

Evaluation of Concentration, RQ, ECR of BTX, MDA Level and DNA Degeneration in Workers Exposed to BTX

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Abstract

Introduction. The use of BTX as a solvent in the production process potentially causes negative impact on workers, especially on DNA degeneration and MDA levels in workers of industrial sector.

Aims. This study aims to determine the relationship between concentration, RQ and ECR of BTX, MDA levels and DNA degeneration in industrial workers exposed to BTX in Surabaya.

Method. This research was conducted in industries in Surabaya that use BTX as a solvent in its production process. It was conducted in Ketintang, Jemursari, Kalijudan, AUP and Romokalisari. The research design used was cross sectional with 81 samples. The variables studied in this study were the concentration, RQ, ECR of BTX, MDA and DNA degeneration. The data were analyzed using the Pearson correlation test.

Result. There was a relationship between the concentration of benzene, toluene and MDA level in workers exposed to BTX with a P value <0.05 . There was a relationship between the concentration of toluene, ECR of toluene and DNA degeneration in workers exposed to BTX in the industry in Surabaya with a P value <0.05 .

Conclusion. There was a relationship between the concentrations of benzene, toluene and MDA levels in workers exposed to BTX. There was a relationship between toluene concentrations, ECR of toluene and DNA degeneration in workers exposed to BTX.

Introduction

Benzene, Toluene and xylene (BTX) are several types of air pollutants which are Polycyclic Aromatic Hydrocarbons (PAH). PAH is formed due to incomplete combustion of organic matter, spreads in the environment and is mixed. The main sources of PAH exposure to humans are working environment, passive and active smoking (Suzuki and Yoshinaga, 2007) as well as food, water and air pollution (Nethery et al., 2012).

BTX is a Volatile Organic Compound (VOC), a compound containing carbon that evaporates at a certain pressure and temperature or has a high vapor pressure at room temperature. The most commonly known VOCs are solvents. Other types of VOCs that are widely used are monomers and fragrances (Tunsaringkarn, Rungsiyothin, et al., 2012). BTX is a chemical including a class of chemicals that are toxic to health, either carcinogenic or a trigger for cancer (Gammon and Santella, 2008; Reid et al., 2012; White et al., 2014) to increase oxidative stress (Bae et al., 2010) and non-carcinogenic, such as affecting the hematopoietic system, central nervous system and reproductive system (Han et al., 2010). Long-term solvent exposure may have an elevated effect on blood pressure, particularly that is not associated with changes in liver fat, yet is attenuated by alcohol use (Kaukiainen et al., 2006).

The toxicity of BTX in high levels of exposure causes neurotoxic symptoms. Mild exposure causes irregular heartbeat, headache, dizziness, nausea and even fainting if exposure is continued for a long time. The initial manifestations of toxicity are anemia, leucocytopenia, and thrombocytopenia (Singh A.K., Tomer Neetu, 2012). Research by Ovrum, Hultengren and Lindqvist, (1978) shows that there is a high correlation between the concentration of toluene in alveolar air and arterial capillary blood in workers after exposure to toluene. Carlsson, (1982) states that there is a close linear correlation between alveolar and arterial toluene concentrations, both during and after exposure. Continuous exposure to high levels of BTX can result in degeneration to human bone marrow, DNA degeneration in mammalian cells and degeneration to the immune system.

Organic solvents such as BTX that enter the body will oxidize against proteins, lipids and will produce Malondialdehyde (MDA). Increased levels of MDA are a sign of an increase in free radicals in the blood. Increased levels of MDA become a measure to determine the risk of cancer that will occur in workers exposed to benzene. MDA can also be used as an index of lipid peroxidation induced by the initial biochemical action of manganese (Yiin, Lin and Shih, 1996). Some evidence suggests that organic solvents can express their toxicity by producing ROS which can cause cell degeneration (Ulakoğlu et al., 1998).

Several studies have demonstrated the toxic potential of genes from organic solvents in humans and other animals (Singh et al., 2011; Mazzeo et al., 2013; Wang et al., 2013). Mattia et al. have shown that toluene or its metabolites can stimulate the formation of reactive oxygen species that cause lipid peroxidation in various organs (Mattia, Adams and Bondy, 1993; Mattia, Ali and Bondy, 1993). The motility of rabbit spermatozoa is reported to decrease with lipid peroxidation (Alvarez and Storey, 1982) and in vitro exposure to toluene impairs sperm motility (Yelian and Dukelow, 1992). This report supports the idea that oxidative DNA degeneration induced by toluene is involved in male reproductive toxicity.

Carcinogenic benzene (Hayes et al., 2001) and nitrobenzene (Iida, Misaka and Naya, 1997) are reproductive toxins like toluene, and their metabolites are also known to cause oxidative DNA degeneration (Hiraku and Kawanishi, 1996; Ohkuma and Kawanishi, 1999; Oikawa; et al., 2001). Although chronic exposure to xylene is associated with harmful effects on human health, the World Health Organization does not consider this chemical to be genotoxic or carcinogenic (WHO, 1996). Results are scarce, but several studies have shown that xylene reduces cell viability and increases DNA degeneration (Al-Ghamdi, Raftery and Yaqoob, 2004; Chen et al., 2008; Singh et al., 2011). Biomonitoring studies should be developed to explain cytological changes in workers exposed to xylene and other organic solvents.

Research regarding to BTX on various human activities has been carried out, such as BTX measurements at gas stations (Tunsaringkarn, Ketkaew, et al., 2012) and in urban areas (Singh A.K., Tomer Neetu, 2012). The high level of exposure to organic solvents such as BTX in industrial workers in big cities such as Surabaya and the length of exposure experienced by workers exposed to BTX cause these workers to have a high health risk. However, there are still few studies and scientific publications regarding BTX

exposure with its effect on DNA degeneration and its effect on MDA levels. Therefore, the aim of this study was to analyze the relationship between the concentration of RQ, ECR BTX, MDA and DNA degeneration in industrial workers exposed to BTX in Surabaya.

Methods

This research is an observational, cross sectional study conducted in five industries in Surabaya that use BTX as a solvent in its production process in the Ketintang, Jemursari, Kalijudan, AUP and Romokalisari areas. The variables studied in this study were concentration, RQ, ECR BTX, MDA and DNA degeneration. This research passed the ethical test and was approved by the Institutional Ethics Council of Airlangga University. Participation, confidentiality, and informed consent were obtained from each respondent. The types of data used in this study are primary and secondary data. Primary data is data obtained directly at the research location in the form of field observations, questionnaire results and sampling which is then analyzed in the laboratory. Secondary data is a literature study to strengthen the analysis in this study.

Subjects

The population in this study were all workers exposed to toluene in five industrial areas in Surabaya. The inclusion criteria in this study were all workers exposed to toluene, aged ≥ 16 years, willing to participate (signing informed consent) and able to communicate. Workers with acute physical or mental illness, those taking medication for medical conditions, and those with infections and/or acute illnesses that might suppress their immune systems as well as pregnant women were excluded from the study. The research sample was taken using accidental sampling method which resulted in 81 respondents who met the inclusion and exclusion criteria.

Questionnaire

Questionnaires were distributed and direct interviews were conducted to obtain information on general characteristics of the subjects including age, gender, body weight, job characteristics including daily working hours, duration of work, and years of service.

Measurement of BTX RQ and ECR concentrations

The air was taken from near the breathing zone during working hours. Sampling was taken using an adsorber tube (charcoal tube) and a low flow pump (0.05-0.1 liter/minute), a personal sampling pump. The adsorber tube was placed between the chest and nose (breathing zone) of the worker. After sampling, the contents of the tube were removed and dissolved in a solution of carbon disulfid (CS₂) and analyzed by Gas Chromatography, Flame Ionized Detector (GC/FID) based on the NIOSH method number 1501.

Assessment of benzene intake dose is measured by determining the dose of risk agent benzene as intake using the equation of $I = C \times R \times tE \times fE \times Dt / Wb \times tavg$ (Lauvan and Louvar, 1998). Assessment of non-carcinogenic characteristics (Risk Qoutient (RQ)) is a comparison between intake or intake (Ink) and reference (RfD or RfC) (Tualeka, 2013).

The risk characteristics for the cancer effect can be determined by multiplying the cancer intake value by the CSF value with the following formula:

$$\text{ECR} = \text{Carcinogenic Intake (CI)} \times \text{CSF}$$

MDA measurement

The examination specimen is 8 ml of serum derived from venous blood. Checking the levels of Malondialdehyde using the TCA (Trichloroacetic acid) method. Internal validation was carried out using daily Quality Control (QC) and normal controls and pathological controls, while external validation was carried out using a comparative test to an independent laboratory.

Comet Assay

Comet Assay was performed in an alkaline version according to the protocol described below which was adapted from Singh et al., (1988). Blood samples are transported to the laboratory in the refrigerator and processed immediately. The slides were prepared by mixing 5 μL of peripheral blood with 95 μL of low melting point agarose (0.75%) stored at 37 ° C in a water bath. The mixture was spread on a microscope slide covered with normal melting point agarose (1.5%) and covered with a cover slip. After 10 minutes in the refrigerator to allow for agarose compaction, the cover was removed and the slide was placed in a lysed solution of [2.5 M NaCl, 100 mM ethylene diamine tetra acetic acid (EDTA) and 10 mM Tris, pH = 10.0, with just 1 mL Triton added. X-100 and 10% dimethyl sulfoxide (DMSO)] for 72 hours. Subsequently, the slides were incubated in a freshly prepared alkaline buffer (300 mM NaOH and 1 mM EDTA, pH> 13) for 15 min to allow DNA release and exposure to alkaline labile sites. Then, DNA was electrophoresed for 300 mA and 20 minutes at 25 V (0.9 V / cm), and the slides were neutralized with 0.4 M Tris (pH 7.5). Then, the slides were fixed and stained with silver nitrate (solution A: 5% sodium carbonate; solution B: 0.1% ammonium nitrate, silver nitrate 0.1% tungstosilicic acid 0.25% and formaldehyde 0.15%) for 35 minutes with agitation (110 rpm; 37 ° C) (Nadin, Vargas-Roig and Ciocca, 2001)

Data processing and statistical analysis

Data processing was carried out after data collection and interviews with respondents were conducted. The next process includes editing, coding, data entry and tabulating. Univariate analysis yields frequency and percentage distribution. Bivariate analysis was used to determine the relationship between two variables, using the Pearson IBM Software Statistical Product and Service Solution (SPSS) version 20 correlation test for the Windows operating system.

Results

Distribution of Respondent Characteristics Frequency

Respondent characteristics include age, gender, level of education, and work area. Below is a table of the distribution of the characteristics of respondents.

Table 1. Frequency Distribution of Respondents' Characteristics Exposed to BTX

Respondents' Characteristics	Frequency	Percentage
Age		
16-25	13	16%
26-35	14	17.3%
36-45	27	33.4%
46-55	18	22.3%
56-65	9	11%
Gender		
Male	63	77.7%
Female	18	22.3%
Level of Education		
Primary	14	17.3%
Junior High	22	27.1%
Senior High	42	51.9%
Undergraduate	3	3.7%
Working Area		
Romokalisari	24	29.6%
Ketintang	19	23.5%
Jemursari	10	12.3%
Kalijudan	17	21%
AUP	11	13.6%

Source: primary data

Based on Table 1, most of the workers (33.4%) are aged 36-45 years, are male (77.7%), have high school/vocational education (51.9%) and work in the Romokalisari area (29.6%).

Comparison of the results of the calculation of concentration, RQ, ECR BTX for respondents exposed to BTX

Table 2. Distribution of Benzene Concentrations (ppm), RQ and ECR of Benzene in Workers Exposed to BTX

Benzene	n	%	Mean ± SD	Min	NAB
C (ppm)					
Abnormal (>0,05 ppm)	53	65.4%	5.78±11.17	0.04	≤0,05 ppm
Normal (≤0,05 ppm)	28	34.6%			
RQ					
Unsafe (≥1)	52	64.2%	27.3±68.81	0.03	<1
Safe (<1)	29	35.8%			
ECR					
Unsafe (> 10 ⁻⁴)	74	91,4%	0.074±0.01	0.00	≤ 10 ⁻⁴
Safe (≤ 10 ⁻⁴)	7	8.6%			

Based on Table 2 and in Figure 1, the majority of workers (65.4%) have benzene concentrations above the Threshold Value (> 0.05 ppm).

The average benzene concentration was 5.78 ppm which has exceeded the specified TLV (> 0.05 ppm) (Ministry of Manpower of the Republic of Indonesia, 1997). Health risk characteristics are expressed as Risk Quotient (RQ, Risk Level) calculated by dividing intake (ink) and reference (RfC). The results of the calculation of Risk Quotients (RQ) can show the level of health risk of workers due to xylene exposure in the work environment. If the RQ value is more than or equal to 1 (RQ > 1), workers exposed to benzene have a health risk due to exposure. If the RQ value is less than 1 (RQ < 1), the exposed worker is safe from health risks due to exposure (Kolluru, 1996). Based on the RQ calculation in Table 2, the majority of workers (64.2%) had a value of RQ ≥ 1, which means that the majority of workers had a health risk impact due to exposure to toluene.

ECR ≤ 10⁻⁴ means the concentration of benzene exposure does not cause the risk of carcinogenic effects. ECR is > 10⁻⁴ means the concentration of benzene exposure may have carcinogenic health effect. From the results of ECR calculations for benzene exposure, the majority of workers (91.4%) had an ECR value > 10⁻⁴ which means the risk of developing cancer.

Table 3. Distribution of Toluene Concentrations (ppm), RQ and ECR of Toluene in Workers Exposed to BTX

Toluene	n	%	Mean ± SD	Min	NAB
C (ppm)					
Unsafe (>20 ppm)	34	42%	30.02±59.07	0.0036	≤20 ppm
Safe (≤20 ppm)	47	58%			
RQ					
Unsafe (≥1)	61	75.3%	15.93±48.19	0.03	<1
Safe (<1)	20	24.7%			
ECR					
Unsafe (> 10 ⁻⁴)	69	85.2%	0.109±0.284	0.00	≤ 10 ⁻⁴
Safe (≤ 10 ⁻⁴)	12	14.8%			

Based on Table 3 and Figure 2, the majority of workers (58%) showed toluene concentrations below the Threshold Value (≤20 ppm). The average concentration of toluene was 30.02 ppm. This exceeds the established TLV (Ministry of Manpower of the Republic of Indonesia, 1997). Based on the RQ calculation in Table 3, the majority of workers (75.3%) had an RQ≥1 value, which means that the majority of workers have a health risk impact due to exposure to toluene. From the results of ECR calculations for toluene exposure, the majority of workers (85.2%) have ECR values> 10⁻⁴, which means they are at risk of cancer.

Table 4. Distribution of Xylene Concentrations (ppm) and RQ Xylene in Workers Exposed to BTX in the Surabaya Industry

Xylene	n	%	Mean ± SD	Min	NAB
C (ppm)					
Unsafe (>100 ppm)	1	1.2	24.85±114.03	0.0015	100 ppm
Safe (≤100 ppm)	80	98.8			
RQ					
Unsafe (≥1)	63	77.8	959±1564	0.0045	<1
Safe (<1)	18	22.2			
ECR					
Unsafe	-	-	-	-	-
Safe	-	-			

Based on Table 4, the majority of workers (98.8%) had xylene concentrations below the Threshold Value (≤ 100 ppm). The average value of xylene concentration is 24.85 ppm not exceeding the specified TLV (Ministry of Manpower of the Republic of Indonesia, 1997). Based on the RQ calculation in Table 4, the majority of workers (77.8%) have an $RQ \geq 1$ value which means they have a health risk due to xylene exposure. The ECR was not calculated for xylene exposure because xylene is not a carcinogenic substance.

Biomarker parameters

Table 5. Frequency Distribution of parameter biomarkers in Workers Exposed to BTX

Biomarker	Number (n)	Percent (%)	Mean \pm SD	Min	Max
MDA (mikron mol/L)					
Abnormal	57	100	7.76 \pm 2.40	4.35	14.4
Normal	0	0			
DNA degeneration					
Degeneration	25	30.9	-	-	-
Non degeneration	56	69.1			

Biomarker parameters examined in this study included DNA degeneration and MDA levels (micron mol / L), each of which was compared with the existing TLV and categorized into 2 types, namely abnormal and normal. Normal MDA levels are 1.076 micron mol / L (Bhutia et al., 2011; Arifin, Ernawati and Prihatini, 2019). Based on Table 5, the majority of workers have MDA levels in the abnormal category while the majority of workers (69.1%) did not experience DNA degeneration.

Relationship between BTX concentrations (ppm), MDA and DNA degeneration (N = 81)

Table 6. Statistical test results between concentrations of BTX, MDA and DNA degeneration

Compound	MDA (mikron mol/L)	DNA
B	-.268*	-0.140
T	-.286*	-.231*
X	-0,119	-.161

* $p < 0.05$

Based on the test results in table 6, there was a relationship between the concentrations of benzene, toluene and MDA levels in workers exposed to BTX with a P value <0.05. There was a relationship between toluene concentration and DNA degeneration in workers exposed to BTX with a P value <0.05. There was no relationship between xylene concentrations and MDA levels in workers exposed to BTX in the industry in Surabaya with a P value > 0.05. There was no relationship between the concentration of benzene, xylene and DNA degeneration in workers exposed to BTX in industries in Surabaya with a P value > 0.05.

Relationship between RQ BTX, MDA and DNA degeneration (N = 81)

Table 7. Statistical Test Results Between RQ BTX, MDA and DNA degeneration

RQ	MDA (mikron mol/L)	DNA degenartion
B	0.063	0.195
T	-0.087	-0.153
X	0.085	-0.077

* $p < 0.05$

Based on the test results in table 7 there was no relationship between RQ BTX, MDA level and DNA degeneration in workers exposed to BTX in industries in Surabaya with a P value > 0.05.

Relationship between ECR BTX, MDA and DNA degeneration (N = 81)

Table 8. Statistical Test Results Between ECR BTX, MDA and DNA degeneration

ECR	MDA (mikron mol/L)	DNA degeneration
B	-0.190	0.002
T	-0.182	-.221*
X	-	-

* $p < 0.05$

Based on the test results in table 5, there was a relationship between Toluene ECR and DNA degeneration in workers exposed to BTX in industries in Surabaya with a P value > 0.05. There was no relationship between ECR Benzene and toluene with MDA levels in workers exposed to BTX (P > 0.05). There was no relationship between ECR Benzene and DNA degeneration in workers exposed to BTX in industries in Surabaya (P > 0.05).

Discussion

Based on the results of the study, there was a significant relationship between the concentrations of benzene, toluene and MDA in workers exposed to BTX ($P < 0.05$). The results of this study are consistent with other studies that show an increase in MDA levels in response to exposure to organic solvents (Rana and Kumar, 1994; Al-Ghamdi, Raftery and Yaqoob, 2003a, 2003b). Benzene that enters the body oxidizes against proteins, lipids and produces Malondialdehyde (MDA). An increase in MDA levels is a sign of an increase in free radicals in the blood, even an increase in MDA levels has become a measure to determine the risk of cancer that occurs in workers exposed to benzene.

Exposure to high levels of benzene causes narcotic effects and is irritating to the eyes and airways. Long-term exposure to low levels can result in bone marrow suppression and may be associated with leukemia or other blood cell disorders. Malondialdehyde (MDA) is produced by lipid oxidation and as a byproduct of prostaglandin and thromboxan synthesis. According to research by Odewabi, Ogundahunsi and Oyalowo in 2014 in Nigeria, exposure to free radicals, especially benzene in gas station workers, increased MDA level in workers. Research by Tambunan (2014) shows that toluene exposure causes the formation of Reactive Oxygen Species (ROS), thereby affecting myocardial MDA values among the study groups. This can be used as a marker of oxidative stress in myocardial degeneration. Ariyani stated that the risk of cancer in gas station workers increases due to DNA degeneration due to exposure to free radicals (Ariyani, 2009).

The structure of toluene and several studies on the metabolic activation of toluene raises the suspicion that toluene can undergo biotransformation into metabolites with relatively low electrophilic reactivity which may react preferentially with proteins having high nucleophilic substituents such as sulfhydryl groups with cysteine residues. The human DNA repair enzyme hOGG1 has eight cysteine residues (Finkenwirth et al., 2009). The inactivation potential of this important repair enzyme can lead to accumulation of 8-oxoguanine. DNA degeneration is a major incorrect code caused by reactive oxygen species produced by exogenous agents or by ubiquitous endogenous processes (Finkenwirth et al., 2009).

Based on the results of the study, there was a relationship between the concentration of toluene, ECR of toluene and DNA degeneration in workers exposed to BTX ($P < 0.05$). Several reproductive toxins have a damaging effect on DNA on the testicular germ epithelium (Bjørge et al., 1996). Previous research (Murata, Tsujikawa and Kawanishi, 1999) showed that small metabolites of toluene have the oxidative degeneration ability of DNA isolated from human genes, and suggested that toluene may exhibit carcinogenicity and reproductive toxicity through oxidative DNA degeneration.

Previous studies have shown that Toluene inhalation alters the hormonal status of the anterior pituitary gland in rodents (Andersson et al., 1980; Hsieh, Sharma and Parker, 1991). A recent study also demonstrated that acute exposure to toluene affects gonadotropin secretion in workers (Svensson et al., 1992; Luderer et al., 1999). This report suggests that one possible means of reproductive toxicity of toluene may be related to changes in endocrine status. Another possible mechanism of toluene's reproductive toxicity may involve its direct effects on the reproductive organs.

A research by Nakai et al., (2003) showed that toluene decreased sperm count and increased 8-oxycodG formation in testicular sperm cells. This in vivo oxidative DNA degeneration can be attributed to the degeneration caused by toluene metabolites as shown by in vitro experiments. In conclusion, toluene has a direct toxic effect on male reproductive organs acting on their DNA rather than disturbing the hypothalamus-pituitary-testis axis. This study is the first to show that the reproductive toxicity of toluene can be associated with oxidative DNA degeneration to the testes (Nakai et al., 2003).

It is recommended to take foods that contain CYP2E1 enzymes such as beef liver, beef brain and salmon (Lieber, 2004; Hodges, RE; Minich, 2015), foods containing sulfation, namely eggs, chicken and tuna and foods containing glutathione, namely broccoli (Forman, Zhang and Rinna, 2009), carrots and tomatoes (Dhivya, 2012) to detoxify BTX exposure in workers' bodies. Research by Tualeka et al., (2020) states that each individual has a different dose of food effect to detoxify organic solvents depending on the amount of inhalation concentration, body weight, and length of work. Using these foods to detoxify contaminants is a cost-effective measure.

The money and time needed to reduce BTX emissions from outside sources is higher than that required for the reduction of active and passive smoking. However, reducing BTX emissions from external sources is of course more beneficial to the public and more effective in terms of reducing atmospheric pollution globally (Carletti and Romano, 2002). Improvement of air quality to minimize health effects in the workplace include increasing ambient and local ventilation, replacing solvent-based products with water-based products, and establishing routines to maintain container closure after use (Martins et al., 2016).

Conclusion

The results of this study indicate a relationship between the concentrations of benzene and toluene with MDA level in workers exposed to BTX in the industry in Surabaya as well as a relationship between concentrations of toluene, ECR of toluene and DNA degeneration in workers exposed to BTX. However, these results need to be interpreted carefully by considering the limitations of the study, i.e a cross-sectional design with a test limited to the Pearson correlation test. Therefore, further studies need to be deeper with a larger and representative sample size.

Recomendation

Accompany the subject for a longer time and focus on other tissues such as the oral mucosa which can also accumulate cellular lesions.

Declarations

Ethics approval and consent to participate: The study was approved by the institutional Ethical Board of the Public Health, Airlangga University

Consent for publication: All authors have agreed if this article is published

Availability of data and materials: All data relating to this article has been approved for publication

Competing interests: All authors have no conflicts of interest to declare.

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Authors' contributions: Abdul Rohim Tualeka and Juliana Jalaludin as authorship conceptors, Noor Fatihah Mohamad Fandi and Syamsiar S Russeng as compilers of articles and looking for reference sources, Ahsan Ahsan as data processors, Indri H Susilowati, Pudji Rahmawati and Muhamad Firdaus contributed in sampling and improving manuscripts. All authors have contributed to improving the article.

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Figures

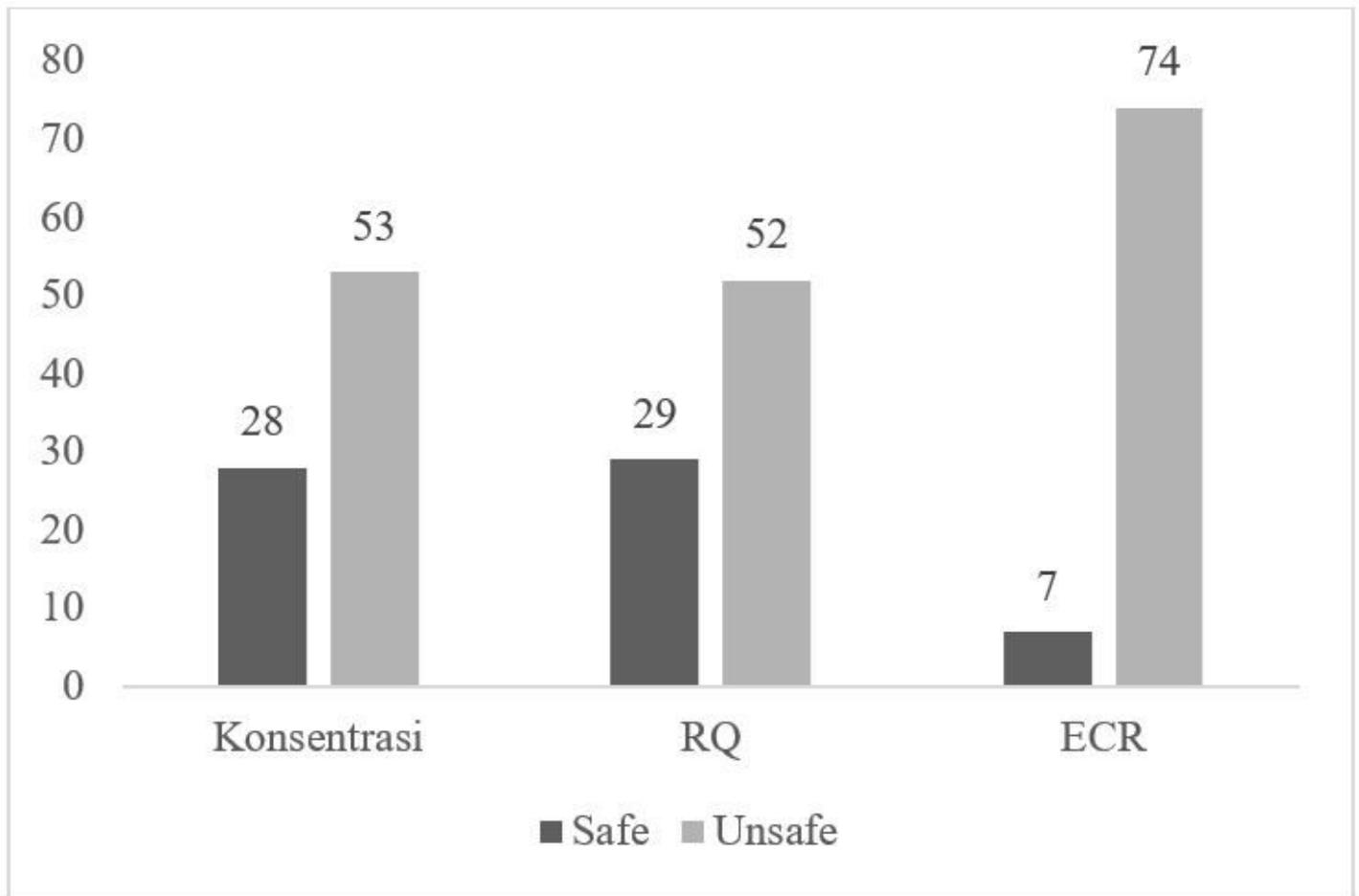


Figure 1

Comparison of the concentration, RQ and ECR of Benzene in Respondents exposed to BTX

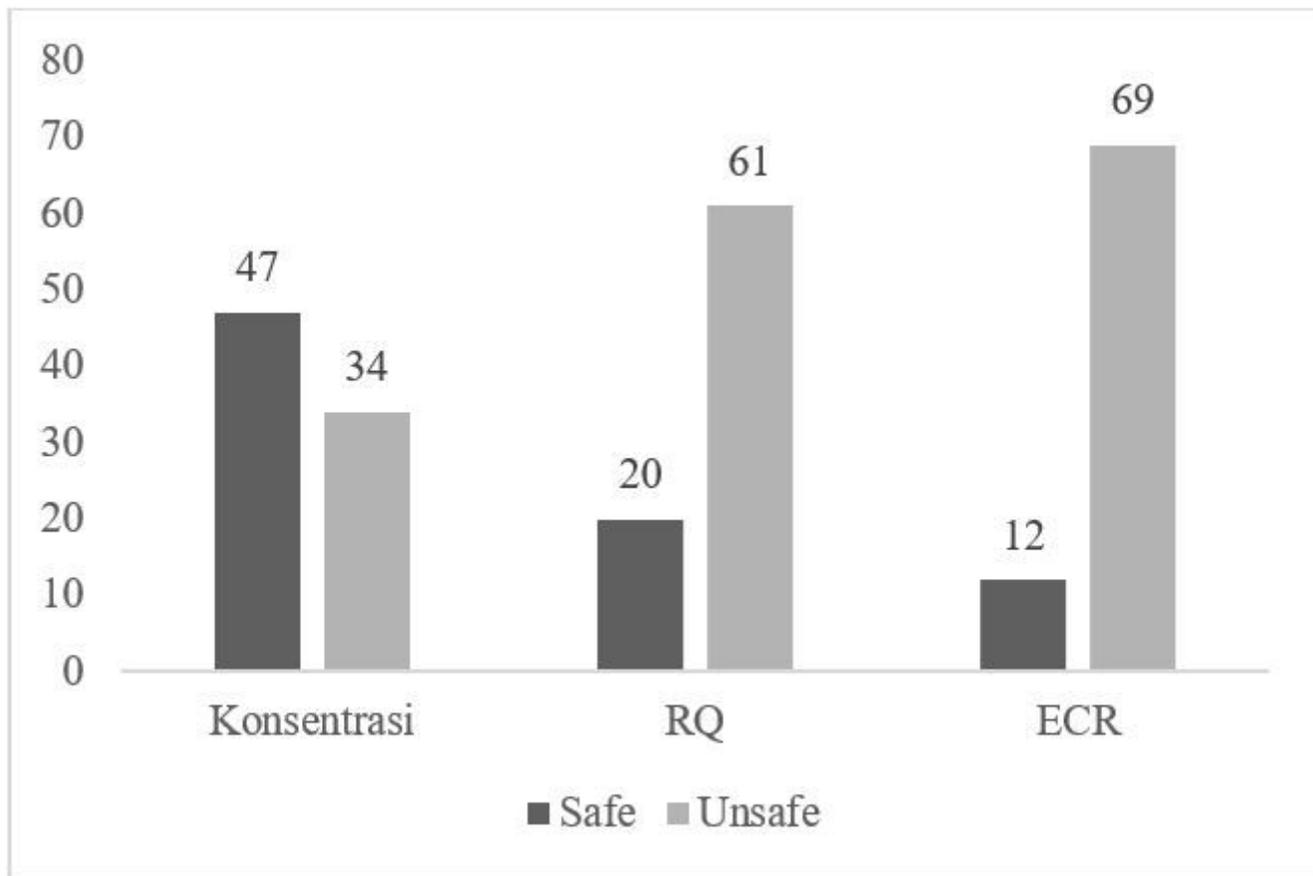


Figure 2

Comparison of the concentration, RQ and ECR of Toluene in the Respondents exposed to BTX

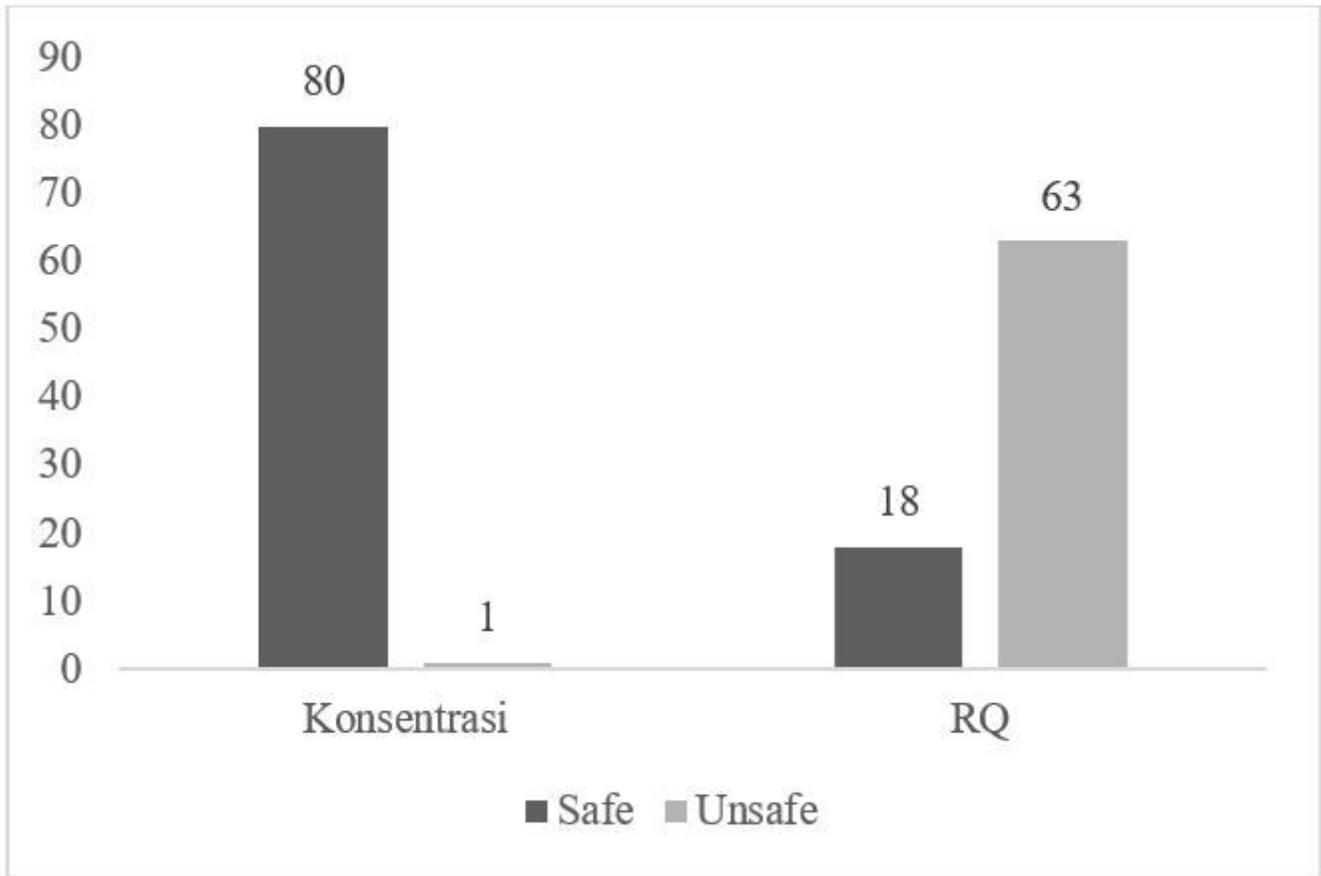


Figure 3

Comparison of Xylene Concentration and RQ in Respondents Exposed to BTX