Industrial Health 2019, **57**, 525–529

**Original Article**

# Biological monitoring of occupational ethylbenzene exposure by means of urinalysis for un-metabolized ethylbenzene

**Toshio KAWAI[1], Haruhiko SAKURAI[2] and Masayuki IKEDA[3]***

[1]Kansai Technical Center for Occupational Medicine, Japan
[2]Japan Association for Work Environments, Japan
[3]Kyoto Industrial Health Association, Japan

*Received August 23, 2018 and accepted November 29, 2018*
*Published online in J-STAGE December 8, 2018*

**Abstract : This study aimed to examine quantitative relation between ethylbenzene (EB) in air (EB-A) and un-metabolized EB in urine (EB-U) for biological monitoring of occupational EB exposure by urinalysis for EB. In total, 49 men in furniture production factories participated in the study. Time-weighted average EB-A was monitored by diffusive sampling. Urinalysis for EB was conducted by head-space gas-chromatography with end-of-shift samples. Data were subjected to regression analysis for statistical evaluation. A geometric mean (GM) and the maximum (Max) EB-A levels were 2.1 and 45.5 ppm, respectively. A GM and the Max for EB-U (observed values) were 4.6 and 38.7 µg/l. A significant linear correlation was observed. The regression equation was Y=3.1+0.73X where X is EB-A (ppm) and Y is EB-U (µg/l) (r=0.91, $p$<0.01). The significant correlation between EB-A and EB-U coupled with a small intercept suggests that biological monitoring of occupational EB exposure is possible by analysis for un-metabolized EB in end-of-shift urine samples. Further validation studies (including those on applicability to women) are envisaged. The feasibility should be examined for biological monitoring and the applicability of the equation among the workers exposed to EB at low levels.**

**Key words:** Biological monitoring, Ethylbenzene, Exposure-excretion relationship, Occupational exposure

## Introduction

Ethylbenzene (EB) is present both in petroleum products[1–3] and in coal distillates[4] as a minor component, and usually detected in combination with major components such as benzene, toluene and xylene isomers. Thus, EB is present in automobile fuel (including diesel oil) and automobile emission[5, 6]. In residential areas, outdoor source of EB may be highway traffic[7, 8]. EB in indoor air[9] might be from varnished materials[10]. Smoking may be an additional source for EB[10]. Thus, it is clear that EB is everywhere in occupational as well as non-occupational setting in life, although usually at low levels even in occupational settings.

Since the pioneer work by Baododej and Bardodejova[11] followed by Gromiec and Piotrowski[12], biological monitoring of ethylbenzene exposure has been conducted by means of urinalysis for EB metabolites such as mandelic acid, phenylglyoxylic acid, or the combination[13–16]. The present study was conducted to detect successfully a significant correlation between air-borne exposure level of EB in workroom air (EB-A) and the level of EB in urine

*To whom correspondence should be addressed.
E-mail: m-71-ikeda@nifty.com

**Table 1. Basic data on ethylbenzene exposure**

| Para-meter | Age[1] (yr) | EB in air (ppm) | EB in urine | | |
|---|---|---|---|---|---|
| | | | OB[2] (μg/l) | CR[3] (μg/g) | SG[4] (μg/l) |
| Min | 18 | 0.24 | 1.1 | 0.95 | 0.59 |
| Max | 60 | 45.45 | 38.70 | 83.38 | 40.13 |
| GM | 39.8[5] | 2.053 | 4.57 | 5.96 | 4.13 |
| GSD | 118[6] | 3.084 | 2.12 | 2.33 | 2.24 |

n=49. [1]Age for 12 men were unknown. [2]As observed (i.e., no correction). [3]Divided by creatinine concentration (g/l). [4]Adjusted for a specific gravity of 1.016. [5]AM. [6]ASD.

of workers involved (EB-U). The success will be an additional example for the use of un-metabolized solvents in urine as indicators of occupational solvent exposures at low levels. To the knowledge of the authors, this is the first study to report quantitative relationship between EB exposure and excretion of un-metabolized EB in urine.

## Materials and Methods

The data on EB exposure, previously reported in short[17], were analyzed in detail in the present report. Male workers (49 subjects) at the ages of 18 to 60 yr were engaged in furniture production, and they were exposed to several solvents such as ethylbenzene, with toluene, xylenes and others[17].

Diffusive samplers with activated carbon-cloth were employed for the measurement of time-weighted average solvent exposures[17]. Urine samples were collected at the end of work of the day, and were immediately transferred to designed vials to be analyzed by head-space gas chromatograpy[17] for EB. The lowest limit for determination was 0.1 ppm for EB-A and 1 μg/l for EB-U. For urine density issues, WHO's quality assurance guidelines for sample exclusion[18] were applied, i.e., <0.3 g/l or >3.0 g/l for creatinine concentration, or <1.010 or >1.030 for a specific gravity of urine.

Regression analyses followed by comparison between two regression lines were employed for statistical evaluation after Ichihara[19].

Each of the workers submitted his informed consent and the study design was approved by an institutional review board[17]. Creatinine and specific gravity of urine were measured by colorimetry and refractometry, respectively.

## Results

The exposure-excretion data are summarized in Table 1. EB exposure was generally low with a GM of 2.1 ppm, but the maximum was as high as 45.5 ppm. Correspondingly, EB excretion in urine (EB-U) (as observed) was in a range of 1 to 39 μg/l with a GM of 4.6 μg/l. Correction for urine density did not induce remarkable changes except that correction for creatinine gave the Max of 83 μg/g. This was due to the 2nd highest EB exposure (40 ppm) resulting in the 2nd highest EB in urine (30.1 μg/l) coupled with relatively low creatinine level (0,36 g/l).

The correlations between EB in air and EB in urine are depicted in Fig. 1. The equations for the regression lines are summarized in Table 2. It should be noted that the correlations were all statistically significant ($p<0.01$), irrespective of correction for urine density.

The figures appear to suggest that two cases at the upper-right corner in each figure may affect over-all correlation coefficients. This concern will be discussed in detail in the Discussion section.

## Discussion

Significant correlation between air-borne solvent and un-metabolized solvent in urine has been reported for two major components of toluene[17, 20] and xylenes[17] in petroleum and coal distillate products. It is now made clear that the same strategy may be applicable to a minor component such as EB. The experience with toluene revealed that the best indicator in urine for solvent exposure varies as a function of exposure intensity and that the level of un-metabolized toluene in biological materials such as urine is the most practical marker of low level exposure. Thus, un-metabolized toluene is much more sensitive than the traditional marker of a metabolite such as hippuric acid in urine[20].

Diffusion is considered as a mechanism for excretion of un-metabolized solvents in urine. The ratio of solvent in urine over solvent in air has a close correlation with a

physico-chemical parameter of $P_{ow}$ (octanol-water partition coefficient)[21, 22]. The observation is on line with the consideration of diffusion. Therefore, the risk of modification due to competitive metabolic interaction[23, 24] to modify the parameter level in urine should be small.

Regarding EB exposures in factory workplaces, Inoue and others[15] previously reported EB exposures at the level of 1.8 ppm as a GM (the Max at 44 ppm). Reports are rather scarce on workroom EB exposure in recent years. Among the few reports, Martins *et al.*[9] observed that the EB exposure was at the level of <100 μg/m$^3$ or well below 0.1 ppm. EB exposures have been confirmed also in association with petroleum distribution works[25]. Rather exceptionally, Mao *et al.*[24] reported EB exposure at 40 ppm (as an arithmetic mean) among spray painters in a shipyard. Further studies are apparently envisaged to confirm applicability of 'EB in urine' approach for EB exposure monitoring in present day industries.

On the chronic toxicities of EB, International Agency for Research on Cancer classifies EB in Group 2B (i.e., possibly carcinogenic to humans)[25]. With regard to occupational exposure limits, both Japan Society for Occupational Health[26] and American Conference of Governmental Industrial Hygienists[27] maintain the occupational exposure limits at the levels the same with that for toluene (i.e., 50 and 20 ppm, respectively). These limits are set in reference to local irritation, suppressive effects on the cenral nervous system and effects on the renal system[26, 27]. The adoptions of 20–50 ppm as occupational exposure limits may suggest that the exposure to EB will stay at the present levels.

A major problem as a study limitation is that the close exposure-excretion correlation (Fig. 1 and Table 2) appears to be strongly affected by the two high exposure cases (at 40 and 46 ppm). In fact no cases were available in the exposure range of 15 to 35 ppm in the present survey. Nevertheless, a tentative regression analysis excluding the 40 and 46 ppm exposure cases (thus with remaining 47 cases) resulted in a regression line equation (Eq. 2 in Table 2) very close to Eq. 1 (with 49 cases). Statistical comparison of Eq. 1 and Eq. 2 revealed no significant differences ($p>0.05$) in the intercepts and slopes although the correlation coefficients were different (0.913 vs. 0.563; Eq. 1 and Eq. 2 in Table 2). The same were the cases when urine density was corrected for creatinine concentration or a specific gravity of urine of 1.016. In the present study, urine samples were collected only at the end of shift. Thus, it was not possible to examine the possibility that un-metabolized EB may be excreted earlier whereas the excretion of metabolites (i.e., mandelic and phenylglyoxylic acids) may take longer time. It was not possible to conduct surveys on the same day of the week, although it was made in the second half of a working week as far as possible.

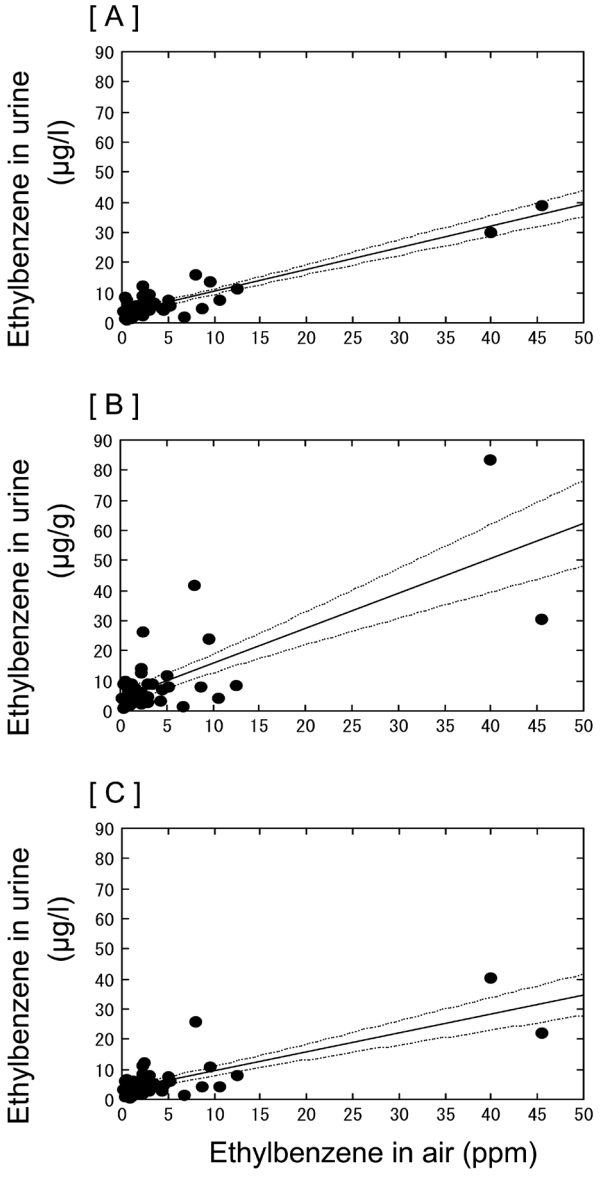


**Fig. 1. Linear regression between ethylbenzene in air (ppm) and ethylbenzene in urine (μg/l or μg/g creatinine).**
The lines in the middle are calculated regression lines, and the curves on both side of the line show 95% confidence ranges. Each dot represents one case studied.
[A] EB in urine as observed (i.e., no urine density correction)
[B] EB in urine as corrected for creatinine concentration (i.e., EB divided by creatinine concentration).
[C] EB in urine adjusted for a specific gravity of 1.016.
The equation for each regression line is given in Table 2, Eq. 1, 3, and 4 for Fig. 1 [A], Fig. [B] and Fig. [C], respectively.

**Table 2. Regression line parameters**

| Correction for | Equation | n | Parameters | | | |
|---|---|---|---|---|---|---|
| | | | Intercept (α) | Slope (β) | r | *p* |
| None | Eq. 1 | 49 | 3.065 | 0.729 | 0.913 | <0.01 |
| Modified[1] | Eq. 2 | 47 | 3.267 | 0.654 | 0.563 | <0.01 |
| Creatine[2] | Eq. 3 | 49 | 4.121 | 1.165 | 0.743 | <0.01 |
| Specific gravity[3] | Eq. 4 | 49 | 3.138 | 0.633 | 0.780 | <0.01 |

The regression lines are calculated for Y=α+βX, where X is [EB-A (ppm)] and Y= [EB-U (μg/l or μg/g creatinine)].

[1]Modification by removal of two higest exposure cases (EB-A=45 ppm and 40 ppm).

[2]Divided by creatinine concentration (g/l).

[3]Adjusted for a specific gravity of 1.016.

Another limitation is the lack of data for women. However, no gender-related difference in exposure-excretion relation was observed in a previous study[15] when exposure conditions are comparable. Therefore, the present men-based conclusion might be applicable also to women. Further studies are necessary to examine the possibility if the present men-based conclusion is applicable also to women.

## Conclusion

Biological monitoring of occupational ethylbenzene exposure is possible by means of urinalysis for un-metabolized ethylbenzene. Confirmation of the applicability to detect low-level ethylbenzene exposure in current day industry is envisaged.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Acknowledgements

The authors are grateful to Osaka Occupational Health Service Center, Osaka, Japan, and Occupational Health Research and Development Center, Tokyo, Japan, for their interest in and support to this study.

## References

1) Hopf NB, Kirkeleit J, Bråtveit M, Succop P, Talaska G, Moen BE (2012) Evaluation of exposure biomarkers in offshore workers exposed to low benzene and toluene concentrations. Int Arch Occup Environ Health **85**, 261–71.
2) Campo L, Rossella F, Mercadante R, Fustinoni S (2016) Exposure to BTEX and ethers in petrol station attendants and proposal of biological exposure equivalents for urinary benzene and MTBE. Ann Occup Hyg **60**, 318–33.
3) Heibati B, Godri Pollitt KJ, Charati JY, Ducatman A, Shokrzadeh M, Karimi A, Mohammadyan M (2018) Biomonitoring-based exposure assessment of benzene, toluene, ethylbenzene and xylene among workers at petroleum distribution facilities. Ecotoxicol Environ Saf **149**, 19–25.
4) He Q, Yan Y, Zhang Y, Wang X, Wang Y (2015) Coke workers' exposure to volatile organic compounds in northern China: a case study in Shanxi Province. Environ Monit Assess **187**, 359–70.
5) Jo WK, Yu CH (2001) Public bus and taxicab drivers' work-time exposure to aromatic volatile organic compounds. Environ Res **86**, 66–72.
6) Moolla R, Curtis CJ, Knight J (2015) Assessment of occupational exposure to BTEX compounds at a bus diesel-refueling bay: a case study in Johannesburg, South Africa. Sci Total Environ **537**, 51–7 ê.
7) Kwon J, Weisel CP, Morandi MT, Stock TH (2016) Source proximity and meteorological effects on residential outdoor VOCs in urban areas: results from the Houston and Los Angeles RIOPA studies. Sci Total Environ **573**, 954–64.
8) Wang BL, Takigawa T, Takeuchi A, Yamasaki Y, Kataoka H, Wang DH, Ogino K (2007) Unmetabolized VOCs in urine as biomarkers of low level exposure in indoor environments. J Occup Health **49**, 104–10.
9) Martins EM, Borba PF, Dos Santos NE, Dos Reis PT, Silveira RS, Corrêa SM (2016) The relationship between solvent use and BTEX concentrations in occupational environments. Environ Monit Assess **188**, 608–18.
10) Skender L, Brcić I, Karacić V (2004) Urine analysis for the evaluation of environmental exposures to aromatic hydrocarbons. Arch Environ Health **59**, 237–44.
11) Bardodej Z, Bardodejova E (1970) Biotransformation of ethyl benzene, styrene, and alpha-methylstyrene in man. Am Ind Hyg Assoc J **31**, 206–9.
12) Gromiec JP, Piotrowski JK (1984) Urinary mandelic acid as an exposure test for ethylbenzene. Int Arch Occup Environ Health **55**, 61–72.
13) Sakai T, Takeuchi Y, Ikeya Y, Araki T, Ushio K (1989) [Method for simultaneous determination of six metabolites

of toluene, xylene and ethylbenzene, and its application to exposure monitoring of workers in a printing factory with gravure machines]. Sangyo Igaku **31**, 9–16 (in Japanese).
14) Kawai T, Yasugi T, Mizunuma K, Horiguchi S, Iguchi H, Uchida Y, Iwami O, Ikeda M (1992) Comparative evaluation of urinalysis and blood analysis as means of detecting exposure to organic solvents at low concentrations. Int Arch Occup Environ Health **64**, 223–34.
15) Inoue O, Seiji K, Kudo S, Jin C, Cai SX, Liu SJ, Watanabe T, Nakatsuka H, Ikeda M (1995) Urinary phenylglyoxylic acid excretion after exposure to ethylbenzene among solvent-exposed Chinese workers. Int J Occup Environ Health **1**, 1–8.
16) Knecht U, Reske A, Woitowitz HJ (2000) Biological monitoring of standardized exposure to ethylbenzene: evaluation of a biological tolerance (BAT) value. Arch Toxicol **73**, 632–40.
17) Kawai T, Zhang ZW, Takeuchi A, Miyama Y, Sakamoto K, Higashikawa K, Ikeda M (2003) Methyl isobutyl ketone and methyl ethyl ketone in urine as biological markers of occupational exposure to these solvents at low levels. Int Arch Occup Environ Health **76**, 17–23.
18) World Health Organization (1996) Biol Monit Chem Expo Workplace **1**, 24. WHO, Geneva.
19) Ichihara K (1995) Bioscience for Statistics. 218, 219 and 233, Nankodo Publishers, Tokyo.
20) 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**, 148–54.
21) Kawai T, Sakurai H, Ikeda M (2015) Estimation of biological occupational exposure limit values for selected organic solvents from logartihm of octarol water partition coefficient. J Occup Health **57**, 359–64.
22) Kawai T, Sakurai H, Ikeda M (2018) Further examination of log $P_{ow}$-based procedures to estimate biological occupational exposure limits. J Occup Health **60**, 453–7.
23) Jang JY, Droz PO, Kim S (2001) Biological monitoring of workers exposed to ethylbenzene and co-exposed to xylene. Int Arch Occup Environ Health **74**, 31–7.
24) Mao IF, Chang FK, Chen ML (2007) Delayed and competitively inhibited excretion of urinary hippuric acid in field workers coexposed to toluene, ethyl benzene, and xylene. Arch Environ Contam Toxicol **53**, 678–83.
25) International Agency for Research on Cancer (2000) IARC Monographs on the Evaluation of Carcinogenic Risks to Huamns, Vol. 77. IARC, Geneva.
26) Japan Society for Occupational Health (2018) Recommendation of occupational exposure limits (2018–2019). J Occup Health **60**, 419–542.
27) American Conference of Governmental Industrial Hygienists (2018) TLVs® and BEIs®. ACGIH, Cincinnati.