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Review Article

Evaluation of Exposure to Mixed Solvents by Analyzing Their Metabolites and Solvents in Urine

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Abstract

The effect of exposure to solvent mixtures on metabolism are reviewed and tolerable biological levels resulting from exposure, with their ranges, are described. The inhibitory effect of each solvent depends on its metabolic pathway and on the degree of exposure. When exposure to solvent mixtures has no or only a minimum effect on biological levels of exposure indicators, the biological hazard index proposed is useful as an indicator for tolerable exposure. The confidence and predictive limits of the biological hazard index are also discussed. When the urinary metabolites of some of the solvents in a solvent mixture are determined in exposed workers and others are not, a procedure is described to estimate the metabolite levels of the unmeasured solvents. When exposure affects biological level of exposure indicators by the interaction of solvent mixtures, a method for estimating the biological hazard index using adjustment factor "f" is described.

Introduction

In the workplace, exposure to mixed solvents is more common than exposure to a single solvents. There has been a need for evaluating exposure to mixed solvents by analyzing the metabolites or parent solvents in the urine. In 1970, Ogata et al.[1] proposed a method for the evaluation of exposure to toluene and m-xylene by measuring the urinary metabolites, hippuric acid and m-methyl hippuric acid. Subsequently, these authors[2-5] developed an equation for evaluating exposure of workers to mixed solvents by measuring the metabolites in the urine and proposed a biological hazard index while assuming that the compounds had no cross effects on the concentrations of urinary metabolites or parent solvents. The biological hazard index indicates biological levels for tolerable exposure to mixtures. Thereafter, Ogata et al. reported an evaluation of exposure to sol-

vents from their urinary excretions in workers coexposed to toluene, xylenes and methyl isobutyl ketone[6]. In order to evaluate the overexposure to mixed solvents, considering personal differences in metabolic activities, calculations for a biological hazard index and its ranges are necessary. Therefore, Ogata et al.[7] described the confidence limits of the biological hazard index and predictive limits for individual specimens. In the case of a solvent mixture containing many solvents as ingredients such as thinners, it is difficult to measure all the solvents and metabolites in urine. In this case, a method was devised for estimating the concentration of urine components not measured using the airborne concentration and threshold limit values of these components[7]. This paper reviews the results related to the evalution of mixed exposure.

The effect of exposure to multiple solvents on their metabolism

The effect depends on the metabolic pathway of each solvent and on the degree of exposure. The effects of various solvent mixtures on biological levels of exposure indicator are summarized in Table 1.

1) Interaction in solvent mixtures has the least effect on urinary level of exposure indicators.[1]

There had been no reports in the literature that described the application of an equation to quantify exposures to mixed solvent vapors till 1970. A study carried out by the authors was the first case in which volunteers were exposed to 67 ppm toluene and 83 ppm xylene. They are exposed for three hours and urinary hippuric acid (HA) and methyl hippuric acid (MHA) were determined. The report indicated that the excretion rates for both metabolites were the same as those for separate exposure.

Tardiff et al.[8] reported the results of a study in which volunteers were exposed to 50 ppm toluene and 40 ppm m-xylene for seven hours. They noted that simultaneous exposure to this low level of solvents did not alter the concentration of unchanged solvents in blood or in exhaled air. In addition, the excretion rate of urinary metabolites was unchanged during the three 3-7 hour exposure period. The simultaneous exposure to 95 ppm toluene and 80 ppm m-xylene also did not affect the concentration of unchanged solvents in blood and in exhaled air. There was a significant delay in the urinary excretion of HA but not of m-MHA. These results indicate that there is a threshold level below which metabolic interaction of solvents does not occur.

Kawai et al.[9] monitored urinary metabolites of workers exposed to a mixture of 11.0 ppm toluene, 7.1 ppm styrene and 32.6 ppm methanol and reported that no modification in metabolism was induced by the combined exposure at these low exposure levels of concentration.

The results[6] of a survey of workers exposed to toluene, xylene, and MIBK indicated that regression lines between concentrations of solvents and metabolites calculated for workers exposed to a single solvent are identical with those from exposure to mixed solvent. The results of our study are similar to the results of the study on controlled exposure to toluene and xylene by Tardiff et al.[8] The data show that the differences in the regression equations were not apparent between exposure to single and mixed solvents, because our study was carried out using a low concentration of solvent mixture.

The metabolic pathways of toluene and xylene are similar and are initiated by oxidation of a methyl residue. Therefore, the possibility of competitive inhibition between these solvents should be considered. Our results

also indicated that the least modification is induced by combined exposure when the concentration of toluene, xylenes and MIBK is relatively low.

These four reports suggest that mutual metabolic interactions among the multiple components of solvents are not likely to occur in humans who are exposed to such mixtures as toluene and xylene, or toluene, xylene and methanol, when the concentra-

Table 1 Relationship between the exposure concentation of each solvent and the concentration of each unchanged solvent in the blood, of each unchanged solvent in exhalation and of metabolites of each solvent in the urine of workers exposed to mixed solvents

solv.	urinary metabo.	exposure condition			sample	inhibit	f_i	Ref.
		ppm	hours	occup or extraoccup				
A. mutual metabolic effects were not found								
tol	HA	67	3	extraoccup	u	neg	1.0	(1)
m-xy	MHA	83				neg	1.0	
tol	HA	50	7	extraoccup	u.bl	neg	1.0	(8)
m-xy	MHA	40			exa	neg	1.0	
tol	HA	11.0	8	occup	u	neg	1.0	(9)
sty	MA	7.1				neg	1.0	
meth	METH	32.6				neg	1.0	
tol	HA, Cr.	14.6	8	occup	u	neg	1.0	(6)
xyls	MHAs	13.6				neg	1.0	
mibk	MIBK	16.7				neg	1.0	
B. mutual metabolic effects were found								
tol	HA	95	4	extraoccup	u	pos	1.23	(8)
					bl	pos	0.68	
					exa	pos	0.81	
m-xy	MHA	80			u	pos	1.07	
					bl	pos	0.90	
					exa	pos	0.80	
ebz	MA	150	4	extraoccup	u	pos	2.0	(10)*
m-xy	MHA	150				pos	1.6	
m-xy	MHA	100	4	extraoccup	u	pos	1.4	(11)*
mek	BUTD	200				neg	1.0	
m-xy	m-xy	100	4		bl	pos	0.5	
mek	BUTD	200				neg	1.0	
bz	PH	92	8	occup	u	pos	2.48	(12)
tol	HA	86				pos	1.43	
tol	Cr	86				pos	1.28	

tol; toluene. xy; xylene, sty; styrene, meth & METH; methnol, mibk & MIBK; methyl isobutyl ketone, bz; benzene, mek & MEK; methyl ethyl ketone, ebz; ethylbenzene, HA; hippuric acid, MHA; methylhippuric acid, MA; mandelic acid, PH; phenol, Cr. cresol, BUTD; 2,3-Butanediol, occup; occupational exposure, extraoccup; extraoccupational exposure, u; urine, bl; blood, exa; exhaled air, neg; negative, pos; positive and *; f_i was estimated from the values of Figs. in the reports of (10) and (11).

tions of the solvents are lower than those reported in the above studies.

2) Interaction of mixture solvents affects biological levels of exposure indicators.

The effect of inhibition of metabolism on biological levels during coexposure are as follows.

In the study of Engstrom et al.[10] volunteer subjects were exposed to 150 ppm ethylbenzene and 150 ppm of m-xylene both separately and in combination. The combined exposure resulted in a mutual inhibition of the metabolism of both solvents which was demonstrated by delayed excretion and decreased amounts of metabolites excreted.

In the study of Liira et al.[11] the volunteers inhaled 100 ppm of m-xylene and 200 ppm of methylethyl ketone simultaneously. The excretion of methylhippuric acid was reduced while excretion of 2, 3-butanediol was enhanced, compared to excretion rate after a similar exposure to a single solvent. Occupational exposure to benzene and toluene[12] reduces excretion of phenol and hydroquinone to a large extent than excretion of catechol.

Calculation of the Biological hazard index

1) *Interaction of mixture solvents has practically no effect on urinary levels of exposure indicators.*

(1) *Calculation of hazard index*

In the relationship between concentrations of toluene in the air and hippuric acid in the urine, lines fitted to the means, the confidence limits of the means and the predictive limits of individual specimens in each group calculated by the least squares method, according to the group mean method of Weisbrot[13], are shown in Fig. 1.[14].

In accordance with a proposal by the American Conference of Governmental Industrial Hygienists (ACGIH)[15], the workplace air containing multiple organic vapors was evaluated with the sum of fractions as follows;

$$\sum_{i=1}^{n} C_i / TLV_i = K \qquad (1)$$

in which C_i denotes the airborne concentration of the component and TLV_i denotes the threshold limit value -time-weighted average (TLV-TWA) for the same component. The symbol K denotes the hazard index. If the K value exceeds unity, the workplace is deemed to exceed the exposure threshold for the mixed vapors.

(2) *The biological hazard index*

Equations were developed from the group means. We used the average values of the concentrations of metabolites and parent solvents in the urine of worker's group classified by exposure concentrations for the airborne concentrations of solvents in the breathing zone. The lines were fitted by the least square method as described above and are shown in Fig. 1.

In this figure, ECM_i and BCM_i denote the mean concentrations of the excretions of an "i" solvent in the urine and that of its background concentration of the individual excrete, respectively, and the symbols, ECM_{TLVi} and BCM_{TLVi}, denote the mean concentration of urinary excretions corresponding to TLV_i and that of its background concentrations as described in Table 2.

In this figure, TLV in equation 1 is replaced by ECM_{TLVi} or biological exposure indices (BEI) in the above equation 1 as follows: In the range within which C (the concentration of solvent vapor) is proportional to $(EC_i - BC_i)$ and TLV_i corresponds to $ECM_{TLVi} - BCM_{TLVi}$ or $BEI_i - BC_{BEIi}$ in the same grade. The equations $C_i = k_1 (ECM_i - BCM_i)$ and $TLV_i = k_1 (ECM_{TLVi} - BCM_{TLVi})$ or $TLV_i = k_1 (BEI_i - BC_{BEIi})$ can be induced.

Thus, the equation

$$C_i / TLV_i = (ECM_i - BCM_i) / (ECM_{TLVi} - BCM_{TLVi}) \qquad (2)$$

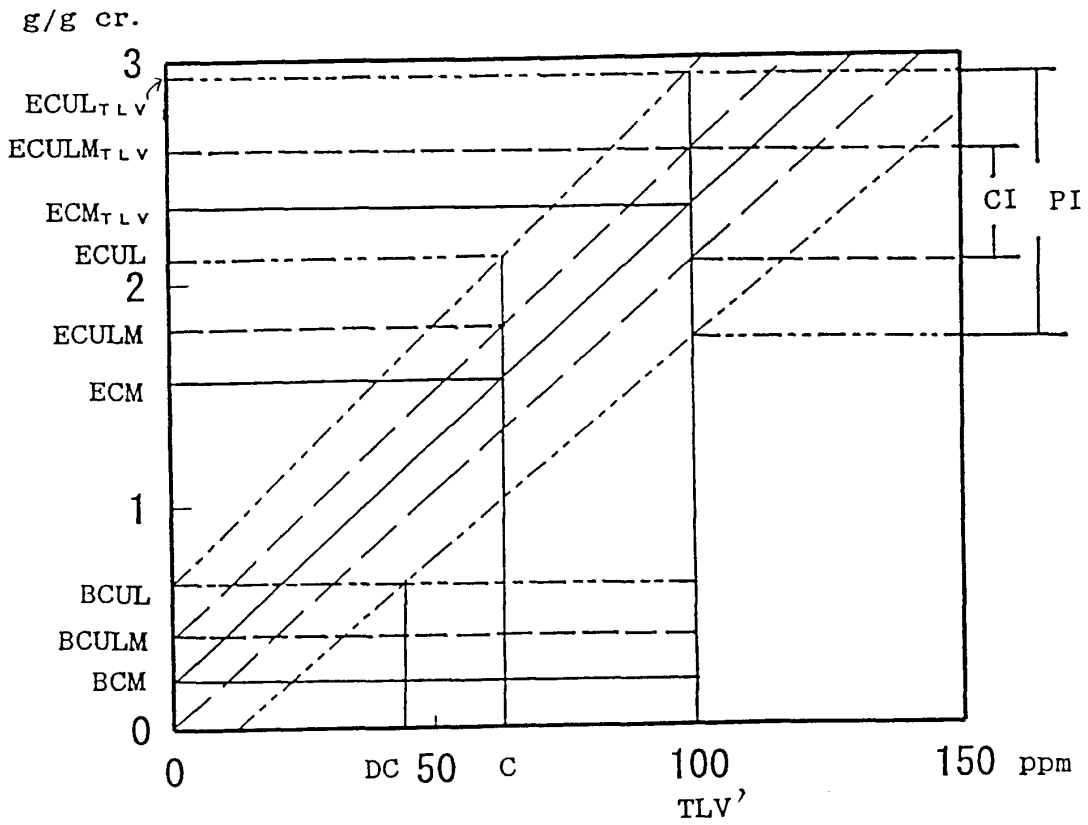

Fig. 1 Relationship between concentrations of toluene in the breathing zone air and concentrations of hippuric acid in the urine. Workers were classified into several groups on the basis of exposure to toluene concentrations and their hippuric acid concentrations were determined. Lines were fitted to the means, confidence limits of the mean and predictive limits of individual specimens in each group. Symbols CI and PI are indicated the 95% confidence interval of group mean and the 95 % predictive interval of individual values.
The symbol TLV' is the TLV of toluene (100 ppm) till 1991 but the current TLV of toluene is 50 ppm.
The symbols ECM_{TLV}, ECM and BCM indicate the mean concentrations of hippuric acid corresponding to TLV' of toluene, that of hippuric acids corresponding to C of toluene and that of background concentrations of hippuric acid, respectively.
The symbols of $ECUL_{TLV}$, ECUL and BCUL indicate the 97.5 % upper predictive limit of individual specimens of hippuric acid concentrations corresponding to TLV' of toluene, that of hippuric acid concentrations corresponding to C of toluene and that of background concentrations of hippuric acid, respectively. The symbols of $ECULM_{TLV}$, ECULM and BCULM indicate the 97.5 % upper confidence limit of the mean of hippuric acid concentrations corresponding to TLV' of toluene, that of hippuric acid concentrations corresponding to C of toluene and that of background concentrations of hippuric acid, respectively.
The symbol DC indicates the discriminant concentration which is considered to discriminate exposure from non-exposure using urinary metabolites at 2.5 % levels as proposed by authors[14].

or

$$C_i/TLV_i=(ECM_i-BCM_i)/(BEI_i-BC_{BEIi}) \quad -(3)$$

is obtained.

The following equations (4) to (7) were derived from the equations (2) and (3).

$$\sum_{i=1}^{n}(ECM_i-BCM_i)/$$

$$(ECM_{TLVi}-BCM_{TLVi})=K_B \quad -(4)$$

or

$$\sum_{i=1}^{n}(ECM_i-BCM_i)/(BEI_i-BC_{BEIi})=K_B' \quad -(5)$$

Thus

$$K=K_B \quad -(6)$$

or

$K = K_B'$ ——————————————(7)

is obtained.

The coefficient, K_B or K_B', stands for the biological hazard index and ideally is equal to the hazard index k in equation (1) for a given mixture.

To maintain occupational safety, the coefficient K_B or K_B' calculated mean values from individual data of urinary determinants or BEI of the determinants of groups surveyed should be equal to or smaller than 1. When the coefficient of K_B or K_B' exceed 1, the cause of the excessive values must be investigated and proper action must be taken

Table 2 A list of the symbols of the concentrations of excretions in the urine of subjects exposed to "i" solvents and values corresponding to 100 ppm toluene (T) and 100 ppm xyline (X), with background levels shown in Fig. 1

ECM_i	: the mean concentrations of urinary excretions
BCM_i	: background concentrations of urinary excretions (T; 0.21)
ECM_{TLVi}	: the mean concentration of urinary excretions corresponding to TLV_i (T; 2.31, X; 2.10).
BCM_{TLVi}	: background concentrations of urinary excretions corresponding to TLV_i (T; 0.21).
$ECUL_i$	: the upper 97.5 % predictive limit of concentrations of individual excretions in urine.
$BCUL_i$	: the upper 97.5 % predictive limit of background concentrations of individual excretions in urine.
$ECUL_{TLVi}$	: the upper 97.5 % predictive limit of the concentration of urinary excrete corresponding to TLV_i (T; 3.05, X; 2.80).
$BCUL_{TLVi}$	: the upper 97.5 % predictive limit of background concentration of urinary excrete corresponding to TLV_i. (T; 0.65).
$ECLL_i$	: the lower 2.5 % predictive limit of concentration of individual excretions.
$BCLL_i$	: the lower 2.5 % predictive limit of background concentration of individual excretions.
$EBLL_{TLVi}$	: the lower 2.5 % predictive limit of the concentration of urinary excretions corresponding to TLV_i.
$BCLL_{TLVi}$	: the lower 2.5 % predictive limit of the background concentration of urinary excretions corresponding to TLV_i.
$ECULM_i$	: the upper 97.5 % confidence limit of the mean of the concentrations of urinary excretions.
$BCULM_i$	: the upper 97.5 % confidence limit of the mean of background concentrations of urinary excretions.
$ECLM_{TLVi}$	: the upper 97.5 % confidence limit of the mean of the concentrations of urinary excretions corresponding to TLV_i (T; 2.61, X; 2.40).
$BCULM_{TLVi}$	: the upper 97.5 % the confidence limit of the mean of background concentrations of urinary excretions corresponding to TLV_i (T; 0.41).
$ECLLM_i$	: the lower 2.5 % confidence limit of the mean of the concentrations of urinary excretions.
$BCLLM_i$	: the lower 2.5 % confidence limit of the mean of background concentrations of urinary excretions
$ECLLM_{TLVi}$	: the lower 2.5 % confidence limit of the mean of the concentration of urinary excretions corresponding to TLV_i.
$BCLLM_{TLVi}$	: the lower 2.5 % confidence limit of the mean of background concentration of urinary excretions corresponding to TVL_i.

Unit; T; ppm X; ppm and excretion in urine, T→HA; g/g creatinine, X→MHA; g/g creatinine.

to reduce exposure.

2) *Compounds have cross effect on urinary metabolites or parent solvents:*

In the case of competitive inhibition, metabolism is reduced during exposure to multiple solvents (coexposure in short). Consequently, the concentrations of urinary excretions (metabolites and parent solvents) are reduced. For including changes in biological levels, equation for deriving K_B, assuming that compounds have no cross effects, is modified to;

$$\sum_{i=1}^{n} f_i(E'C_i - BC_i)(BEI_i - BC_{BEIi}) = K_B \quad \text{——————(5')}$$

Where K_B is the biological hazard index. EC_i is the concentrations of excretions in the urine of workers exposed to a single solvent "i" and $E'C_i$ is the concentrations of excretions in the urine of workers exposed to each solvent of "i" in mixed solvents. $f_i=(EC_i-BC_i)/(E'C_i-BC_i)$ and f_i is the adjustment factor. If the biological levels of the determinants of an "i" solvent are unaffected by exposure to multiple solvents, $f_i=1$; if the biological levels of the indicators are reduced as a results of coexposure (e.g., urinary excretions); $f_i>1$ and if the biological levels of the indicators are increased as a result of coexposure (e.g., parent solvents in blood), $f_i<1$. Therefore, the f_i is changed by the degree of exposure and mechanism of the effect. The values of the adjustment factor are described in Table 1.

Other indicators besides the biological hazard index and a method of estimating biological levels of exposure indicators which are not determined, when interaction of mixture solvents has no toxicokinetic effects[7].

1) Other indicators

(1) Upper predictive limits of individual samples

The symbols of $ECUL_i$, $BCUL_i$, $ECUL_{TLVi}$ and $BCUL_{TLVi}$ are explained in Table 2. In the range where C_i is proportional to $ECUL_i$-$BCUL_i$, and TLV_i is proportional to $ECUL_{TLVi}$-$BCUL_{TLVi}$ in the same grade (Fig. 1),

$C_i=K'(ECUL_i-BCUL_i)$ and $TLV_i=K'(ECUL_{TLVi}-BCUL_{TLVi})$ are formulated.

Then the following equation (8) can be derived;

$$\sum_{i=1}^{n}(ECUL_i-BCUL_i)/(ECUL_{TLVi}-BCUL_{TLVi}) = \sum_{i=1}^{n} C_i/TLV_i \quad \text{——————(8)}$$

When the following equation

$$\sum_{i=1}^{n}(ECUL_i-BCUL_i)/(ECUL_{TLVi}-BCUL_{TLVi}) = K_{ULB} \quad \text{——————(9)}$$

is set up, the equation

$$K=K_{ULB} \quad \text{——————(10)}$$

can be derived from equation (8).

The value of the coefficient K_B was calculated from the means of specimens and standard using equation (4). On the other hand, the value of the coefficient K_{ULB} was obtained from the 97.5 % upper predictive limits of concentrations of determinants, in the individual urine specimens and standard using equation(9). The practical evaluation of K_{ULB} in exposure to mixed solvents is described in the item, example of calculation.

(2) Lower predictive limits

Symbols $ECLL_i$, $BCLL_i$, $ECLL_{TLVi}$ and $BCLL_{TLVi}$ are described in Table 2.

In a manner similar to that used for derivation of the equation (8), the following equation (11) can be derived, in the range where C_i is proportional to $ECLL_i$-$BCLL_i$, and TLV_i correspond to $ECLL_{TLVi}$-$BCLL_{TLVi}$ in the same grade (Fig. 1).

$$\sum_{i=1}^{n}(ECLL_i-BCLL_i)/(ECLL_{TLVi}-BCLL_{TLVi}) = \sum_{i=1}^{n} C_i/TLV_i \quad \text{——————(11)}$$

When following equation of

$$\sum_{i=1}^{n}(ECLL_i-BCLL_i)/(ECLL_{TLVi}-BCLL_{TLVi}) = K_{LLB} \quad (12)$$

is formulated, then

$$K = K_{LLB} \quad (13)$$

is derived from equation (11).

The lower 95 % predictive limit of concentrations of individual metabolites or parent solvents ($ECLL_{TLVi}$), corresponding to TLV of a single solvent, has been used as the screening level for workers exposed to TLV[7]. Similarly in workers exposed to mixed solvents, the equation with coefficient K_{LLB} equal to 1, can be used as the screening level for selecting the workers whose exposures are exceeding the TLV. In order to derive these equations, it is assumed that the interaction of mixed components has no effect on the biological levels of urinary metabolites or solvents.

(3) Equations derived from 95 % confidence limits of a mean of each group:

a) Upper confidence limit.

Symbols, $ECULM_i$, $BCULM_i$, $ECULM_{TLVi}$ and $BCULM_{TLVi}$ are described in Table 2. In the range where C_i is proportional to $ECULM_i$-$BCULM_i$, and TLV_i is proportional to $ECULM_{TLVi}$-$BCULM_{TLVi}$ in the same grade (Fig. 1), the equations C_i=K'($ECULM_i$-$BCULM_i$) and TLV_i=K'($ECULM_{TLVi}$-$BCULM_{TLVi}$) are obtained.

Therefore, equation (14) can be derived.

$$\sum_{i=1}^{n}(ECULM_i-BCULM_i)/(ECULM_{TLVi}-BCULM_{TLVi}) = \sum_{i=1}^{n} C_i/TLV_i \quad (14)$$

When

$$\sum_{i=1}^{n}(ECMULM_i-BCULM_i)/(ECULM_{TLVi}-BCULM_{TLVi}) = K_{ULMB} \quad (15)$$

is formulated then,

equation $K = K_{ULMB}$ (16)

can be obtained.

b) Lower confidence limit.

Symbols of $ECLLM_i$, $BCLLM_i$, $ECLLM_{TLVi}$ and $BCLLM_{TLVi}$ are described in Table 2. In a manner similar to that for derivation of equation (14), the following equation (17) was obtained.

$$\sum_{i=1}^{n}(ECLLM_i-BCLLM_i)/(ECLLM_{TLVi}-BCLLM_{TLVi}) = \sum_{i=1}^{n} C_i/TLV_i \quad (17)$$

When the following equation of

$$\sum_{i=1}^{n}(ECLLM_i-BCLLM_i)/(ECLLM_{TLVi}-BCLLM_{TLVi}) = K_{LLMB} \quad (18)$$

is derived, the equation of

$$K = K_{LLMB} \quad (19)$$

is obtained.

2) Making a comparison between the hazard index (K) and biological hazard index (K_B):

The following equations were set up when biological levels of exposure are not affected by the exposure to multiple solvents. An equation derived from equation (4) for group mean is as follow;

(1) When K is higher than K_B, or K is higher than K_{ULMB}, the following equations

$$\sum_{i=1}^{n} C_i/TLV_i > \sum_{i=1}^{n}(ECM_i-BCM_i)/(ECM_{TLVi}-BCM_{TLVi}) \quad (20)$$

or

$$\sum_{i=1}^{n} C_i/TLV_i > \sum_{i=1}^{n}(ECM_i-BCM_i)/(BEI_i-BC_{BEIi}) \quad (21)$$

is derived.

In using equation (20) or (21), if the solvents have no cross effects, then the possibility that the workers drank alcohol before exposure or wore masks during exposure, should be examined.

In the case that the equation (20) is set up using some examples, the following equation (22) should be checked.

When the symbol $ECLL_i$, indicating the

lower 2.5 % predictive limit of the concentration of urinary determinants, was used instead of ECM_i, the following equation (22) is derived.

$$\sum_{i=1}^{n} C_i/TLV_i > \sum_{i=1}^{n} (ECLL_i - BCLL_i)/(ECLL_{TLVi} - BCLL_{TLVi}) \quad (22)$$

Equation (21) is derived from ECM_i of group means of urinary determinants of solvents, and equation (22) is from $ECLL_i$ the lower 95 % predictive limit of the concentration of urinary determinants. Therefore, Equation (21) is based on the comparison of C_i with ECM_i, and equation (22) is based on the comparison of C_i with $ECLL_i$.

(2) K lower than K_B or K lower than K_{ULMB}.

The following equation (23) is derived from equation (4) and equation (24) is from equation (5).

$$\sum_{i=1}^{n} C_i/TLV_i < \sum_{i=1}^{n} (ECM_i - BCM_i)/(ECM_{TLVi} - BCM_{TLVi}) \quad (23)$$

or

$$\sum_{i=1}^{n} C_i/TLV_i < \sum_{i=1}^{n} (ECM_i - BCM_i)/(BEI_{BEIi} - BC_{BEIi}) \quad (24)$$

Microsomal enzyme activity is induced by chronic alcohol intake, or dermal absorption is considered in equations (23) and (24).

When equation (23) is set up using some examples, the following equation of (25) should be checked. When the symbol $ECUL_i$, indicating the lower 2.5 % predictive limit of the concentration of urinary determinants, was used instead of ECM_i in equation (23), the following equation is derived.

$$\sum_{i=1}^{n} C_i/TLV_i < \sum_{i=1}^{n} (ECUL_i - BCUL_i)/(ECUL_{TLVi} - BCUL_{TLVi}) \quad (25)$$

Figure 2 is an example evaluating exposure to a solvent mixture using equation (3), (21) and (24).

3) Only some components of a mixture are biologically determined:

In n kinds of solvents (i), the concentration of urinary metabolites or parent solvents derived from m kinds of solvents (j) are measured and the concentration of urinary determinants from the remaining (n-m) kinds of solvents (k) are not measured, the following equations are useful for the evaluation of mixed exposure under the assumption that the compounds have no effect on the concentration of urinary metabolites or parents solvents.

(1) The ratio of the airborne concentration to TLV is used instead the ratio of the concentration of urinary metabolites or parents solvents to biological exposure indices for the remaining n-m kinds of solvents

$$\sum_{i=1}^{n} (ECM_i - BCM_i)/(BEI_i - BC_{BEIi}) = \sum_{j=1}^{m} (ECM_j - BCM_j)/(BEI_j - BC_{BEIj}) + \sum_{k=m+1}^{n} C_k/TLV_k \quad (26)$$

(2) Estimation of the ratio of urinary concentrations of metabolite or solvent to BEIs for n-m kinds of undetermined solvents

The ratio of the remaining solvents is estimated under the assumption that a linear relationship existed between each concentration of n-m kinds of remaining solvents in the air and each metabolite or solvent in the urine.

$$\sum_{i=1}^{n} (ECM_i - BCM_i)/(BEI_i - BC_{BEIi}) = \sum_{j=1}^{m} (ECM_j - BCM_j)/(BEI_j - BC_{BEIj}) + \sum_{k=m+1}^{n} (ECM_k - BCM_k)/(BEI_k - BC_{BEIk}) \quad (27)$$

From the equation of

$$\sum_{j=1}^{m} C_j/TLV_j = \sum_{j=1}^{m} (ECM_j - BCM_j)/(BEI_j - BCM_{BEIj}) \quad (28)$$

and

$$\sum_{k=m+1}^{n} C_k/TLV_k = \sum_{k=m+1}^{n} (ECM_k - BCM_k)/(BEI_k - BC_{BEIk}) \quad (29)$$

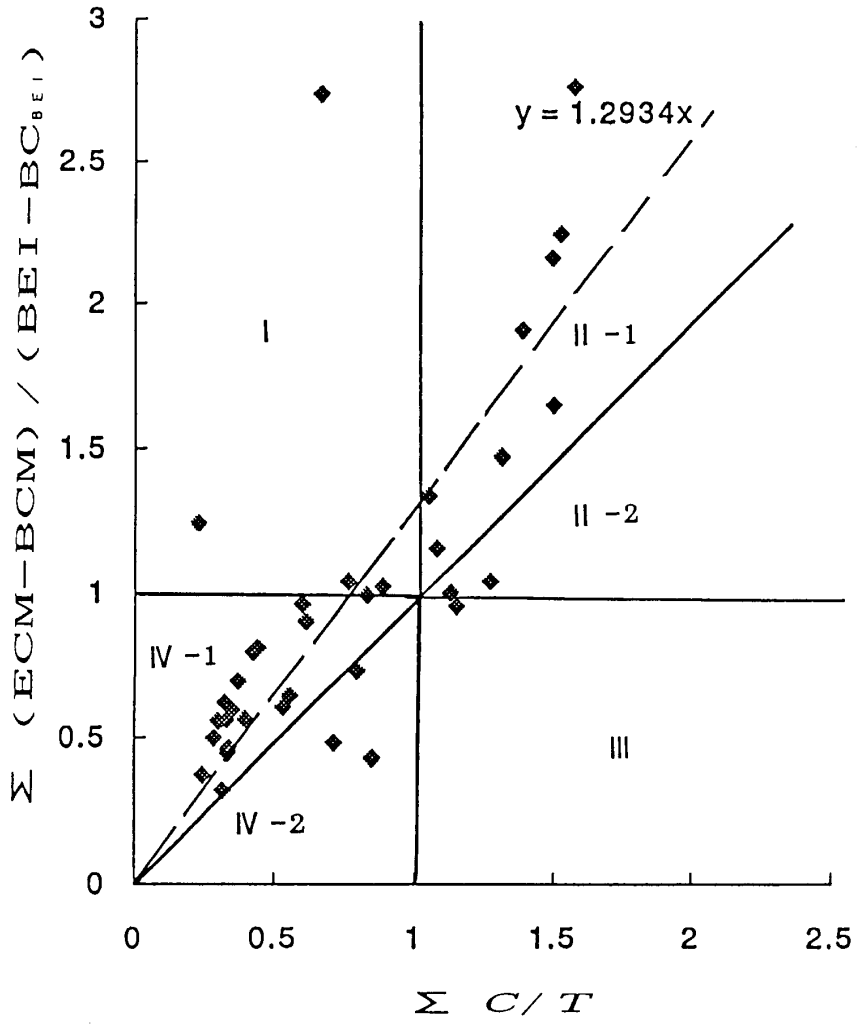

Fig. 2 Relationships between the K and K_B values in workers exposed to solvent mixtures. Data are based on urinary biological monitoring of workers exposed to a solvent mixture of toluene, xylene and methyl isobutyl ketone (MIBK)[6].

For calculating the K and K_B values in the workers exposed to xylene, MIBK and toluene vapors, 100 ppm xylene of the TLV and 1.5 g/g cr. urinary methylhippuric acid of the BEI for xylene exposure, 50 ppm MIBK of the TLV and 2 mg urinary MIBK/mL of the BEI for MIBK exposure and 50 ppm toluene of TLV and 0.5 mg urinary o-cresol/g cr. corresponding to the TLV for toluene exposure are used as the standard values. The concentration of urinary o-cresol corresponding to the TLV was used as a standard value instead of the BEI. In depicting this figure, the background concentration of o-cresol is generally less than 0.1 mg/g cr. which is omitted to calculate K_B for convenience.

The figure is divided into four quadrants of I, II, III and IV by the line of ΣC/T and the line of Σ (ECM-BCM)/(BEI-BC_{BEI}) and divided into II-1, II-2, IV-1 and IV-2 et al. for each quadrant by the line of $K=K_B$ are as follows:

I ($K_B>1$, $K_B>K$), II-1 ($K_B>1$, $K_B>K$), II-2 ($K_B>1$, $K>1$, $K_B<K$), III ($K_B<1$, $K>1$, $K_B<K$), IV-1 ($K_B<1$, $K<1$, $K_B>K$), IV-2 ($K_B<1$, $K<1$, $K_B<K$).

(1) $K_B>1$: A worker on different occasions exceeds the BEI, then the cause of the excessive values must be investigated and proper action taken to reduce the exposure.

(2) $K>1$: The value exceeds the TLV, which indicates that workers have adverse health effect.

(3) $K_B>K$: Presence of dermal absorption or nonoccupational exposure should be considered.

(4) $K_B<K$: Possibility that wore a protective mask, or drank ethanol prior to exposure to solvents should be investigated.

Therefore, the workers belonging to each division from I to IV-2(4) are indicated as follows; I =(1) and (3); II-1=(1), (2) and (3); II-2=(1), (2) and (4); III=(2) and (4), IV-1=(3) and IV-2 (4).

The following equation of (30) is derived from equations (28) and (29).

$$\sum_{j=1}^{m} C_j/TLV_j : \sum_{k=m-1}^{n} C_k/TLV_k = \sum_{j=1}^{m} (ECM_j-BCM_j)/(BEI_j-BC_{BEIj}) : \sum_{k=m+1}^{n} (ECM_k-BCM_k)/(BEI_k-BC_{BEIk}) \quad —(30)$$

From equations (27) and (30), the following equation

$$\sum_{i=1}^{n} (ECM_i-BCM_i)/(BEI_i-BC_{BEIi}) = \sum_{j=1}^{m} (ECM_j-BC_j)/(BEI_j-BC_{BEIj}) \times \left(\sum_{k=m+1}^{n} C_k/TLV_k + \sum_{j=1}^{m} C_j/TLV_j\right)/\sum_{j=1}^{m} C_j/TLV_j \quad ————(31)$$

is derived.

The following equation (32) was derived in a simpler way.

$$\sum_{j=1}^{m} (ECM_i-BCM_i)/(BEI_j-BC_{BEIj}) \times \sum_{i=1}^{n} C_i/TLV_i \div \sum_{j=1}^{m} C_j/TLV_j = K_B' \quad —(32)$$

Similar to the equations of (32) and also (4) as follows

$$\sum_{i=1}^{n} (ECM_i-BCM_i)/(ECM_{TLVi}-BCM_{TLVi}) = K_B \quad —(4),$$

the following equation of

$$\sum_{j=1}^{m} (ECM_j-BCM_j)/(ECM_{TLVj}-BCM_{TLVj}) \times \sum_{i=1}^{n} C_i/TLV_i \div \sum_{j=1}^{m} C_j/TLV_j = K_B \quad —(33)$$

was obtained.

(3) m kinds of solvents out of a total of n kinds of all solvents are used for evaluation:

In this instance, the ratios of the concentrations of only m kinds of solvents to their TLVs are compared with the ratios of urinary excretions to their BEIs:

$$\sum_{j=1}^{m} C_j/TLV_j > \sum_{j=1}^{m} (ECM_j-BCM_j)/(ECM_{TLVj}-BC_{TLVj}) \quad ————(34)$$

or

$$\sum_{j=1}^{m} C_j/TLV_j > \sum_{j=1}^{m} (ECM_j-BCM_j)/(BEI_j-BC_{BEIj}) \quad ————(35)$$

In equation (34) or (35), ethanol intake several hours prior to or just before exposure to organic solvents should be taken into consideration as described in equations (18) or (19).

$$\sum_{j=1}^{m} C_j/TLV_j < \sum_{j=1}^{m} (ECM_j-BCM_j)/(ECM_{TLVj}-BC_{TLVj}) \quad ————(36)$$

or

$$\sum_{j=1}^{m} C_j/TLV_j < \sum_{j=1}^{m} (ECM_j-BCM_j)/(BEI_j-BC_{BEIj}) \quad ————(37)$$

In equations (36) and (37), the possibility of dermal absorption of solvents and/or use of masks to minimize inhalation should be considered as described in equation (23).

When equation (34) is set up using some examples, the following equation

$$\sum_{i=1}^{n} C_i/TLV_i > \sum_{i=1}^{n} (ECLL_i-BCLL_i)/(ECLL_{TLVi}-BCLL_{TLVi}) \quad ————(38)$$

namely $K > K_{LLB}$, should be checked next. When equations (36) is set up using some examples, the following equation

$$\sum_{i=1}^{n} C_i/TLV_i < \sum_{i=1}^{n} (ECUL_i-BCUL_i)/(ECUL_{TLVi}-BCUL_{TLVi}) \quad ————(39)$$

namely $K < K_{ULB}$, should be checked next. Personal differences in the concentration of urinary determinants are taken into consideration in equations (37) and (38).

Example of calculation:

In a previous report[1], the authors described the urinary excretion of hippuric acids (ECM; 1.41, BCM; 0.25 g/g. cr) and m-methyl hippuric acids (ECM; 1.91 g/g. cr) in the persons coexposed to vapors of 67 ppm toluene and 83 ppm m-xylene. The report also indicated the least effect of coexposure was on the excretion rates of metabolites. The values of this example were substituted into equations (1), (4) and (5), and the following biological haz-

ard index was calculated.

1) Hazard index (K):

The index was calculated by equation (1) as follows;

(83/100)+(67/100)=1.5

2) Biological hazard index (K_B):

The index was calculated using equations (4) and (5) as follows: The average values of metabolite concentrations in the urine of a group of specimens, ECM_{TLV_i} (HA; 2.31., MHA; 2.10 g/g.cr) and BCM_{TLV_i} (HA; 0.21 g/g. cr) from Fig. 1, and BEI (HA; 2.5, MHA; 1.5 g/g. cr) were used for the calculation. The TLV_s of toluene and xylene used were 100 ppm and 100 ppm, respectively. From equation (4) using ECM_{TLV_i} as a standard, K_B is as follows;

(1.41-0.25)/(2.31-0.21)+1.91/2.10=1.46.

From equation (5) using BEI as a standard K_B' is as follows; (1.41-0.25)/(2.5-0.21)+1.91/1.5=1.78.

The values of the coefficients K_B and K_B' were 1.46 and 1.78, respectively. In this instance, the values K and K_B exceed 1, therefore, the cause of the excessive values must be investigated and proper action should be taken to reduce the exposure to mixed solvents.

3) Confidence and predictive limits of concentrations of urinary determinants:

(1) Upper confidence limits of mean.

When ECUML (HA; 2.00, MHA; 2.19 g/g. cr) and BCUML (HA; 0.30 g/g cr.) of the specimens[1] corresponding TLV and $ECUML_{TLV}$ (HA; 2.61, MHA; 2.40 g/g. cr) and $BCUML_{TLV}$ (0.41 g/g. cr) in Fig. 1 were substituted into equation (17), K_{ULMB} was calculated as follows;

(2.00-0.30)/(2.61-0.41)+2.19/2.40=1.69

The data indicate that the upper 97.5 % predictive limit of the mean in this instance also exceeds 1.

(2) Upper predictive limits of individual specimens:

Comparison of a test group of workers to those from the standard group of workers.

When ECUL (HA; 2.70, MHA; 3.10 g/g. cr) and BCUL (HA; 0.41 g/g. cr) of the specimens[1] corresponding to the TLV_S, and also $ECUL_{TLV}$ (HA; 3.05, MHA; 2.80 g/g. cr) and $BCUL_{TLV}$ (HA; 0.65 g/g. cr) in Fig. 1 were substituted into equation (9), K_{ULB} was calculated as follows;

(2.70-0.41)/(3.05-0.65)+3.10/2.80=2.06

The data indicate that the upper 97.5 % predictive limit of individual specimens in this instance also exceed 1.

(3) Comparison of datum from a test worker to data from the standard group of workers.

The other methods of comparison are as follows: The values of the coefficient K_B (1.46) and K_B' (1.78) exceed 1 as described above, which suggests that K_B values calculated from the mean value of a group of specimens was larger than the K_B values of the standard.

Then the comparion between the mean values of examples and the upper 97.5 % predictive limit of individual specimens of standards was taken into consideration. When data of 1.41 g/g cr. of hippuric acid and 1.91 g/g cr. of methyl hippuric acid obtained from the mean value of a group of specimens is substituted into equation (15), a value of (1.41 - 0.3)/(2.61 - 0.41)+1.91/2.4=1.30 for K_{ULMB} was obtained and when the data are substituted into equations (9) a value of (1.41 -0.41)/(3.05-0.65)+1.91/2.8=1.1 for K_{ULB} was obtained. This indicates that the mean values exceed not only the mean or upper 97.5 % confidence limit of the mean or BEI but also the upper 97.5 % predictive limit of the individual samples.

(4) Comparison of data from a test group to BEI instead of ECM_{TLV_i}.

The BEI only represented the mean value

of determinants of specimens of workers who have been exposed to the TLV in which the confidence range of the mean and the predictive range of each specimen were not indicated.

The K_B of each worker in a worker group is calculated by the equation (3). Therefore, the mean, the 97.5 % confidence limit of the mean and the 97.5 % predictive limit of each specimen are inserted in equation (5) and these values can be compared to 1 for K_B.

For example, in the case of the data of 2) K_B, described above the mean K_B value was 1.78. Using the same data, the only approximate values of the 97.5 % upper confidence limit of the mean and the 97.5 % upper predictive limit of each specimens are calculated to be (2.00-0.25)/(2.5-0.21)+2.19/2.1=2.22 and (2.70-0.25)/(2.5-0.21)+3.1/1.5=3.13, respectively, from the data of the same group. Similarly, the 0.25 % lower confidence range of the mean and 0.25 % lower predictive range of each specimen are calculated to be 1.34 and 0.43, respectively. Therefore, not only the mean K_B value but also the 0.25 % lower confidence limit of the mean is exceed the 1.0 value for K_B. In addition, if a simple evaluation method based on BEI, using equation (3), indicated that when the majority of the data from the workers exceeds 1 by the equation (3), the cause of the excess value must be investigated and proper action taken to reduce the exposure.

4) Complementary method:

When subjects are exposed to 67 ppm toluene and 83 ppm m-xylene[1] and urinary concentrations of methyl hippuric acid, but, not hippuric acid, are measured, the complementary method can be used by substituting 1.91 g/g. cr of average concentration of methyl hippuric acids into equation (32) and (33). When 2.1 g/g cr. was used for ECM_{TLVi} in equation (32), (1.91/2.1)x(83/100+67/100)/(83/100)=1.64 was obtained. The value was similar to 1.46 from equation 4. When 1.5 g/g cr. of BEI_i in equation (33) was used, K'_B was (1.91/1.5)x(83/100+67/100)/(83/100)=2.30

The value was not markedly different from the value of 1.78 from equation (5).

5) Biological hazard index using 50 ppm toluene as TLV

Currently, the TLV-TWA of toluene was changed from 100 ppm to 50 ppm, and the BEI is under consideration. The DC in Fig. 1, to be 42 ppm[14], was considered to discriminate the exposure from the non-exposure at a 2.5 % margin of error. This suggests that hippuric acid can be useful for BEI. The biological hazard index corresponding to 50 ppm toluene from equation (4) is as follows; (1.41-0.25)/(1.19-0.21)+1.91/2.10=2.09.

Ogata et al.[6] reported that the concentrations, 0.21 mg/g cr., o-cresol, 0.32 mg/g methylhippuric acid and 0.54 mg/L MIBK were recognized in the urine of workers coexposed to 14.6 ppm toluene, 13.9 ppm xylenes and 16.7 ppm MIBK. The background level of o-cresol was about 0.04 mg/g cr. In this instance, the concentration, 0.5 mg o-cresol/g cr. corresponding to 50 ppm toluene (TLV)[16] is used as standard. The biological hazard index (K_B) using equation (4) is as follows; (0.21-0.04)/(0.5-0.04)+0.32/1.5+0.54/2.0=0.85. and the hazard index (K) using equation (1) is 14.6/50+13.9/100+0.54/50=0.77. The result shows K_B is higher than K.

Discussion

In the present paper, the author reported a fundamental method for the evaluation of biological monitoring of a mixed exposure. The methods for the calculation of the biological hazard index and its ranges are described. When the concentrations of some metabolites and/or solvents in urine are not determined, a complementary method using

their airborne concentrations is described. Its application is limited to cases where the kinds of solvents and the concentration range of the solvents have been reported. A more generalized application of the equation will be reported after further experimental and field data have been taken into account.

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