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Equation for Calculating the Concentration of Solvent in Air That Discriminates between Exposure and Non-exposure Based on Biomarker Concentrations in the Urine of Workers

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To develop a new method for evaluating the intensity of workers' exposures to toluene alone or toluene in mixed solvents, regression equations were calculated between the concentrations of toluene to which workers were exposed and the concentrations of hippuric acid or toluene in workers' urine samples taken at the end of their shifts. Thereafter, the discriminant exposure concentration of the solvents in air, which was the concentration considered to discriminate exposure from non-exposure within a fixed level of error using fiducial ranges of individual specimens (DEC-I) or using confidence ranges of regression equation (DEC-R), was measured by a scale. The devised equations were applied to calculate DEC-I or DEC-R accurately using the formulas expressing a regression line and its fiducial ranges or confidence ranges. The equations can calculate not only more precise values of DEC-I or DEC-R than can be measured by a scale, but can also calculate values corresponding to any level of error. Moreover, DEC-I and DEC-R can be defined by the equations. The concentration capable of discriminating TLV (threshold limit value) exposure from non-TLV exposure was estimated using fiducial ranges (DTL-I) and then using confidence ranges of the regression equation (DTL-R).

Key words: biological monitoring, exposure to toluene, discriminant exposure concentration, biomarker, urinary toluene

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Discriminant exposure concentration (DEC) is defined as the concentration discriminating between exposure and non-exposure of workers, and is calculated using regression lines and their confidence ranges (DEC-R) and/or fiducial ranges (DEC-I) between the exposure concentrations of solvents and the concentrations of their biomarkers.

The discriminant exposure concentration using concentration calculated using regression lines and fiducial ranges of individuals (DEC-I) between the concentration of toluene and of urinary hippuric acid was first described by Ogata in 1984 [1], and in a subsequent work the DEC-I was applied to the exposure intensity of toluene and the concentrations of toluene biomarkers [2]. The discriminant exposure concentration calculated using both confidence ranges (DEC-R) and fiducial ranges (DEC-I) was first described by Ogata *et al.* in 1986 [3] in a study

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examining urinary excretions of phenol, and in a later study DEC-I and DEC-R were determined by the use of 5 kinds of biomarkers of toluene [4].

In the previous reports [1-4], regression lines used to calculate DEC-I and/or DEC-R were estimated by the least squares methods as described by Snedecor [5].

With respect to the relation between the concentration of metabolites and the exposure concentration of solvents, there is a tendency indicating the value of standard deviation tends to increase with the increase in the mean value. As regards this tendency, the DEC-I of toluene exposure was calculated using concentrations of urinary hippuric acid [6] by the modified group mean method of Weisbrot [7].

The indicator DEC-I was applied to the selection of available biomarkers in a single exposure of toluene alone and to the selection of biomarkers in toluene exposure to mixed solvents composed of toluene, xylene and methyl isobutyl ketone in relatively low concentration [2].

The DEC-R and DEC-I calculated from the regression line and its confidence and fiducial ranges between the concentrations of each solvent and the concentrations of its biomarker have been described as follows. The results of estimating suitable biomarkers of toluene were reported using DEC-I from hippuric acid and o-cresol in urine [2] and using DEC-I and DEC-R from hippuric acid in urine, o-cresol concentrations in urine and toluene concentrations in exhaled air, blood and urine of workers [4]. The indicator DEC-I of biomarkers was available for evaluation of exposure to each solvent using urinary excretion in workers co-exposed to mixed solutions of toluene, xylene and methyl isobutyl ketone [2]. In addition, an evaluation of urinary phenol phenyl sulfate and glucuronide in the urine of workers exposed to phenol using DEC-I and DEC-R has been reported [3].

To date, methods in which the DEC-R or DEC-I is estimated from the value measured by a scale using confidence or fiducial lines of a regression equation have been used. However, the values obtained in this way were not accurate. Therefore, more precise methods for calculating DEC-R and DEC-I are needed.

In the present study, a new method using a quadratic equation was devised for calculating the concentration of a solvent discriminating exposure from non-exposure (DEC-I and /or DEC-R).

We also devised a method of estimating the exposure concentration that discriminates between TLV exposure and non-TLV exposure based on the confidence interval (DTL-R) or fiducial intervals (DTL-I) of the regression equation.

In addition, the interval between DCE-R and DTL-R was determined from the biomarker concentrations and taken as the available exposure concentration.

The mean concentration of the biomarkers (BT), the confidence range (BTL-R) and the predictive range (BTL-I), corresponding the TLV and of the presumed ranges of biological exposure indices (BEI) are also described.

Materials and Methods

The analytical procedures for workers exposed to toluene gas alone were different from those for workers exposed to toluene in mixed solvents.

Workers. Exposure to toluene alone: During the latter half of the work week, a factory survey was carried out on 79 male workers who were exposed to 1.5-50.0 ppm of toluene gas collected by personal samplers in a painting factory in Japan. They were not equipped with protection masks against organic solvents. To determine the time-weighted average (TWA) of the vapor concentration in the breathing zone, each worker was equipped with a personal sampler Organic Vapor Monitor #3500, 3 M (Sumitomo 3 M Ltd. Tokyo, Japan) from 8 AM. to the 4 PM. Monitors were collected at 4 PM. The solvents adsorbed in the activated carbon felt of monitors were desorbed by carbon sulfide, and the extracts were analyzed by gas chromatography coupled with a flame ionization detector (GC-FID) as described previously [8].

Exposure to toluene in solvent mixture: Fourteen male workers were exposed to 2.4-64.2 ppm of toluene, 0.33-15.5 ppm of isopropyl alcohol and 9.5-106.1 ppm of methyl ethyl ketone. They were equipped with a passive organic vapor monitor #3500, 3 M (Sumitomo 3M Ltd.), from 8 AM to 4 PM. The methods used to analyze solvents in the monitors were the same as those used for the single exposure.

Before the survey, the employer and then the workers were informed that the individual sampler would be attached to the collar of workers to survey the personal exposure to solvents, that urine samples would be taken to evaluate the amount of solvents absorbed in the body. In the case of publication, the type of work conducted in the shop would be described, but without disclosing the name of the shop. The results for the group of workers would be reported, but those for the individual workers would not be disclosed in any published article. Thereafter, workers were given a personal sampler and asked to provide a urine specimen.

On October 1, 1989, the Ministry of Labor of Japan passed a law making it mandatory for workers exposed to specified 8 other organic solvents including toluene to undergo periodical monitoring. The biological monitoring of toluene alone in the present study was conducted in 1985, and was performed in preparation for large scale biological monitoring.

The survey of toluene in mixed solvents was also, conducted in 1992 as part of the biological monitoring mandated by the government, which requires employers to report the results of the monitoring to both the workers and the chief of the Labor Standard Inspection Office. The present survey of toluene in mixed solvents was carried out under biological monitoring in Audience of Protection of Organic Solvent Poisoning. Our results of biological monitoring of mixed exposure were presented at 66 th annual meeting of the Japan Association of Industrial Health in 1993, held at Yokohama.

The Japan Society for Occupational Health (JSOH) proposed its Occupational Exposure Limits (OELs) for 50 ppm of toluene in 1994. The JSOH recommended the OELs as reference value for preventing adverse effect on workers caused by occupational exposure to chemical substances [9]. The OEL value is referred to the threshold limit value (TLV) recommended by ACGIH in USA [10], Maximum Concentration at Workplace (MAK) recommended by German Research Society and various reports on biological monitoring. The proposed OEL value of toluene has changed 50 ppm from 100 ppm as described in Documentation of the proposed reason for provisional OELs, considering adverse effect on central nerve system [9].

Gas chromatography. Exposure to toluene alone: The GC apparatus used was a Hitachi Model 163, (Hitachi High Technologies, Tokyo, Japan) equipped with FID and glass column ($\phi 3$ mm × L2 m) packed with Benton 34 (5%) + DIDP (5%) on Shimalite 60–80 mesh. The temperature in the oven and the injection part were 100 °C and 150 °C, respectively. The rate of supply of N_2 as a carrier gas was 50 ml/min, while H_2 and air were 0.6 and 1.5 kg/cm^2. The methods used to analyze the solvents were similar to those reported previously [6].

Exposure to toluene in solvent mixture: The GC apparatus used was a Hewlett Packard 5890 Series 2 (Agilent Technologies Japan Ltd, Tokyo, Japan) equipped with FID and wide bore capillary column ($\phi 0.53$ mm × 30 m) packed with DB wax. Column temperature was set 40 °C for 2 min, then heated to 200 °C with rate of 10 °C/min. Injection temperature was 200 °C. Detector temperature was 250 °C. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. Methods used to analyze the solvents in monitor were the similar to those described previously [4, 6].

Urinary analysis. Exposure to toluene alone: All urine samples from workers were collected at the end of the work shift. One ml of methanol was added to 1 ml of each urine specimen, mixture was centrifuged at 2,000 rpm for 5 min and 5 μl of supernatant obtained used for HPLC.

Analyses of urinary hippuric acid were performed by HPLC as described in the previous report [4, 6]. An HPLC (Hitachi 633, Hitachi High Technologies) equipped with a spectrophotometer measuring the absorbance from 200 nm to 320 nm was used. A 10 μm column (Sumipax) packed with silica gel having dinitrophenyl residue and UV detector at 225 nm wave length were used throughout this investigation.

A mixed solution of methanol/water/acetic acid (80/20/0.2) containing tetra-n-butyl ammonium bromide (0.2% w/v) was a favorable mobile phase for the separation of hippuric acid. The flow rate was 1.2 ml/min, produced pressure was 30 Kg/cm^2 and the column temperature was kept at 25 °C.

Exposure to toluene in solvent mixture: All urine specimens were collected at the end of shift. Urinary toluene was analyzed by the head space gas method. Deionized water (4 ml) was injected and then 1 ml of urine was injected into Teflon sealed vials. Urinary solvents were analyzed by the space gas method with

FID using a head space sampler.

Simultaneous analyses of urinary hippuric acid and creatinine were performed by HPLC as described in the previous report [8].

Statistical analysis. The general regression analysis was carried out with a Windows version of the Statistical Package for Social Science (SPSS), 11.0 J (SPSS Inc Chicago, IL, USA). The systematic calculations of DEC-I, DER-I, DTL-I and DTR-R were carried out using computer software: designed by the authors in C language.

Results

The intensity of exposure to toluene, and concentrations of hippuric acid in urine of workers.

1. Workers exposed to toluene alone: Table 1 summarizes the monitored concentrations of exposure to toluene and hippuric acid concentrations corrected for specific gravity in the urine samples collected from workers at the end of their shifts.

2. Workers exposed to toluene in mixed solvents: Table 1 also shows the exposure concentrations of toluene and those of toluene and hippuric acid in the urine of workers who were exposed to the mixture of toluene, isopropyl alcohol and methyl ethyl ketone.

Correlation between the concentration of toluene in the air and the concentration of its biomarkers in the urine of workers exposed to solvents.

1. Workers exposed to toluene alone: The results of the correlation analyses are shown in Table 1. The correlation coefficient between the levels of toluene concentrations in the air and corrected hippuric acid concentrations for specific gravity of 1.024 in the urine at the end of their shift was high at 0.66.

2. Workers exposed to toluene in mixed solvents: Correlation coefficient between exposed concentrations of toluene and hippuric acid concentrations corrected for creatinine was high at 0.94. Correlation coefficient between concentrations of toluene in the air and toluene concentrations in urine was the highest at 0.95 as shown in Table 1.

Regression line, confidence bands line of population regression line and fiducial lines of individual specimens. Table 1 summarizes the regression equations of exposure to concentrations of toluene and concentrations of toluene biomarkers.

Typical results of regression analyses are shown in (Fig. 1A, B and C). Two kinds of predictive lines are used in this study. ① The confidence range of the regression line (-R) define the region within which the population regression line lies at given level of error. ② The fiducial range of individuals (-I) is generally used to predict number of new specimens with known value of x [5].

1. Workers exposed to toluene alone: The regression equation between toluene concentrations in air (x, ppm) and hippuric acid concentrations corrected for specific gravity (1.024) in urine (y, g/l) in Fig. 1A was $y = 0.262 + 0.021x$, which was almost identical to regression equation of $y = 0.55 + 0.022x$ as described previously [8].

2. Workers exposed to toluene in mixed solvents: ① The regression equation between toluene concentrations in air (x, ppm) and actual concentrations of toluene in urine (μg/g) is $y = -6.92 + 2.63x$ (Fig. 1B), which is almost identical to the regression equation $y = -1.4 + 2.25x$ by Ghittori [11]. ② The regression equation between toluene concentrations in air (x, ppm) and hippuric acid concentrations in urine (y, g/g.cr.) of workers exposed to toluene in mixed solvents was $y = 0.30 + 0.025x$ (Fig. 1C). The regression equation in this study was identical to regression equation of $y = 0.21 + 0.025x$ [2] in the case of the exposure to toluene in mixed solvents, and regression equations of $y = 0.36 + 0.021x$ [8] and those of $y = 0.23 + 0.023x$ [3] which were representing the exposure to toluene alone.

Comparison among the concentrations of biomarkers corresponding to 50 ppm (TLV and OEL in 2006) of toluene in the present report and those in previous reports [8, 11, 12] is given in the discussion.

Reverse estimation of the exposed concentration of solvents and ranges from the urine samples of workers. For reverse estimation, a significant high correlation coefficient between concentrations of solvent gas and concentrations of urinary excretions is needed. The calculated regression lines and their confidence and fiducial lines at the 90 % levels depicted in Fig. 1.

① In the case of workers exposed to toluene alone: A typical regression line between concentrations of toluene gas and those of urinary hippuric acid corrected for specific gravity is shown in Fig. 1A. ② In the case of workers exposed to toluene in mixed solvents: A regression equation between concentrations of toluene in air and those of toluene in urine (Fig. 1B) and a regression equation between concentrations of toluene and those of hippuric acid corrected for creatinine (Fig. 1C) are shown.

1. Discriminant concentration in air for exposure and non-exposure using fiducial lines of individual specimens and confidence bands of regression line.

In order to determine the intensity of exposure from concentrations of biomarkers, following indicators of DEC-I and DEC-R are required.

1) Workers exposed to toluene alone:

When calculating with fiducial limits of individuals, on the regression line and fiducial lines of individual samples, a straight line parallel to the x axis was drawn from the upper fiducial limit of individual samples of the non-exposed level ($x = 0$) to the lower fiducial line of the individual specimens, and the concentrations of the x axis corresponding to the vertex crossing the lower fiducial line was used as discriminant exposure concentration (DEC-I) of toluene in air for exposure and non-exposure from individual samples at 5% levels of significance on one-tailed test by reference to the individual specimens (Fig. 1A).

When calculating with confidence limits, the confi-

Table 1 Correlation coefficients and regression equations between toluene concentrations in the breath-zone air of workers and urinary biomarker levels. And the indicators for exposure concentration from biomarker levels including discriminant exposure concentration (DEC)

	HAUCor.SG: Sin	TOLUAct: Mix	HAU
Cor.CR: Mix			
Exp-Tol-conc ($m \pm SD$)	12.2 ± 10.9 ppm	33.54 ± 22.53 ppm	33.54 ± 22.53 ppm
Exc-conc. ($m \pm SD$)	0.522 ± 0.351 (g/l)	81.27 ± 62.20 (μg/l)	1.155 ± 0.610 (g/gCr)
Cor coef. (*r*)	0.66	0.95	0.94
t. test (*r*)	$p < 0.01$	$p < 0.01$	$p < 0.01$
Reg.equa. $x =$ ppm	$0.262 + 0.0214x$	$-6.92 + 2.63x$	$0.303 + 0.254x$
INDICATOR			
From fiducial lines of individuals (-I)			
DEC-I (ppm)	42.5 (43.1)	28.8 (27.4)	33.2 (33.7)
DTL-I (ppm)	7.68 (5.86)	22.8 (21.9)	18.7 (15.8)
DEC-I/TLV	0.85	0.58	0.66
DTL-I/TLV	0.15	0.46	0.37
From confidence lines of regression equation (-R)			
DEC-R (ppm)	6.13 (6.67)	11.7 (11.8)	13.2 (13.3)
DTL-R (ppm)	36.6 (35.6)	42.6 (41.3)	41.8 (39.8)
DEC-R/TLV	0.12	0.24	0.26
DTL-R/TLV	0.73	0.85	0.84
D-DEC-I (ppm)		(57.5)	
(DTL-R)-(DEC-R)	30.5 (28.9)	30.9 (29.5)	28.6 (26.5)
BT	1.32	124.8	1.57
BTL-R	1.14	109.8	1.44
BTL-I	0.85	87.2	1.16

The values of indicators in parenthesis were measured levels using the scale in Fig. 1. HAUCor.SG: Sin, Hippuric acid concentrations corrected for a specific gravity of urine (1.024) in the urine of workers exposed to toluene singly. TOLUAct: Mix, Actual concentrations of toluene in the urine of workers exposed to toluene gas in mixed solvents. HAUCorr.Cr: Mix, Hippuric acid concentration corrected for creatinine in urine of workers exposed to toluene gas in mixed solvents. Cor.Coef, Correlation Coefficient, Reg.equation, Regression equation $y=a'+b'x$ ($x =$ toluene concentration and $y =$ concentration of toluene excretions in urine).

DEC-I and DEC-R, Discriminant exposure concentration from individual samples and from the regression equation; DTL-I and DTL-R, Lower discriminant value of TLV exposure from individual samples and from the regression equation; D-DEC-I, Discriminant concentration for DEC-I exposure and non-DEC-I exposure; BT, Biomarker level corresponding to TLV of solvent. Numbers of workers, $n = 79$ in the single-exposure group and $n = 14$ in the mixed exposure group. The TLV and OEL of toluene were 50 ppm (2006) as adopted and documented by ACGIH during the year 1992–2006, and proposed by the Japan Society of Occupational Health during the year 1994–2006.

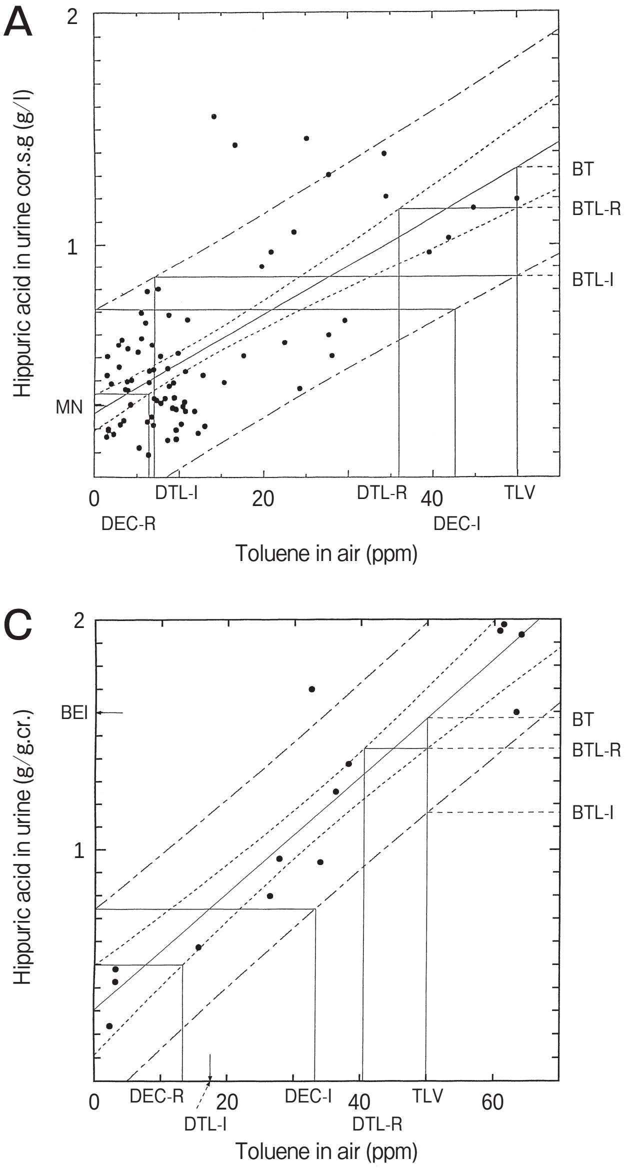

Fig. 1 Regression lines and confidence and fiducial lines between concentrations of toluene in breathing zone air and concentrations of corresponding urinary biomarkers of workers exposed to toluene alone or toluene in mixed solvents. **A**, Concentration of toluene in air versus concentrations of hippuric acid in urine corrected with a specific gravity of urine (1.024). Workers had a single exposure to toluene. The symbol MN is the mean of the normal value from non-exposed persons; **B**, Concentrations of toluene in air versus concentrations of toluene in urine. Workers were exposed to mixed solvents containing toluene; **C**, Concentrations of toluene in air versus concentration of hippuric acid in urine corrected for creatinine. Workers were exposed to mixed solvents containing toluene.

The thick line is a regression line, the thin dotted lines represent the 90% confidence range of the regression line and the chain lines represent the 90% fiducial line of individual samples, respectively. ① The symbols of DEC-I and DEC-R on the abscissa are the discriminant exposure concentrations of exposure from non-exposure using the fiducial and confidence lines, respectively. ② The symbols of DTL-I and DTL-R are the discriminant TLV exposure and non-TLV exposure in the lower TLV concentration from the fiducial and confidence lines, respectively. ③ The symbols of D-DEC-I in Fig. 1B are the discriminant DEC-I exposure and non-DEC-I exposure concentrations in the higher DEC level. ④ The symbols of BT, BTL-R, and BTL-I are the concentrations of biomarkers corresponding to 50 ppm TLV of toluene (ACGIH in 2006) and /or 50 ppm OEU (Occupational exposure unit) of toluene (Japan Association of Industrial Health in 2006) in the regression line, its lower confidence line and lower fiducial line, respectively. The symbol of BEI in Fig. 1C is the biological exposure index of hippuric acid (1.6 g/g creatinine) with notation; background and nonspecific value, documented by ACGIH (American Conference of Governmental Industrial Hygienist) in 2006.

dence lines were used instead of fiducial lines. A straight line paralleled to the x axis was also drawn from the upper confidence line of regression equation ($x = 0$) to the lower confidence line and the concentration of the x axis corresponding to the vertex crossing the lower confidence line was used as another discriminant exposure concentration (DEC-R) for exposure or non-exposure by reference to the confidence ranges of the regression line (Fig. 1A).

The values of DEC-I and DEC-R between toluene and hippuric acid in workers exposed to toluene alone were measured by a scale and then computed to be 42.5 and 6.13 ppm, respectively (Table 1).

2) Workers exposed to toluene in mixed solvents:

Measurements of DEC-I and DEC-R were similar to those of indicators obtained from the case of exposure to toluene alone as described above.

The indicators DEC-I and DEC-R were computed from the regression equation and its confidence and fiducial ranges between concentrations of toluene in air and concentrations of toluene in urine (Fig. 1B), *i.e.*, 28.8 ppm for DEC-I and 11.7 ppm for DEC-R were obtained (Table 1).

The DEC-I and DEC-R are calculated from regression line and its ranges between intensity of toluene and concentrations of urinary hippuric acid corrected by creatinine in workers exposed to toluene in mixed solvents (Fig. 1C) *i.e.*, 33.2 ppm of DEC-I and 13.2 ppm of DEC-R were obtained (Table 1).

These values of DEC-I and DEC-R in Fig. 1 A, B and C were measured by a scale and then computed by the newly devised equations as will be described later.

2. Discriminant concentration for TLV exposure and non-TLV exposure at a fixed level of error from biological specimens in the lower TLV levels.

In order to measure the available concentrations of toluene estimated from toluene biomarkers, TLV exposure or non-TLV exposure at $x = \mathrm{TLV}$ is required beside exposure or non-exposure at $x = 0$. In the case of estimating TLV exposure from concentrations of biomarkers, following indicator is required.

1) Workers exposed to toluene alone: When calculating with fiducial ranges of individuals, on the regression line, and fiducial lines of individual specimens between toluene concentration and hippuric acid concentration in Fig. 1A, a straight line paralleled to the x axis at TLV was drawn from the lower fiducial limit of individual specimens of the threshold limit value ($x = \mathrm{TLV}$) of ACGIH [10] and/or OEL of JSOH to the upper fiducial line, and the concentration of the x axis corresponding to the vertex crossing the upper fiducial line was used as an indicator, which is the discriminant solvent concentration in air for TLV exposure and non-TLV exposure in the lower TLV level (DTL-I) in lower TLV level at a 5% level of error in one tail (Fig. 1A).

When calculating using confidence ranges of regression equation. the confidence lines were used instead of fiducial lines. A straight line also paralleled to the x axis was also drawn from the lower confidence line of the regression equation ($x = \mathrm{TLV}$) to the upper confidence line and x axis corresponding to the vertex crossing the upper confidence line was used as another indicator which was considered to discriminate between TLV exposure and non-TLV exposure in the lower TLV side (DTL-R) by referring to the ranges of the population regression line. The values of the indicators DTL-I and DTL-R were calculated to be 7.68 and 36.6 ppm, respectively.

2) Workers exposed to toluene in mixed solvents: When calculating with fiducial ranges of individuals: indicator DTL-I was calculated using fiducial lines of the regression equation between the concentration of toluene in air and the concentrations of toluene or hippuric acid in urine (Fig. 1B, C and Table 1).

When calculating with confidence ranges of the regression equation, the indicator DTL-R was calculated using confidence ranges instead of fiducial ranges used for measurements of DTL-I described above (Fig. 1 and Table 1).

3. Available range of exposure concentration estimated from biomarkers.

1) Available range of exposure estimated from DTL-R and DEC-R: The range of exposure concentrations from the level of DTL-R to the lower side of DEC-R level (DTL-R minus DEC-R or DTL-R～DEC-R) indicates the available exposure range, which is estimated from biomarker concentrations using the confidence range of the population regression equation (Fig. 1A B and C). The range can be calculated under the assumption that the level of DTL-R is higher than the level of DEC-R (Table 1).

2) Available range of exposure estimated from DTL-I and DEC-I: In the present study of hippuric acid or toluene concentrations in the urine of workers exposed to toluene (Fig. 1A, B and C), the level of DTL-I is smaller than that of DEC-I, therefore making it difficult to calculate the available range from the fiducial range of individual specimens in this study.

4. Discriminant solvent concentration (D-DEC-I) for DEC exposure from non-DEC exposure at a fixed level of error from biological specimens in higher DEC levels, including estimation of DECMN-I.

The indicator D-DEC-I can be taken as the discriminant concentration for DEC-I exposure and non-DEC-I exposure at the higher DEC-I side. The upper fiducial limit of individuals at $x = \text{DEC}$ is equal to the lower fiducial limit of $x = \text{D-DEC-I}$. The indicator D-DEC-I is not related to TLV for calculation and values can be compared with TLV.

On the regression line and fiducial lines of individual samples, a straight line parallel to the x axis was drawn from the upper fiducial limit of individual samples of DEC to the lower fiducial line of the individual specimens, and the concentrations on the x axis corresponding to the vertex crossing the lower fiducial line were used as D-DEC-I (Fig. 1B). The value of D-DEC-I using urinary toluene as a biomarker (Fig. 1B) was measured to be 57.5 ppm of toluene gas. The symbol x_{DN} is exposure concentration corresponding to (y) mean of normal value (MN) by regression equation. The DECMN-I is discriminant concentration for x_{DN} exposure and non-x_{DN} exposure. in higher x_{DN} level from biomarkers and obtained as x_2 value from x_1 when x_1 is x_{DN} value in Fig. 2 (see, Footnote of Table 2).

5. The ratios of indicators for the threshold limit values (2006).

DEC-R, DTL-R and DEC-R～DTL-R were divided by the threshold limit value in 2006 and got the indicators DTL-I/TLV, DTL-R/TLV and DTL-R～DEC-R/TLV which are listed in Table 1. Indicators are useful for biomarkers among solvents having different TLV. The level of TLV of industrial chemicals is checked by committee yearly.

Biomarker concentrations corresponding to TLV exposure and their ranges. Concentrations of biomarkers on the regression line ($y = \text{BT}$) corresponding TLV ($x = \text{TLV}$) in 2006, the lower confidence range of the regression equation (BTL-R) and the lower fiducial ranges of individual specimens (BTL-I) should be taken into consideration with reference to the confidence and fiducial ranges of the regression equations (Fig. 1A, B and C). The y value of the lower fiducial range of individual specimens (BTL-I) was first reported by Ogata in 1984. In 1986 we described the indicators BTL-I and BTL-R [6] and in 1987 Ghittori *et al.* described the indicator BTL-R [11]. The indicators BTL-I and BTL-R are available for the biomarker levels corresponding TLV, referred to ranges of regression lines. When the mean concentration of the biomarkers (BT) is different from their biological exposure indices (BEI), BEI is inserted into the regression line and its lower confidence and fiducial values of regression line will be useful as approximate values in the case of evaluation using BEI. The definitions of indicators are listed in Table 2.

Newly devised equation for calculating concentrations of toluene in air that can discriminate between exposure and non-exposure (DEC-I or DEC-R) and between TLV exposure and non-TLV (DTL-I and DTL-R) exposure based on the urinary excretions in workers exposed to toluene. The devised equations were described in detail in Appendix A and B. Explanations of the abbreviations used in this study were provided in the beginning of Appendix. Examples of the order of calculation were given in Appendix C.

In Appendix A, formulas for determining the discriminant exposure concentration of DEC-I(x_{DI}), and DEC-R(x_{DR}), and in Appendix B, general formulas for calculating DEC-I(x_{DI}), DEC-R(x_{DR}), D-DEC-I(x_{DIILV}) and DTL-I(x_{DITLV}) were described. Principals of equations referred to Fig. 1A, B and C are following.

The formulas used to calculate DEC-I, DEC-R, BTL-I, BTL-R were derived as follows. Toluene in air was used as a representative solvent and hippuric acid or toluene in urine were used as representative biomarkers as shown in (Fig. 1 A, B and C).

The concentration that discriminates between exposure and non-exposure was then calculated. The principles applied to the calculation of discriminant concentration are as follows.

In Fig. 2, x represents the exposure concentrations of industrial chemicals, and y is the concentra-

Table 2 Relationship among the indicators

Objects of Discrimination.	Confidence range of population regression line (-R).	Fiducial range for prediction of new individuals (-I).
†Exposure or Non-exposure.	DEC-R.	DEC-I.
TLV-exposure or NonTLV-exposure in lower TLV conc.	DTL-R	DTL-I
Available range.	DEC-R～DTL-R.	*
DEC or non-DEC	D-DEC-R (ne).	D-DEC-I

(ne) not examined. *DEC-I～DTL-I was a negative value in the present cases. †DECMN-I: mean of the normal value from the non-exposed group (Fig. 1A) is inserted into the regression line and its x value (x_{DN})$^{1)}$ is obtained. Symbol $x = x_{DN}$ was used instead of x = DEC-I x value (x_{DN}) in the method for calculating D-DEC-I from DEC-I (Fig. 1B) with fiducial lines, and then DECMN-I$^{2)}$ was obtained. Similarly, DECMN-R is obtained using confidence lines.

1) The exposure concentration corresponding to MN was expressed as x_{DN} and obtained by formula of x_{DN} = (MN-b) / a, which was calculated from regression equation y = ax + b between the concentrations of solvent (x) and of its biomarkers (y). The mean was used as a stable indicator. 2) For determining DECMN-I using Fig. 2, the method from x_1 (x_{DN}) to x_2 (DECMN) by way of $x_1 \rightarrow y_1 \rightarrow [x_1 \rightarrow x_2] \rightarrow y_2 \rightarrow x_2$ was appled. And levels between x_{DN} and DECMN can be estimated x_{DN} exposure from biomarkers at fixed level of error. The approximate value of DECMN-I was DEC + x_{DN} (or DEC-x_{DN}, in MN < b) when x_{DN} value was not so high (see DECMN-I in Results. 4. D-DEC-I).

tion of biomarkers derived from industrial chemicals.

1) The principals of calculation of DEC-I and DEC-R ($x_1 \rightarrow x_2$):

(1) Calculation of DEC-I.

From the figure containing the regression line and its fiducial lines, the following formulas is devised with.

①{the upper fiducial line of regression line (y value) at x = 0 } is equal to

②{the lower fiducial line (y value) at x = DEC-I}.

This leads to the following equation:

①{(the y value at x = 0) plus (fiducial range at x = 0)} is equal to

②{(the y value of regression equation at x = DEC-I minus (fiducial range at x = DEC-I}

(2) Calculation of DEC-R.

In the formula of the regression equation and its confidence and fiducial ranges, the formula of confidence ranges was used instead of the formula of fiducial ranges used to calculating DEC-I, and then the formula estimating DEC-R was estimated.

2) Calculation of DTL-I and DTL-R: We next calculated the concentrations of solvents capable of discriminating between TLV-exposure and non-TLV exposure at the fixed level of error in the lower TLV level.

Theory for calculation of discriminate concentration for TLV exposure (DTL-I and DTL-R) were

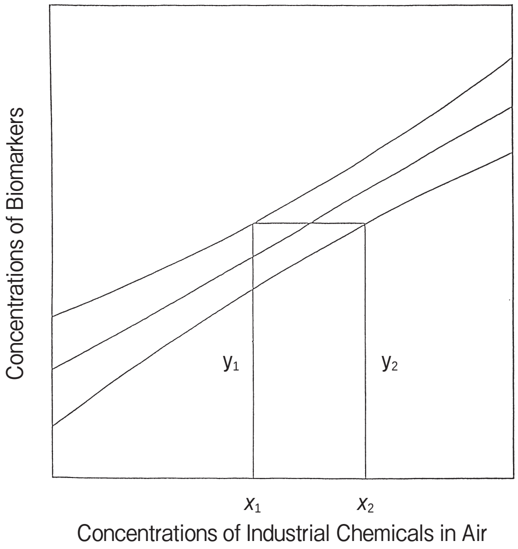

Fig. 2 Graphic explanation of the representative equation (4') in the Appendix B: One example of a regression line and confidence or fiducial lines between concentrations of industrial chemicals in air and concentrations of biomarkers. The value of y_1 on the left side of the equation is expressed by the equation y_1 = regression equation + confidence (or fiducial) range, and the value of y_2 on the right side of the equation is expressed by the equation y_2 = regression equation-confidence (or fiducial) range. The value of y_1 is equal to the value of y_2; therefore, Equation (4') can be written as follows;

$$\hat{a}x_1 + \hat{b} + t_{n-2}(\alpha)\gamma(x_1) = \hat{a}x_2 + \hat{b} - t_{n-2}(\alpha)\gamma(x_2) \qquad (4')$$

Equation (4') in the item of Appendix can be used in the cases from x_1 to x_2 and from x_2 to x_1.

shown in Fig. 1A, B and C ($x_2 \rightarrow x_1$).

In the figures,

x = exposure concentration of solvents;

y = concentration of biomarkers derived from solvents.

(1) Calculation of DTL-I.

In the figure of the regression line and its fiducial lines, following formulas is recognized.

①{the lower fiducial line of regression line (y value) at x = TLV } is equal to

②{the upper fiducial line (y value) at x = DTL-I.}.

Then following equation is recognized.

①{(the y value in regression equation at x = TLV) minus (fiducial range at x = TLV)} is equal to

②{(the y value of regression equation at x = DTL-I) plus (fiducial range at x = DTL-I)}

(2) Calculation of DTL-R

In the formula of regression equation and its confidence and fiducial ranges, the formula of confidence ranges were used instead of the equation of fiducial ranges for DTL-I, and then the formulas estimating DTL-R was obtained.

Discussion

In order to reverse-estimate of the exposure concentration from the biomarker concentration, the presence of individual differences must be considered.

In this study, several indicators based on personal differences have been improved for the application of reverse estimation to biological monitoring.

Selection of an appropriate evaluation method is necessary for the biological monitoring of workers exposed to industrial chemicals such as solvents.

The indicator DEC-I is useful for the evaluation of biomarkers of industrial chemicals found in individual specimens, because DEC-I was calculated from fiducial line which was used to predict new specimens with an x axis of known value. Thus DEC-I was used as the concentration of solvents and other industrial chemicals in the air with the ability to discriminate between exposure and non-exposure at a fixed level of error.

In addition, confidence bands define a region within which the population regression lines lie. Then we use DEC-R as indicators focused on the range of the regression line at a given level of error. In the present study, we obtained accurate DEC-I or DEC-R estimates by using a quadratic equation involving fiducial or confidence ranges of equation at fixed error.

In the previous method [1-4], discriminant exposure concentrations under at any level of error, fiducial lines of a regression equation at any level of error were depicted and then the discriminant concentration was measured by a scale using the regression line and its fiducial lines.

The present method also simplified the previous method for determining the discriminant concentrations by equations at any level of discriminant errors, without depicting fiducial lines as in the previous method. The method also gave a more accurate result than the previous one.

In the present experiment, the new indicators DTL-I and DTL-R were studied for their potential use in estimating TLV exposure from the concentrations of biomarkers. The discriminant exposure concentrations for TLV exposure and non-TLV exposure in lower TLV level (DTL-I) referred to individual specimens at any levels of errors and DTL-R was referred to the region of population regression. The results indicating that range of regression equation or individual specimens should be taken into consideration estimating TLV exposure from the concentrations of biomarkers.

The discriminant exposure concentrations and the ratios of these concentrations to the TLV (DEC-I / TLV or DEC-R/TLV) should serve as excellent indicators for the selection of appropriate method on measuring the biomarkers in the biological specimens from workers exposed to solvents that have different TLV concentrations in the case of biological monitoring.

It should be noted that the regression equation and its fiducial ranges used in this study were generally applied by using the least square method as described by Snedecor [5]. This method can be used for either small or large number of cases, as reported previously [1-4].

It has been reported that the value of standard deviation tend to increase with the increase in mean value of biomarker concentration in a group of workers when workers are classified into the several groups by the levels of exposure concentrations [6].

In these cases, group mean (average) method of Weisbrote [7] could be useful, though it requires a relatively large number of workers. In the case of method of Weisbrot [7], workers were classified into several groups based on the concentration of toluene to which they were exposed and the concentrations of toluene metabolites and the concentration of toluene in urine. In each group the mean value, the confidence limit of mean and rejection limit of the individual samples were calculated. Then a regression equation between the solvent concentration and the mean value of urinary biomarkers in each group, that between the solvent concentration and the confidence ranges of excretions in group and that between solvent concentration and the rejection limits of biomarkers in group were calculated by the least square method [7]. In these cases, the indicators (DECG-I, DECG-R, DTLG-I and DTLG-R) can be calculated using each regression equation without measuring indicators from regression line by means of a scale. For example, in the case of the reference [6], the regression equation between toluene concentration (x) and upper rejection limit of hippuric acid concentration (y) in the groups was $y = \boldsymbol{a}_U + \boldsymbol{b}_U x$, ($y = 0.66 + 0.021x$) and that of between the toluene concentration and the lower rejection limit of hippuric acid in the groups was $y = \boldsymbol{a}_L + \boldsymbol{b}_L x$, ($y = -0.24 + 0.0199x$). Then $\boldsymbol{a}_u = \boldsymbol{a}_L + \boldsymbol{b}_L x_{DG}$, $x_{DG} = (\boldsymbol{a}_U - \boldsymbol{a}_l)/\boldsymbol{b}_L$ and x_{DG} (DECG-I) indicated DEC-I was calculated by group mean method [7]. Using equation of $x_{DG} = (0.66 + 0.24)/0.0199$, the value of $x_{DG} = 45.2$ ppm was obtained by the use of rejection lines. In the case using confidence lines instead of rejection lines, DECG-R can be obtained.

The urinary hippuric acid concentration corrected by specific gravity of 1.024 for 50 ppm toluene alone is 1.34 g/l in the present report, which is less than 1.6 g/l of hippuric acid as described previously [8].

Toluene concentration in the urine of workers who were exposed to 50 ppm of toluene was 124.6 μ g/L in the mixed exposure trial in this study, which is a little higher than the value of 110 μg/L by Ghittori *et al.* [11]. These findings suggest no pronounced effect was observed by either single or mixed exposure.

In the present study, the urinary hippuric acid concentration for 50 ppm toluene exposure was calculated to be 1.57 g/g creatinine from a regression equation between toluene concentration in mixed solvent and hippuric acid concentration in urine. The value was a little higher level of 1.4 g/g creatinine of hippuric acid for 50 ppm toluene in the previous report [2] and slightly higher than the level of hippuric acid (1.5 g/g creatinine) calculated from the regression equation report by De Rosa *et al.* [12]. The biological exposure indices (BEI) for hippuric acid for 50 ppm of toluene of TLV documented by the American Conference of Industrial Hygienists (ACGIH) is 1.6 g/g creatinine and this value included the notation having background levels and nonspecific (10). The value of the hippuric acid in the present study was similar to the values reported by other scientists, which belongs to the distribution land 2 in the legal biological monitoring.

In the biological monitoring of mixed exposure, an additive effect has been expected in the lower levels of exposure to solvents such as central nerve system depressants [13], and our results in concentration of toluene excretions from workers exposed to toluene in mixed solvents were not substantially different compared to the intensity of excretions of workers exposed to toluene alone.

Mixed solvents used in this study were composed of toluene, isopropyl alcohol, and methyl ethyl ketone. Other solvents than toluene in the mixture will be described in a future report.

The threshold limit value (TLV) of toluene documented by ACGIH was changed from 100 ppm to 50 ppm in 1992–1993, and that by the Japan Society for Occupational Health was changed from 100 ppm to 50 ppm in 1994. The TLVs were based on personal exposure and the legal administered concentrations in the air of workplaces were then changed to 50 ppm.

More detailed evaluations of biological monitoring will be needed in the near future.

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Appendix

Explanation of abbreviations
(xi, yi): sample data
x: mean of x
y: mean of y
Sxx: sum of squares of deviations of x
Sxy: sum of products of deviations of x and y
Syy: sum of squares of deviations of y
y = ax + b: regression line
$t_{n-2}(\alpha)$: critical value for t statistic at level α with (n − 2) degrees of freedom.
Ve: sample standard deviation of regression line
$r_I(x)$: standard error of observation y
$r_R(x)$: standard error of mean observation y

A. Formula of determining the discriminant exposure concentration on the abscissa x_{DI}. Based on the sample data of (x_i, y_i) for $i = 1, 2, \Lambda, n$, the regression equation can be written as follows:

$$y = \hat{a}x + \hat{b}$$

where

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i,\ \bar{y} = \frac{1}{n}\sum_{i=1}^{n} y_i$$

$$S_{xx} = \sum_{i=1}^{n}(x_i - \bar{x})^2,\ S_{xy} = \sum_{i=1}^{n}(x_i - \bar{x})\,(y_i - \bar{y}),\ S_{yy} = \sum_{i=1}^{n}(y_i - \bar{y})^2$$

$$\hat{a} = \frac{S_{xy}}{S_{xx}},\ \hat{b} = \hat{y} - \hat{a}\bar{x}.$$

If the $100\alpha\%$ point in t − distribution with df $n-2$ is $t_{n-2}(\alpha)$, then the fiducial interval of 100 $(1-\alpha)\%$ of y that corresponds to an arbitrary given value x_0 is

$$\hat{a}x_0 + \hat{b} \pm t_{n-2}(\alpha) r_I(x_0),$$

where the function of $r_I(x)$ as follows:

$$r_I(x) = \sqrt{\left\{1 + \frac{1}{n} + \frac{(x - \hat{x})^2}{S_{xx}}\right\} V_e}\ ,$$

$$V_e = (S_{yy} - {S_{xy}}^2/S_{xx})/(n-2), \tag{1}$$

and the confidence interval of $100(1-\alpha)\%$ of y that corresponds to an arbitrary given value x_0 is

$$\hat{a}x_0 + \hat{b} \pm t_{n-2}(\alpha) r_R(x_0),$$

where the function $r_R(x)$ is defined by

$$r_R(x) = \sqrt{\left\{\frac{1}{n} + \frac{(x - \hat{x})^2}{S_{xx}}\right\} V_e}\ . \tag{2}$$

The discriminant exposure concentration on the abscess x_{DI} is calculated under the condition that the upper limit of the fiducial range of the regression equation at $x_0 = 0$ is coincident with the lower limit of the fiducial range of x_{DI} at $\alpha\%$ of the error.

The equation can then be written as follows.

$$(\hat{a}x_0 + \hat{b})_{x_0=0} + t_{n-2}(\alpha) r_I(0) = (\hat{a}x_0 + \hat{b})_{x_0=x_{DI}} - t_{n-2}(\alpha) r_I(x_{DI})$$

After subtracting $\hat{b}$ from both side formulas from the formulas on both sides of the above equation, both formulas are squared. Considering x_{DI} is not equal to zero, the following expression for x_{DI} is obtained:

$$x_{DI} = 2 \cdot \frac{\hat{a} r_I(0) t_{n-2}(\alpha) - \bar{x} t_{n-2}(\alpha)^2 V_e/S_{xx}}{\hat{a}^2 - t_{n-2}(\alpha)^2 V_e/S_{xx}}. \tag{3}$$

Formula of discriminant exposure concentration from the regression equation and its confidence ranges of the abscess x_{DR} .

In the equation (2), using $r_R(x)$ defined by (4) instead of $r_I(x)$, the abscess x_{DR} is given by

$$x_{DR} = 2 \cdot \frac{\hat{a} r_R(0) t_{n-2}(\alpha) - \bar{x} t_{n-2}(\alpha)^2 V_e/S_{xx}}{\hat{a}^2 - t_{n-2}(\alpha)^2 V_e/S_{xx}}. \tag{4}$$

B. General formula for x_{DI} and x_{DR}

The condition that the upper limit of fiducial (or confidence) range of the regression equation at x_1 is coincident with the lower limit of fiducial (or confidence) range at x_2, is

$$\hat{a}x_1 + \hat{b} + t_{n-2}(\alpha) r(x_1) = \hat{a}x_2 + \hat{b} - t_{n-2}(\alpha) r(x_2), \tag*{(4)'}$$

where the function $r(x)$ is given by $r_I(x)$ for the fiducial range and given by $r_R(x)$ for the confidence range.

The above equation is reduced to

$$\hat{a}(x_2 - x_1) - t_{n-2}(\alpha) r(x_1) = t_{n-2}(\alpha) r(x_2), \tag{5}$$

and also reduced to

$$\hat{a}(x_1 - x_2) + t_{n-2}(\alpha) r(x_2) = -\,t_{n-2}(\alpha) r(x_1), \tag{6}$$

Squaring both sides of equation (5) and considering that x_1 is not equal to x_2, the expression for x_2 with x_1 is obtained as follows:

$$x_2 = \frac{2(\hat{a} t_{n-2}(\alpha) r(x_1) - \bar{x} t_{n-2}(\alpha)^2 V_e/S_{xx}) + (\hat{a}^2 + t_{n-2}(\alpha)^2 V_e/S_{xx}) x_1}{\hat{a}^2 - t_{n-2}(\alpha)^2 V_e/S_{xx}}. \tag{7}$$

Similarly, from equation (6), the expression for x_1 with x_2 is obtained as follows: These relations are held for both the case $r(x) = r_I(x)$ and the case $r(x) = r_R(x)$.

$$x_1 = \frac{2(\hat{a} t_{n-2}(\alpha) r(x_2) + \bar{x} t_{n-2}(\alpha)^2 V_e/S_{xx}) + (\hat{a}^2 + t_{n-2}(\alpha)^2 V_e/S_{xx}) x_2}{t_{n-2}(\alpha)^2 V_e/S_{xx} - \hat{a}^2}. \tag{8}$$

Application

1) In the equation (7), in the case of $x_1 = 0$ and $x_2 = x_{DI}$ for $r(x) = r_I(x)$ the equation (3) is obtained again.
2) In the equation (7), in the case of $x_1 = 0$ and $x_2 = x_{DR}$ for $r(x) = r_R(x)$ the equation (4) is obtained again.
3) In the equation (7), in the case of $x_1 = x_{DI}$ and $x_2 = x_{DIILV}$ for $r(x) = r_I(x)$ the following relation is derived.

$$x_{DIILV} = \frac{2(\hat{a}t_{n-2}(\alpha)r_1(x_{DI}) - \bar{x}t_{n-2}(\alpha)^2 V_e/S_{xx}) + (\hat{a}^2 + t_{n-2}(\alpha)^2 V_e/S_{xx})x_{DI}}{\hat{a}^2 - t_{n-2}(\alpha)^2 V_e/S_{xx}} \tag{9}$$

4) In the equation (8), in the case of $x_2 = x_{TLV}$ and $x_1 = x_{DITLV}$ for $r(x) = r_I(x)$, the following relation is derived.

$$x_{DITLV} = \frac{2(\hat{a}t_{n-2}(\alpha)r_1(x_{TLV}) + \bar{x}t_{n-2}(\alpha)^2 V_e/S_{xx}) - (\hat{a}^2 + t_{n-2}(\alpha)^2 V_e/S_{xx})x_{TLV}}{t_{n-2}(\alpha)^2 V_e/S_{xx} - \hat{a}^2} \tag{10}$$

C. Examples of the order of calculation

1) The example of calculation of DEC-I (x_{DI}) from the regression line and its fiducial lines of individuals ($\alpha = 0.1$) between the toluene concentration in air and toluene concentration in urine (Fig. 1B) using equation (3) in Appendix is demonstrated as follows. The values of factors in equation (3) of Fig. 1B are given as $a = 2.63$, $r_I(0) = 22$ calculated, $t_{n-2}(0.1) = 1.782$, $\bar{x} = 33.54$, $V_e = 389.9$, $S_{xx} = 6601$.

Then x_{DI} was calculated as follows:

x_{DI}: $2x(2.63 \times 22.0 \times 1.782 - 33.54 \times 1.782^2 \times 389.9/6601)/(2.63^2 - 1.782^2 \times 389.9/6601) = 28.8$ (ppm) is obtained

2) The example of calculation of DEC-R (x_{DR}) from the regression line and its confidence lines in the case of (Fig. 1B) using equation (4) in appendix A is as follows;

The value of the factor of $r_I(0) = 22$ in equation (3) is replaced by $r_R(0) = 9.69$ in equation (3). Then,

x_{DI}: $2x(2.63 \times 9.69 \times 1.782 - 33.54 \times 1.782^2 \times 389.9/6601)/(2.63^2 - 1.782^2 \times 389.9/6601) = 11.6$ (ppm) is obtained.

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