, in biological media, is the current trend in biological monitoring nowadays, as they are more specific than metabolites and less influenced by dietary or other interference factors.

In the present study, we aim to evaluate the exposure of eight workers to toluene in all the workstations of a paint industry, during four working days. The exposure levels will be assessed by the measurement of toluene in air (Tol-A), using both AS and PS methods, and by the measurement of the urinary hippuric acid (HA) and *ortho*-Cresol (o-Cr), blood (B-Tol) and urinary xylenes (U-Tol) concentrations. The results will be compared with the time weighted average occupational exposure limits (TWA-OELs) and the biological exposure indices (BEIs), respectively depicted in Table 1 and Table 2. This study will also examine the correlation between the concentrations measured with both air sampling methods and the four studied toluene biological indicators (HA, o-Cr, B-Tol and U-Tol).

However, before the lowering of the ACGIH-TLV to 20 ppm, the recommended ACGIH-BEI value for HA in urine was < 1.6 g/g creatinine (ACGIH, 2007).

Table 2
Biological limit values.

	ACGIH - BEIs	DFG - BAT	FIOH - BAL
HA	–	–	–
o-Cr	0.3[a] mg/g.c.	1.5[b] mg/l	–
B-Tol	20[c] µg/l	600[d] µg/l	46[c] µg/l
U-Tol	30[a] µg/l	75[a] µg/l	–

ACGIH: American Conference of Governmental Industrial Hygienists; BEIs: Biological exposure indices.
DFG: Deutsche Forschungsgemeinschaft; BAT: Biologischer Arbeitsstoff-Toleranz-Wert (biological tolerance value).
FIOH: Finnish Institute of Occupational Health; BAL: Biological Action Level.
[a] At the end of shift in mg/ g creatinine.
[b] At the end of shift after several shifts.
[c] Before the last shift of the week.
[d] at the end of exposure.

2. Materials and methods

2.1. Workers

The subjects of this study were eight, male workers, between 29- and 52-year old (mean = 49.9 ± 8.5), working at different workstations in two paint production plants (Table 3). A questionnaire was completed for each worker, by oral interview, after informed consent, in order to collect information about working conditions in the current and former jobs, smoking habits, alcohol consumption, personal medical antecedents and medicine intake.

2.2. Air sampling

All workers were equipped with a passive sampling dosimeter (SKC-VOC Chek 575) and with a charcoal tube (SKC 100/50 mg), connected to an SKC AirChek XR5000 sampling pump which flow rate was set at 50 ml/min. Air samples were collected every day from Monday to Thursday, and for six to seven hours. The air flow rate was checked at the beginning and at the end of sampling. The sample was excluded in case the variation of the flow was greater than 5 %.

2.3. Blood and urine sample collection

Urine samples were collected at the end of shift, during four days of the working week (Monday to Thursday). Samples were collected in 50-mL propylene bottles and workers were asked to fill the bottle completely. 1 ml of the sample was immediately transferred into a headspace vial, sealed for the determination of urinary toluene (U-Tol), and analyzed the same day with urinary creatinine. The remainder of the sample was stored at − 20 °C until hippuric acid (HA) and *ortho*-cresol (o-Cr) analysis.

Blood samples were withdrawn at the end of the shift in vacuum EDTA tubes, and 1 ml of the sample was immediately transferred into a headspace vial and sealed for the determination of blood toluene (B-Tol).

2.4. Atmospheric sample analysis

The front and back sorbent sections of the charcoal tubes were placed in separate vials. One milliliter of carbon disulfide was added to each vial and to dosimeters. The determination of toluene was performed using GC-FID according to the NIOSH aromatic hydrocarbons analytical method 1501 (NIOSH, NMAM, Method 1501). The correlation coefficient obtained for the calibration curve was R^2 = 0.9998; the limit of quantification was 4.4 µg (on the sampling device; n = 10), with a relative standard deviation of 4.8 %.

Table 3
Worker's tasks and working conditions.

Workers	Factory	Workstation	Tasks
Worker 1 (W1)	F1	Technical supervisor	Responsible for the operational control and the management of technical procedures
Workers 2,3,4 (W2, W3, W4)	W2 F1 W3 F1 W4 F2	Colorist	Prepares tints by mixing pigments with solvents and other raw materials; checks and adjusts the tint The worker W3 performs also other tasks, mainly product packaging
Workers 5 and 6 (W5, W6)	W5 F1 W6 F2	Washing Vats	Washes the used vats with solvents
Worker 7 (W7)	F2	Loading	Loads raw material (resin, additives, solvents…)
Worker 8 (W8)	F2	Lab technician	In charge of the quality control of the final products and the development of new formula

The identification of the compounds in the sample was checked with GC-MSD using the NIOSH volatile organic compounds screening method 2545 (NMAM, 1996).

2.5. Blood and urine analyses

B-Tol and U-Tol were analyzed by Headspace gas chromatography mass spectrometry (HS-GC-MSD) according to the method developed by Ogawa and Sasahara (2012). Urinary HA and o-Cr determinations were performed using previously published analytical methods using respectively high performance liquid chromatography with a diode-array detector (HPLC-DAD) (Chakroun et al., 2006) and GC-FID (Chakroun et al., 2008).

2.6. Statistical analysis

Statistical analysis was performed using Statgraphics Centurion 19 software. The relationship between toluene concentrations in air samples and the biological markers of exposure was examined using linear regression. A p value of 0.05 was considered as significant.

3. Results

3.1. Atmospheric toluene measurements

Time weighted average toluene concentrations (TWA-C) in air samples are presented in Table 4. The toluene concentrations measured using active sampling (Tol A) and the values obtained with the passive sampling method (Tol P) ranged from 1.2 to 410.0 ppm (median = 18.7 ppm, mean = 62.5 ± 106.8 ppm) and from 0.2 to 414.0 ppm (median = 20.0 ppm, mean = 57.1 ± 101.9 ppm), respectively (Table 4).

The toluene concentration levels in personal air samples of the studied workers are depicted in Table 5. The measured concentrations ranged from 0.2 to 414 ppm, with a mean value of 59.8 ppm.

The highest time weighted average (TWA) toluene concentrations were found for the workers W6 (factory F2) and W5 (factory F1) who were both assigned to the washing vats workstation, with concentrations as high as 414.0 and 189.8 ppm respectively. The mean exposure level during the four working days of the study was as high as 264.6 and 106.0 ppm, respectively.

Relatively high toluene exposure values were also recorded for the worker W7 in the raw materials loading workstation, with exposure levels ranging between 22.4 and 87.3 ppm and a mean TWA-C of 52.5 ppm (Table 5).

The workers W2 and W4 (colorists) were found to be exposed to toluene concentrations varying from 0.2 to 22.3 ppm (Table 5). The lab technician (W8) was found to be also highly exposed to toluene. The measured concentrations ranged from 12.2 to 29.8 ppm with a mean value of 22.1 ± 5.9 ppm (Table 5). Finally, the technical supervisor (W1) was found to be exposed to toluene concentrations varying from 2.5 to 28.9 ppm (mean = 11.3 ± 10. 6 ppm).

For a given worker, toluene exposure levels varied from one day to another during the work week (Fig. 1). For most of the workers, the highest concentrations were measured during the second or the third working day (Fig. 1). Nevertheless, exposure levels were higher on the second day for W5 and on the fourth day for W6 who both work in the wash workstation. This may be explained by the fact that this workstation is the last link in the process chain. The different exposure profile between W5 and W6 is probably due to the different procedures employed and level of industrial activity in the two studied factories. These results highlight the importance of the choice of the sampling day, as the exposure may fluctuate depending on the industrial activity.

A positive and strong correlation between the time weighted average toluene concentrations in the samples taken by the two sampling methods was registered, with a statistically significant relationship between the two variables ($R = 0.97$; $p < 0.0001$) at 95 % confidence level (Fig. 2).

However, for most measurements, Tol A was higher than Tol P (Fig. 3).

Table 4
Toluene concentrations in air samples for the studied workers.

	Median [min–max]	Mean ± SD
Tol A (ppm)	18.7 [1.2–410.0]	62.5 ± 106.8
Tol P (ppm)	20.0 [0.2–414.0]	57.1 ± 101.9
All samples (ppm)	20.0 [0.2–414.0]	59.8 ± 103.6

3.2. Biological measurements

Urinary hippuric acid (HA) was measured each day of the study at the end of the work shift. The results are depicted in Tables 6 and 7. HA concentrations varied from 217.2 to 4045.5 mg/g creatinine.

HA concentrations as a function of the workstations are presented in Table 7. HA values higher than the reference values were found for the workers W2 (colorist) and the workers W5 and W6 (Washing Vats). The highest HA values were found in the urine samples of the worker W6 (manual washing of the used vats).

The results of the Pearson correlation analysis are presented in Table 8. A positive and significant correlation was found between air toluene concentrations and urinary HA concentrations expressed in mg/l or mg/g creatinine.

The correlation was better when the urinary HA concentrations were corrected for creatinine. Moreover, the correlation between atmospheric toluene levels higher than 50 ppm and urinary HA concentrations ($R = 0.931$) was far better than for exposure levels that are less than 50 ppm ($R = 0.101$) (Fig. 4).

Given the good correlation between the results obtained from active and passive sampling methods and the better correlation between the atmospheric concentrations and HA concentrations

Table 5
Toluene concentrations in air samples for the studied workers.

Workers	Factory	Workstation	Toluene (ppm)	
			Median [min–max]	**Mean ± SD**
W1	F1	Supervisor	6.7 [2.5–28.9]	11.3 ± 10.6
W2	F1	Colorist	8.2 [5.2–15.2]	8.9 ± 3.5
W3	F1	Colorist/packaging	2.7 [0.7–5.7]	2.8 ± 1.8
W4	F2	Colorist	9.3 [0.2–22.3]	10.2 ± 7.5
W5	F1	Washing Vats	97.3 [41.2–189.8]	106.0 ± 53.8
W6	F2	Washing Vats	336.3 [1.2–414.0]	264.6 ± 169.6
W7	F2	Loading	54.2 [22.4–87.3]	52.5 ± 21.1
W8	F2	Lab technician	23.6 [12.2–29.8]	22.1 ± 5.9

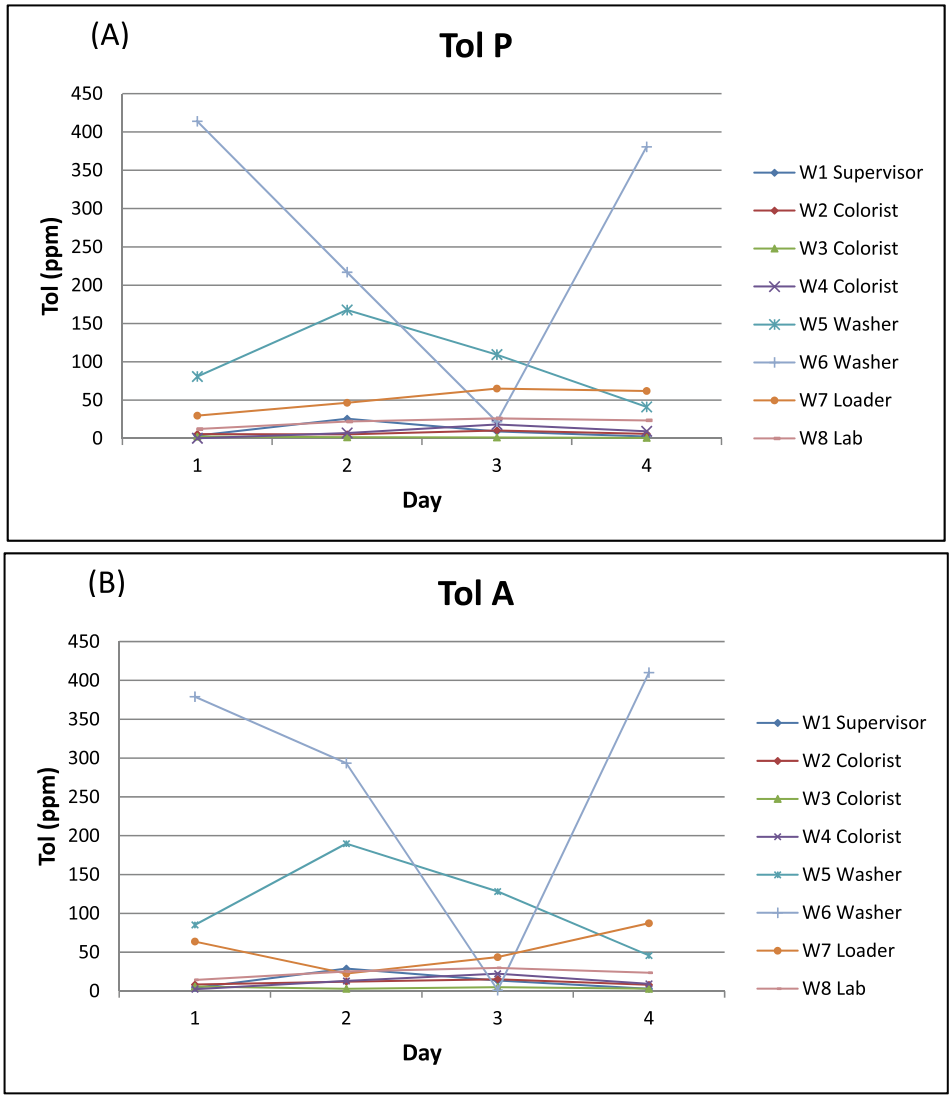

Fig. 1. Day to day variation of toluene concentrations in air samples sampled by (A) passive sampling and (B) by active sampling methods.

corrected for creatinine, we will consider these parameters for the second toluene metabolite studied in this work (o-Cr).

The o-Cr concentrations measured in the current study are shown in Table 9. O-Cr levels varied from 0.03 to 5.09 mg/g creatinine, with a mean value of 1.11 ± 1.25 mg/g creatinine. Compared to atmospheric toluene and urinary hippuric acid levels, it is possible to notice a comparable profile of exposure in the different workstations.

A positive and significant correlation was recorded between toluene concentrations in air sampled on dosimeters and urinary o-Cr concentrations corrected for creatinine ($r = 0.691$; $P < 0.01$). A better correlation was obtained for the exposure levels below 300 ppm ($r = 0.827$; $P < 0.01$) (Fig. 5).

Blood (B-Tol) and urinary toluene (U-Tol) concentrations are presented in Table 10. B-Tol ranged from 6.1 to 687.2 µg/l, with a mean concentration of 186.0 ± 145.2 µg/l. Lower values were

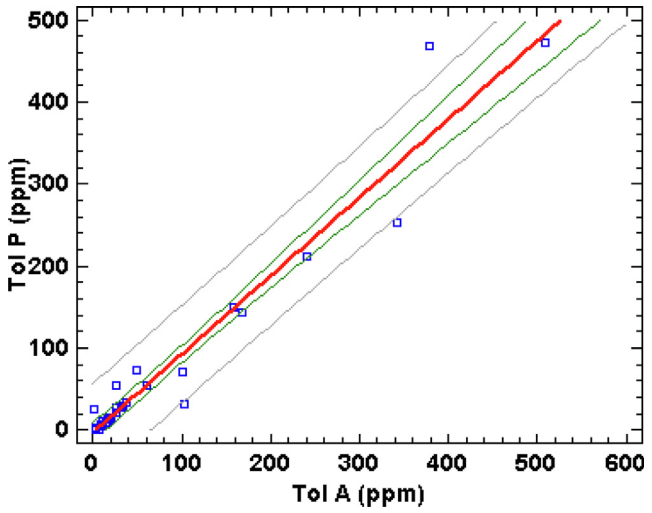

Fig. 2. Correlation between toluene concentrations in air samples sampled by passive sampling (Tol P) and by active sampling methods (Tol A). The inner bounds show 95.0% confidence limits for the mean Tol P of many observations at given values of Tol A. The outer bounds show 95.0% prediction limits for new observations.

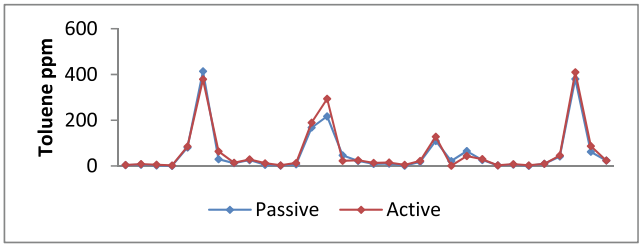

Fig. 3. Toluene concentrations in air samples sampled by passive and by active sampling methods.

Table 6
Hippuric acid concentrations (HA) in urine samples.

	Median [min–max]	Mean ± SD
HA (mg/l)	857.1 [230.9–5923.1]	1498.4 ± 1520.9
HA (mg/g creatinine)	625.6 [217.2–4045.5]	1022.3 ± 1003.4

Table 8
Correlation between passive and active atmospheric toluene (ppm) and urinary HA concentrations in mg/l and in mg/g creatinine.

Sampling mode	Correlation r (P)	
	Toluene - HA (mg/l)	**Toluene - HA (mg/g creatinine)**
Passive	0.891 (<0.01)	0.902 (<0.01)
Active	0.884 (<0.01)	0.915 (<0.01)

recorded for U-Tol, with concentrations ranging from 4.9 to 467.7 μg/l, and a mean value of 115.7 ± 100.6 μg/l.

A positive correlation was found between atmospheric toluene concentrations sampled on dosimeters and urinary toluene concentrations (μg/l) ($r = 0.607$; $p < 0.05$) (Fig. 6). The correlation between atmospheric toluene concentrations sampled on dosimeters and blood toluene concentrations (B-Tol) (μg/l) was $r = 0.820$ ($p < 0.01$).

4. Discussion

The time weighted average toluene concentration (TWA-C) in air samples ranged from 0.2 to 414 ppm, with a mean value of 59.8 ppm. This mean TWA-C value is higher than the ACGIH TLV-TWA and the OSHA-PEL (20 and 10 ppm, respectively). Moreover, with either active or passive sampling methods, 65.63 % of the measured TWA-C toluene concentrations were higher than the OSHA-PEL and 53.13 % were higher than the ACGIH TLV-TWA, indicating high exposure levels for workers. The TWA-C in the present study are far higher than those reported for paint industries in other studies (Sahri and Widajati, 2013; Jafari et al., 2008).

The highest time weighted average exposure levels were found for the workers in the washing vats workstation (W5 and W6) with mean exposure levels during the four working days of the study (106.0 and 264.6 ppm) higher than the highest guideline limit value of 100 ppm (NIOSH-REL). Even if the task of cleaning the used vats is achieved in open air, a large volume of solvent mixture is used to remove the paint from the containers. W5 who performs the same tasks in the first factory (F1) was found to be less exposed to Toluene. This may be explained by the different volume of production between the two factories. Also, in the factory F1, the cleaning of the used vats is performed by an automatic machine. However, the worker still can be considered to be highly exposed to toluene, especially at the opening of the machine door. Moreover, the machine cleaning is not complete and workers have to complete the washing process manually. Moreover, the washing

Table 7
Hippuric acid concentrations (HA) in workers urine samples at the different workstations.

Workers	Factory	Workstation	HA (mg/l)		HA (mg/g creatinine)	
			Median [min–max]	**Mean ± SD**	**Median [min–max]**	**Mean ± SD**
W1	F1	Supervisor	278.1 [258.5–624.8]	359.9 ± 177.2	231.7 [217.2–381.0]	265.4 ± 77.7
W2	F1	Colorist	784.8 [417.3–2305.2]	1073.0 ± 852.8	733.8 [390.0–1921.0]	944.7 ± 671.4
W3	F1	Colorist/ packaging	475.6 [240.7–868.1]	515.0 ± 274.8	352.8 [248.1–449.2]	350.8 ± 85.2
W4	F2	Colorist	1551.3 [619.1–2398.5]	1530.0 ± 756.3	654.0 [412.7–1175.7]	724.1 ± 322.5
W5	F1	Washing Vats	3448.3 [760.9–3807.2]	2366.1 ± 1311.9	1684.9 [1086.9–2126.9]	1645.9 ± 427.2
W6	F2	Washing Vats	4976.6 [2137.1–5923.1]	4503.4 ± 1694.6	3457.2 [1444.0–4045.5]	3101.0 ± 1139.0
W7	F2	Loading	532.0 [230.9–683.6]	494.6 ± 216.2	551.3 [445.3–712.1]	565.0 ± 124.1
W8	F2	Lab technician	1216.8 [846.0–1301.6]	1145.3 ± 212.3	603.7 [347.0–772.3]	581.6 ± 176.0

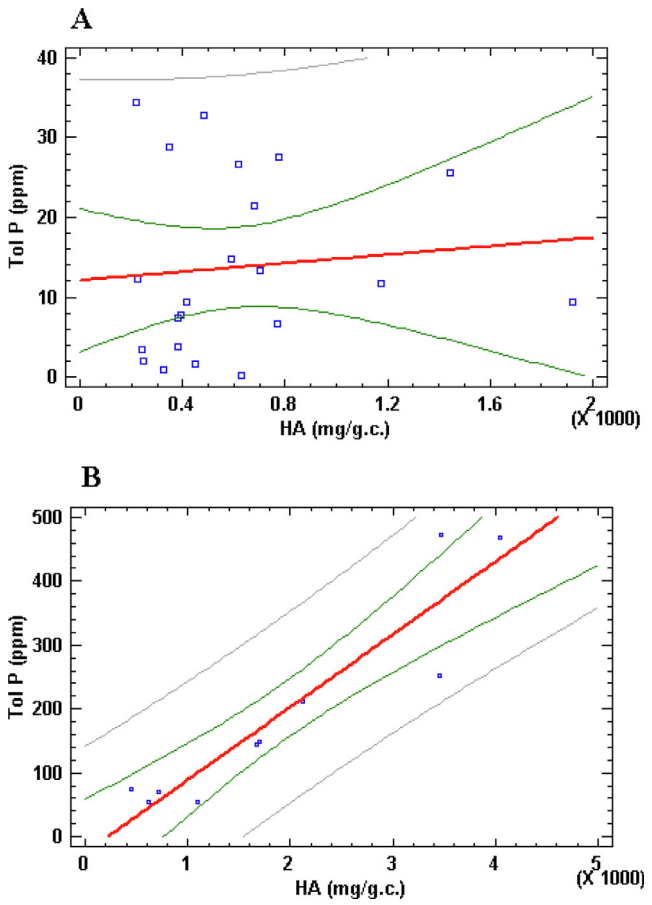

Fig. 4. Correlation between toluene concentrations (passive sampling) < 50 ppm (A) and greater than 50 ppm (B) with urinary HA concentrations. The inner bounds show 95.0 % confidence limits for the mean Tol P of many observations at given values of HA. The outer bounds show 95.0 % prediction limits for new observations.

machine is dedicated to large vats. The small used containers are washed manually with a mixture of solvents. The worker W7 (F2) in the raw material loading workstation was also found to be highly exposed to toluene. The minimum measured concentration was 22.4 ppm, which is higher than the ACGIH TLV-TWA (20 ppm) and more than double of the OSHA-PEL (10 ppm). In the factory F2 the solvents used in the manufacturing of thinners and varnishes are mainly pumped automatically into the mixing tanks. However, an adjustment by manual addition of solvents is often necessary. The worker manually fills the barrel placed on the scale using pump handles similar to those used in gas stations. Besides, this workstation is not equipped with a local ventilation system.

The workers W2 and W4 (colorists) were found to be less exposed to toluene than W5 and W6. However concentrations reached values as high as 22.3 ppm (Table 5). The main tasks of this worker are the paint tint adjustment performed in mechanical mixers. However, supplementary manual agitation is sometimes needed. Moreover, the colorist moves through the workshop and goes to the lab to check for the color. Furthermore, the worker W4 in the factory F2 was found to be more exposed to toluene than the worker W2 (factory F1). This is probably due to the fact that the workstation is equipped with local exhaust ventilation over the mixing tanks in the factory F1.

Both workers were found to be more exposed than the worker W3 who spent more time performing other tasks such as product packaging. All the atmospheric measurement made for the lab technician (W8) suggested that he is highly exposed to toluene. TWA-C exceeded the OSHA-PEL (10 ppm) the first day of exposure (12.2 ppm) and was higher than the ACGIH TLV-TWA (20 ppm) during the three following working days (22.1–29.8 ppm).

Finally, the technical supervisor (W1) was found to be also highly exposed to toluene even though he is not in direct contact with the solvent (mean = 11.3 ± 10.6 ppm). This may indicate that the air is contaminated in the entire factory.

A good correlation ($R = 0.97$; $p < 0.0001$) at 95 % confidence level) was as well recorded between the time weighted average toluene concentrations in the samples taken by the two sampling methods (Fig. 2). Nevertheless, Tol-A was higher than Tol-P in the majority of the samples (Fig. 3). This can be explained by the difference in sampling mechanisms. The passive sampling is based on the principle of diffusion according to Fick's first law of diffusion from the most concentrated medium to the less concentrated one (Górecki and Namieśnik, 2002). On the other hand, in the active sampling method, the air is withdrawn continuously as long as the pump is functioning. Moreover, the sampling flow rate for toluene given by the provider of the dosimeters used in this study is 14.5 ml/min, while the pumps were set at 50 cc/min for the active sampling procedure, which results in higher samples' volumes. Batterman et al. (2002) reported consistent results obtained with passive sampling of volatile organic compounds including toluene, compared with concentrations from active sampling at low flow rates (r = 0.95).

Toluene concentrations measured using the passive sampling method in this study were higher than those found by Thetkathuek et al. (2015) who reported an average toluene concentration of 9.5 ± 10.4 ppm in air samples taken on dosimeters from a paint industry in Thailand. High toluene concentration levels have been reported in air personal samples taken by active sampling on charcoal tubes, with a flow rate of 50 ml/min, for workers in a paint factory in Iran. Mean toluene exposure levels were reduced after implementing local exhaust systems, from 105.82 ± 74.88 to 44.5 0 ± 24.39 ppm (Jafari et al., 2008).

Table 9
o-Cr concentrations in urine samples.

Workers	Factory	Workstation	o-Cr (mg/g creatinine)	
			Median [min–max]	**Mean ± SD**
W1	F1	Supervisor	0.48 [0.03–1.20]	0.55 ± 0.53
W2	F1	Colorist	0.84 [0.40–1.63]	0.93 ± 0.53
W3	F1	Colorist/packaging	0.17 [0.12–0.56]	0.25 ± 0.21
W4	F2	Colorist	0.18 [0.11–0.67]	0.29 ± 0.26
W5	F1	Washing Vats	1.54 [0.74–5.09]	2.23 ± 1.99
W6	F2	Washing Vats	2.77 [2.22–4.88]	3.16 ± 1.19
W7	F2	Loading	0.79 [0.61–1.19]	0.84 ± 0.28
W8	F2	Lab technician	0.62 [0.34–0.99]	0.64 ± 0.29
All W	F1&F2	All Workstations	0.71 [0.03–5.09]	1.11 ± 1.25

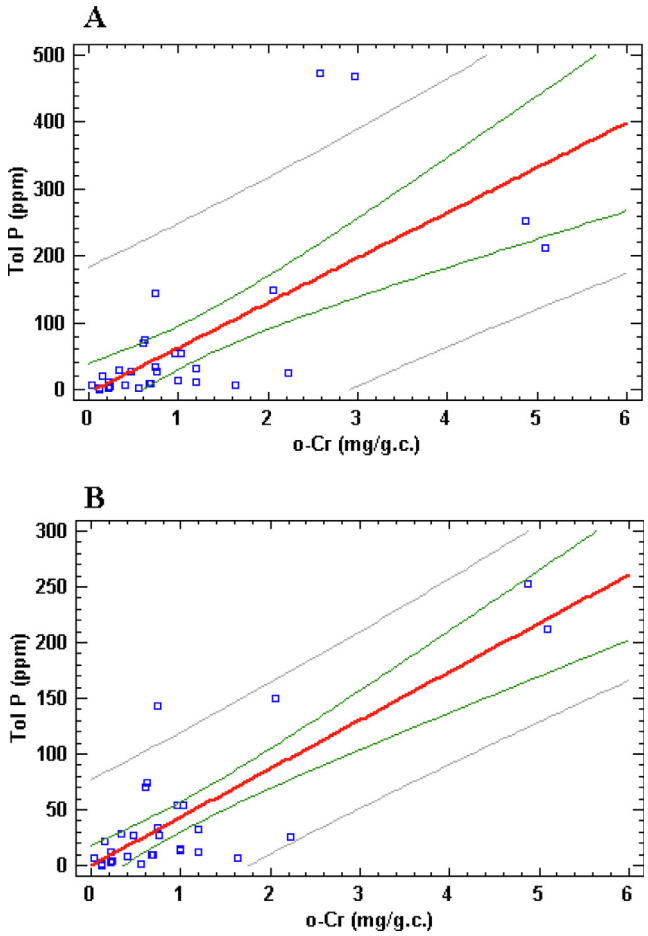

Fig. 5. Correlation between all toluene concentrations (passive sampling) (A) and < 300 ppm (B) with urinary o-Cr concentrations. The inner bounds show 95.0 % confidence limits for the mean Tol P of many observations at given values of o-Cr. The outer bounds show 95.0 % prediction limits for new observations.

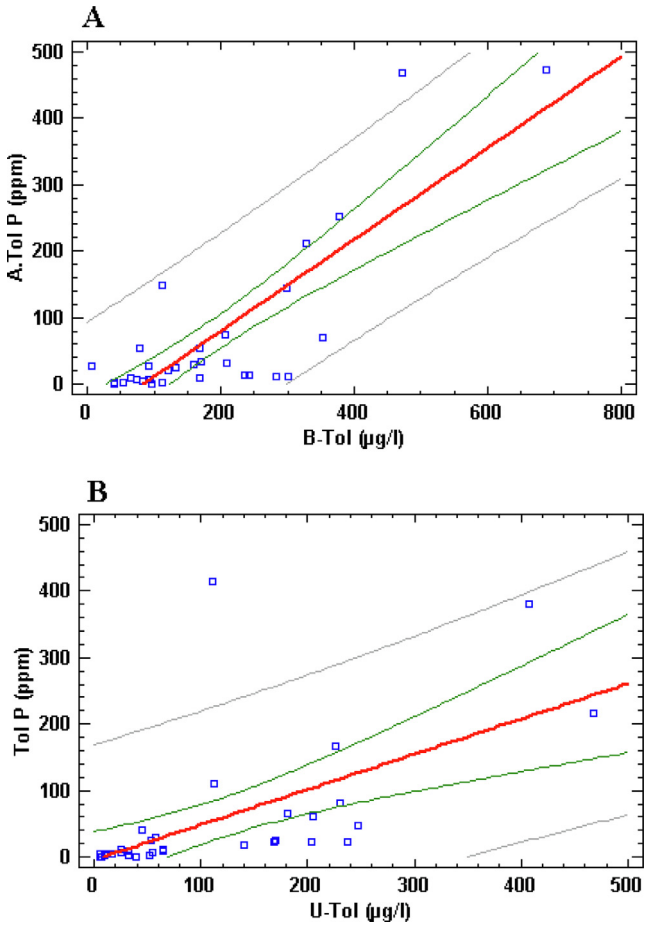

Fig. 6. Correlation between toluene concentrations (passive sampling) with B-Tol (A) and with U-Tol (B) concentrations. The inner bounds show 95.0% confidence limits for the mean Tol P of many observations at given values of B-Tol or U-Tol. The outer bounds show 95.0% prediction limits for new observations.

Table 10
Toluene concentrations in blood (B-Tol) and urine (U-Tol) samples.

	Median [min–max]	Mean ± SD
B-Tol (µg/l)	145.9 [6.1–687.2]	186.0 ± 145.2
U-Tol (µg/l)	75.6 [4.9–467.7]	115.7 ± 100.6

Biological monitoring is, nowadays, one of the main exposure assessment tools used in the industrial hygiene and occupational health management systems. In the present study, we measured urinary hippuric acid (HA) and *ortho*-Cresol (o-Cr), blood (B-Tol) and urinary xylenes (U-Tol) concentrations at the end of shift during four successive working days.

HA concentrations ranged from 217.2 to 4045.5 mg/g creatinine. Currently, there are no biological limit values for urinary HA after the lowering of the ACGIH-TLV to 20 ppm, however, as shown in Table 7, values higher than the former ACGIH - BEI (<1.6 g/g creatinine) (ACGIH, 2007) were recorded for the workers W5 and W6 (washing used vats workstation in both studied factories), with higher HA concentrations for W6 who accomplishes all the tasks manually. Relatively high HA values were also obtained for W2 (colorist in the factory F1). In contrast with air monitoring results, the recorded HA values were higher than those found in the factory F2 (W4). This may indicate that this biological indicator of exposure to toluene is not reliable enough at these exposure levels.

Comparison with values in non-occupationally exposed subjects may also provide information on exposure levels. In the current study, 21.9 % of the measured HA concentrations were higher than the reference values in general population non-occupationally exposed to toluene (i.e. < 1500 mg/g creatinine) (Lauwerys et al., 2001), suggesting that workers in these industries are exposed to toluene.

A better correlation was found between air toluene concentrations and urinary HA concentrations when corrected for creatinine. This confirms the importance of the creatinine correction to avoid the bias from urine dilution and reduce intra-individual variation (German Federal Environment Agency, 2005). The adjustment of urinary HA to creatinine was shown to be reliable (Nicolli et al., 2014). In the present study, we only considered the urine samples with a creatinine concentration within the interval 0.3–3 g l^{-1} as recommended by the world health organization for the biological monitoring of exposure at the workplace (World Health Organization, 1996).

Lower correlations have been reported for workers exposed to 12–198 ppm atmospheric toluene with urinary HA concentrations ($r = 0.548$, $p < 0.01$) (Decharat, 2014). However, Nise (1992) stated that HA was an unreliable biomarker for airborne concentrations lower than 200 mg/m^3 (≈ 53 ppm). Nevertheless, in a recent study, Hormozi et al. (2019) reported a relatively good correlation between atmospheric toluene levels ranging between 19.33 and 132.67 ppm (mean = 37.64 ± 24.09 ppm) in samples taken on charcoal tubes and the urinary HA concentrations ($r = 0.567$; $P < 0.01$).

In the present study, the correlation between atmospheric toluene levels lower than 50 ppm and urinary HA concentrations was very low, suggesting that this metabolite would be reliable only for exposure levels higher than 50 ppm (Fig. 4). This may explain the lower HA values found for the worker W4 (factory F2), although he was more exposed to toluene than the worker W2 in the factory F1. In both factories, the toluene exposure levels were lower than 50 ppm at the colorist workstation.

The ortho-Cresol (o-Cr) concentrations measured in the current study were far lower than HA values. The highest o-Cr concentration didn't exceed 5.09 mg/g creatinine, with a mean value of 1.1 1 ± 1.25 mg/g creatinine. ortho-Cresol (o-Cr) has been considered as more sensitive than HA for the biological monitoring of exposure to toluene concentrations lower than 10 ppm (Bahrami et al., 2005). The authors reported that urinary HA concentrations started increasing only when atmospheric toluene concentrations were approximately at 25 to 35 ppm, while workers exposed to toluene concentrations as low as 2 ppm could be distinguished from non-occupationally exposed subjects. This may also explain the better correlation between o-Cr and atmospheric toluene concentrations lower than 300 ppm obtained in the current study (i.e. r = 0.827), while for HA, the correlation with atmospheric concentrations was better for the high toluene exposure levels. Nevertheless, the profile of exposure in the different workstations monitored by o-Cr measurements was found to be similar to that determined by HA measurements. Both biological indicators indicated high exposure levels for W5 and W6 (washing vats), followed by W2 (colorist). However, although o-Cr is a sensitive biomarker of toluene exposure, its concentration may be reduced up to 30 % in the case of simultaneous exposure to xylenes (Tardif et al., 1998), which is the case in the current study. The worker W6 who was exposed to toluene concentrations above 400 ppm uses a mixture of solvents to clean the used vats including xylenes. In this study, blood (B-Tol) and urinary toluene (U-Tol) concentrations, which are the most specific biological indicators of exposure to toluene, were also measured. U-Tol concentrations were not corrected for creatinine because toluene is excreted in the urine by passive diffusion in the renal tubules. Its elimination is therefore determined by the equilibrium of the partial pressures of the solvent between blood and urine (Ducos et al., 2008).

B-Tol concentrations were higher than U-Tol values, suggesting that B-Tol could be a more sensitive biomarker in end of shift samples. The mean values were respectively 186.0 ± 145.2 and 115.7 ± 100.6 µg/l. These results are consistent with Fustinoni et al. (2000) findings. The authors reported a median B-Tol concentration of 0.36 µg/l versus 0.20 µg/l U-Tol for workers exposed to atmospheric toluene median value of 80 mg/m^3 (21.2 ppm).

Urinary toluene is known for being a sensitive and specific biomarker especially at low exposure levels. Hopf et al. (2012) found a high and significant correlation between air toluene at exposure levels as low as 0.05 ppm and urinary toluene ($r = 0.73$, $p < 0.001$). Although the exposure levels in the current study are much higher, a positive correlation was found between atmospheric toluene concentrations sampled on dosimeters and urinary toluene concentrations (µg/l) ($r = 0.607$; $p < 0.05$). These results are consistent with Kawai et al. (2008) and Inoue et al. (2008) findings. The authors reported correlation coefficients of r = 0.6 and r = 0.61 respectively. However, the correlation coefficient obtained in the current study was lower than the values reported by Takeuchi et al. (2002) (r = 0.73) and Fustinoni et al. (2007) (r = 0.73). Moreover, Ukai et al. (2007) and Ducos et al. (2008) found correlation coefficients as high as r = 0.83 and r = 0.92 respectively. A better correlation was obtained between atmospheric toluene concentrations sampled on dosimeters and blood toluene concentrations (B-Tol) (µg/l) ($r = 0.820$; $p < 0.01$). Similar correlation coefficient (r = 0.90) has been reported by Foo et al. (1988) for workers exposed to atmospheric toluene concentrations ranging from 8.5 ppm to 262.7 ppm. In the opposite, Ikeda et al. (2008) reported lower correlation coefficient (r = 0.424) between end of shift B-Tol and atmospheric toluene concentrations ranging from 4.6 ppm to 120.8 ppm. These discrepancies are probably due to the different exposure levels. Indeed, the correlation coefficient between atmospheric toluene and its biomarkers including HA, o-Cr, U-Tol and B-Tol has been shown to change according to exposure levels (Ikeda et al., 2008). The authors reported that the correlation coefficient for U-Tol and B-Tol were higher than 0.6 when the upper toluene concentration in air was 80 ppm, in accordance with our results.

5. Conclusion

Monitoring chemical pollutants exposure is essential to protect the worker's health and the workplace environment. Biological monitoring is an important tool for occupational exposure evaluation and workers' health surveillance as it integrates all routes of exposure. In this work, we tried to assess exposure to toluene in two paint industries and evaluated the commonly used environmental and biological monitoring techniques. Given the high correlation found between toluene concentrations in samples taken on dosimeters and those actively sampled on charcoal tubes, it may be assumed that both sampling methods are valuable. The atmospheric toluene levels showed high variability between activities and between days, highlighting the importance of the sampling time according to the industrial activity and the work organization. When considering all the results, the correlation between air concentrations and the biomarkers was the best for HA, followed by B-Tol, o-Cr and U-Tol. However, the studied BEIs showed a variable correlation depending on the atmospheric Toluene concentration. According to our results, HA is the best biological indicator when Toluene air concentrations are above 50 ppm, and o-Cr showed a strong correlation up to 300 ppm. Therefore, the atmospheric exposure levels should be considered among the criteria of choice of the biological indicator to be used for the surveillance of occupational exposure to Toluene.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under the grant no. G: 514-155-1439. The authors, therefore, acknowledge with thanks DSR for technical and financial support.

References

American Conference of Governmental Industrial Hygienists (ACGIH), 2007. TLVs® and BEI® based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure indices, Cincinnati, Ohio, 104.

Agency for Toxic Substances and Disease Registry, 2017. Toxicological profile for toluene, U.S. Department of Health and Human Services, Public Health Service, Atlanta, USA.

Bahrami, A., Jonidi-Jafari, A., Folladi, B., Mahjub, H., Sadri, Q., Motamedzadeh, M., 2005. Comparison of urinary O-cresol and hippuric acid in drivers, gasoline station workers and painters exposed to toluene in west of Iran. Pak. J. Biol. Sci. 8 (7), 1001–1005.

Batterman, S., Metts, T., Kalliokoski, P., Barnett, E., 2002. Low-flow active and passive sampling of VOCs using thermal desorption tubes: Theory and application at an offset printing facility. J. Environ. Monit. 4, 361–370.

Bukowski, J.A., 2001. Review of the Epidemiological Evidence Relating Toluene to Reproductive Outcomes. Regul. Toxicol. Pharm. 33, 147–156.
Chakroun, R., Hedhili, A., Faidi, F., Nouaigui, H., Ben Laiba, M., 2006. Simultaneous HPLC determination of urinary metabolites of Toluene, Xylenes, and Ethylbenzene; and its application to biological monitoring of Tunisian workers exposure. Anal. Lett. 39 (1), 83–97.
Chakroun, R., Faidi, F., Hedhili, A., Charbaji, K., Nouaigui, H., Laiba, M.B., 2008. Inhalant abuse detection and evaluation in young Tunisians. J. Forensic Sci. 53 (1), 232–237. https://doi.org/10.1111/j.1556-4029.2007.00623.x.
Chung, S.Y., Kang, S.K., Seong Kim, D., Lee, S.H., 2004. Assessment of neurobehavioral performance among rotogravure printing workers exposed to toluene. Korean J. Occup. Environ. Med. 16 (2), 115–128.
Decharat, S., 2014. Hippuric Acid levels in paint workers at steel furniture manufacturers in Thailand. Saf. Health Work 5 (4), 227–233. https://doi.org/10.1016/j.shaw.2014.07.006.
Ducos, P., Berode, M., Francin, J.M., Arnoux, C., Lefèvre, C., 2008. Biological monitoring of exposure to solvents using the chemical itself in urine: application to toluene. Int. Arch. Occup. Environ. Health 81 (3), 273–284.
Foo, S.C., Phoon, W.O., Khoo, N.Y., 1988. Toluene in Blood after Exposure to Toluene. Am. Ind. Hyg. Assoc. J. 49 (5), 255–258. https://doi.org/10.1080/15298668891379701.
Foo, S.C., Jeyaratnam, J., Koh, D., 1990. Chronic neurobihavioural effects of toluene. Brit. J. Ind. Med. 47, 480–484.
Fustinoni, S., Buratti, M., Giampiccolo, R., Brambilla, G., Foà, V., Colombi, A., 2000. Comparison between blood and urinary toluene as biomarkers of exposure to toluene. Int. Arch. Occup. Environ. Health 73 (6), 389–396. https://doi.org/10.1007/s004200000156.
Fustinoni, S., Mercadante, R., Campo, L., Scibetta, L., Valla, C., Consonni, D., Foà, V., 2007. Comparison between urinary o-cresol and toluene as biomarkers of toluene exposure. J. Occup. Environ. Hyg. 4 (1), 1–9.
German Federal Environment Agency - Human Biomonitoring Commission (2005) Standardisation of substance concentrations in urine – creatinine. Bundesgesundheitsbl – Gesundheitsforsch – Gesundheitsschutz 48 : 5 : 616-618.
Górecki, T., Namieśnik, J., 2002. Trends Anal. Chem. 21, 276–291.
Hammer, K.D., 2002. Metabolite ratio of toluene-exposed rotogravure printing plant workers reflects individual mutagenic risk by sister chromatid exchanges. Mutation Research/Genetic Toxicol. Environ. Mutagenesis 519 (1), 171–177. https://doi.org/10.1016/S1383-5718(02)00134-1.
Hopf, N.B., Kirkeleit, J., Bråtveit, M., Succop, P., Talaska, G., Moen, B.E., 2012. Evaluation of exposure biomarkers in offshore workers exposed to low benzene and toluene concentrations. Int. Arch. Occup. Environ. Health 85 (3), 261–271. https://doi.org/10.1007/s00420-011-0664-1.
Hormozi, M., Mirzaei, R., Nakhaee, A., Payandeh, A., Izadi, S., et al., 2019. Quantification of urinary metabolites of toluene and xylene isomers as biological indices of occupational exposure in printing industry workers. Health Scope 8 (1), e82962.
Hydrocarbons, Aromatic: Method 1501, Issue 3. 4th ed. Washington, DC: NIOSH; March 15, 2003:3.
Ikeda, M., Ukai, H., Kawai, T., Inoue, O., Maejima, Y., Fukui, Y., Ohashi, F., Okamoto, S., Takada, S., Sakurai, H., 2008. Changes in correlation coefficients of exposure markers as a function of intensity of occupational exposure to toluene. Toxicol. Lett. 179 (3), 148–154. https://doi.org/10.1016/j.toxlet.2008.05.003.
Inoue, O., Kawai, T., Ukai, H., Maejima, Y., Fukui, Y., Ohashi, F., Okamoto, S., Takada, S., Sakurai, H., Ikeda, M., 2008. Limited validity of o-cresol and benzylmercapturic acid in urine as biomarkers of occupational exposure to toluene at low levels. Ind. Health 46 (4), 318–325.
Jafari, M.J., Karimi, A., Azari, M.R., 2008. The role of exhaust ventilation systems in reducing occupational exposure to organic solvents in a paint manufacturing factory. Indian J. Occup. Environ. Med. 12 (2), 82–87. https://doi.org/10.4103/0019-5278.43266.
Kawai, T., Ukai, H., Inoue, O., Maejima, Y., Fukui, Y., Ohashi, F., Okamoto, S., Takada, S., Sakurai, H., Ikeda, M., 2008. Evaluation of biomarkers of occupational exposure to toluene at low levels. Int Arch Occup Environ Health 81 (3), 253–262.
Toluene. In: Lauwerys RR, Hoët P. Industrial chemical exposure: Guidelines for biological monitoring, third ed.. Boca Raton: Lewis Publishers, CRC Press LLC; 2001: 218-41, 638 p.
Nicolli, A., Chiara, F., Gambalunga, A., Carrieri, M., Bartolucci, G.B., Trevisan, A., 2014. Reliability of urinary excretion rate adjustment in measurements of hippuric acid in urine. Int. J. Environ. Res. Public Health 11 (7), 7036–7044. https://doi.org/10.3390/ijerph110707036.
Nise, G., 1992. Urinary excretion of o-cresol and hippuric acid after toluene exposure in rotogravure printing. Int. Arch. Occup. Environ. Health 63 (6), 377–381. https://doi.org/10.1007/BF00386931.
Volatile organic compounds (screening): Method 2549, Issue 1. 4th ed. Washington, DC: NIOSH; 15 May 1996.
Ogawa, M., Sasahara, T., 2012. A pilot study on the stability of toluene in blood from workers. J. Occup. Med. Toxicol. 7, 24. https://doi.org/10.1186/1745-6673-7-24.
Sahri, M., Widajati, N., 2013. Evaluation of toluene exposure in workers at industrial area of Sidoarjo, Indonesia by measurement of urinary hippuric acid. Asia Pac. J. Med. Toxicol. 2, 145–149.
Shih, H.-T., Yu, C.-L., Wu, M.-T., Liu, C.-S., Tsai, C.-H., Hung, D.-Z., Wu, C.-S., Kuo, H.-W., 2011. Subclinical abnormalities in workers with continuous low-level toluene exposure. Toxicol. Ind. Health 27 (8), 691–699. https://doi.org/10.1177/0748233710395348.
Takeuchi, A., Kawai, T., Zhang, Z.W., Miyama, Y., Sakamoto, K., Higashikawa, K., Ikeda, M., 2002. Toluene, xylenes and xylene isomers in urine as biological indicators of low-level exposure to each solvent; a comparative study. Int. Arch. Occup. Environ. Health 75 (6), 387–393.
Tardif, R., Truchon, G., Brodeur, J., 1998. Comparison of hippuric acid and O-Cresol in urine and unchanged toluene in alveolar air for the biological monitoring of exposure to toluene in human volunteers. Appl. Occup. Environ. Hyg. 13 (2), 127–132. https://doi.org/10.1080/1047322X.1998.10389137.
Thetkathuek, A., Jaidee, W., Saowakhontha, S., Ekburanawat, W., 2015. Neuropsychological symptoms among workers exposed to toluene and xylene in two paint manufacturing factories in Eastern Thailand. Adv. Prev. Med. 183728. https://doi.org/10.1155/2015/183728.
Ukai, H., Kawai, T., Inoue, O., Maejima, Y., Fukui, Y., Ohashi, F., Okamoto, S., Sakurai, H., Ikeda, M., 2007. Comparative evaluation of biomarkers of occupational exposure to toluene. Int. Arch. Occup. Environ. Health 81, 81–93.
National Institute for Occupational Safety and Health (NIOSH). Manual of Analytical Methods (NMAM). Hydrocarbons, Aromatic: Method 1501, Issue 3. 4th ed. Washington, DC: NIOSH; 15 March 2003.
World Health Organization, 1996. Biological monitoring of chemical exposure in the workplace. Vol. 1. Geneva: WHO.