RISK EVALUATION OF TRICHLOROETHYLENE EXPOSURE AMONG WORKERS IN INDUSTRY

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ABSTRACT: The health risks of twenty five workers exposed to trichloroethylene in three factories were studied. Trichloroethylene in the breathing zone of the workers was collected for a full shift following the NIOSH method number 1022 and urine samples were also collected for analysis of trichloroacetic acid metabolite by Gas Chromatography/Mass Spectrometry. The results showed that average concentrations of trichloroethylene in the breathing zone of workers were 15.85 ppm. Thirteen workers (52%) exposed to higher trichloroethylene concentrations than the 10-ppm Threshold Limit Value-Time Weighted Average (TLV-TWA) recommended by the American Conference of Governmental Industrial Hygienists (ACGIH). The average urinary trichloroacetic acid of workers was 90.92 mg/g creatinine (Non-detectable to 349.33 mg/g creatinine). Most workers (88.0 %) had a lower concentration of urinary trichloroacetic acid than the BEI (100 mg/g creatinine) recommended by the ACGIH. Cigarette smoking (p-value = 0.028) had relationship with urinary trichloroacetic acid. There was a fairly high significant correlation between trichloroethylene in the breathing zone of workers and urinary trichloroacetic acid (r = 0.657; p-value < 0.01). This health risk assessment of trichloroethylene exposure found that the workers had excessive cancer risk when using trichloroethylene. The estimated lifetime cancer risk of trichloroethylene exceeded the range of 10-6-10-4 recommended by U.S.EPA.

Keywords: Trichloroethylene, trichloroacetic acid, risk assessment, Gas Chromatography/Mass Spectrometry

INTRODUCTION: Trichloroethylene (TRI) is a colorless liquid with a strong odor like ether1). Trichloroethylene is widely used in metal cleaner and degreaser because of its properties, such as low flammability, high solvency, non-corrosiveness, high stability, low specific heat, low boiling point2). The main route of absorption is inhalation; skin absorption occurs when contact with liquid trichloroethylene. Approximately 30 % to 50 % of trichloroethylene absorbed is excreted as trichloroethanol while 10 % to 30 % of amount taken up is excreted as trichloroacetic acid. Trichloroethanol has maximum concentration in blood and urine almost directly after exposure whereas trichloroacetic acid in blood and urine increases up to 20 to 40 hours after exposure. The half-life of trichloroethanol and trichloroacetic acid are approximately 10 to 15 hours and 80 to 100 hours, respectively3).

International Agency for Research on Cancer (IARC) and the American Conference of Governmental Industrial Hygienists (ACGIH) also classified trichloroethylene in group 2A (Probably carcinogenic to humans)4,5). The metabolites of trichloroethylene were trichloroacetic acid and trichloroethanol in urine. The sampling period of trichloroacetic acid differs from trichloroethanol in urine; trichloroacetic acid in urine was collected at end of shift and end of workweek while trichloroethanol in urine was collected at end of workweek.

The measurement of trichloroacetic acid in urine is one of several recommended biological determinations for evaluating trichloroethylene exposure, and is the best indicator of integrated exposure over the workweek. ACGIH recommended that trichloroacetic acid in urine should not exceed 100 mg/g creatinine when urine samples were collected at the end of the workweek8). The objective of this study was to assess health risk of workers exposed to trichloroethylene in industries.

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MATERIALS AND METHODS: The design of this study was a cross sectional design to evaluate the health risk of 25 workers exposed to trichloroethylene in working area and to study relationship between personal factors, working duration, concentrations of trichloroethylene in breathing zone of workers with concentrations of trichloroacetic acid in urine. This study was reviewed and approved by the Ethics Committee on Human Rights Related to Human Experimentation, Mahidol University, No. MUPH 2008-036.

Subjects
The subjects in this study were twenty five workers from three factories (factory A, B and C). The task of workers in these three factories was not much different. The factory A has eight workers; used trichloroethylene for cleaning parts of computers such as flexible printed circuits (FPC), electronic wire and semiconductor device packaging products. The factory B has eight workers; six workers used trichloroethylene for cleaning parts of computer such as computer case and two workers works in the area close to cleaning parts of computer. The factory C has nine workers; four workers used trichloroethylene for cleaning product such as LPG cylinder valves, regulators and brass fitting and five workers works in the area close to cleaning products.

Air and urine sample collection
Trichloroethylene concentrations in breathing zone of workers were collected following NIOSH method 10222. Air at breathing zone of workers was collected for full shift using a personal air sampling pump (SKC Inc., Eighty Four, Pa., USA) connected with a charcoal tube (SKC-226-01, SKC Inc., Eighty Four, Pa., USA) at a flow rate of 0.1 litters per minute. After the sampling, the charcoal tubes were capped and packed securely with plastic caps and were stored at -35°C until analysis. The air sample was collected on Monday for 8-hr full shift. Urine samples were also collected for two times at a pre-shift on Monday morning and at the end of workweek on Friday evening. The urine samples were frozen in a refrigerator at a temperature of -35°C until analysis. The subjects were also interviewed using questionnaire consisting of general characteristics, working duration, personal protective equipment used and health symptoms.

Chemicals and reagents
Trichloroethylene was AR grade from Lab scan, Ireland. Carbon disulfide, trichloroacetic acid, butyric acid, sulfuric acid, dichloromethane and methanol were purchased from Merck, Germany. Hexane was HPLC grade purchased from J.T. Baker, USA.

Instrumentation
The gas chromatograph-mass spectrometer (Gas chromatograph, Hewlett Packard 5890 Series II) equipped with mass selective detector (Hewlett Packard 5972 Series) and integrator was used. The analytical column was a HP-5 capillary column (30.00 m × 0.25 mm inner diameter). The carrier gas was helium at a flow rate of 1 ml/min. The GC condition for analysis of trichloroethylene in air was set as follows; injector, 225°C; Oven temperature, initial temperature of 35°C for 2 min; then ramped at 5°C/min to 50°C and ramped at 25°C/min to 140°C and hold for 1 min; detector, 250°C. The GC condition for analysis of trichloroacetic acid in urine was set as follows; injector, 225°C; Oven temperature, initial temperature of 40°C for 2 min; then ramped at 2°C/min to 46°C and ramped at 25°C/min to 175°C and hold for 1 min; detector, 250°C.

Preparation of solutions
Stock standard trichloroethylene (14.62 mg/ml) was prepared in CS2 by pipetting 100 µl trichloroethylene and diluted with 0.005% (v/v) ethyl benzene in CS2 to 10 ml.

A stock standard solution of trichloroacetic acid (10.0214 mg/ml) was prepared by weighing 1.0021 g trichloroacetic acid and dissolving in 75 ml methanol and diluting to 100 ml.

A stock internal standard solution of 0.5M butyric acid was prepared by pipetting 0.46 ml butyric acid and diluted to 10 ml with methanol.

The derivatization reagent for trichloroacetic acid was prepared by mixing solutions of water: conc. H2SO4: methanol at 6:5:1 by volume.

Calibration curves of trichloroethylene in air
The trichloroethylene standard solutions ranging 0.0205-0.204 mg/ml was prepared and one µl was injected to the GC/MS. The calculated
peak area ratio of trichloroethylene and ethyl benzene (internal standard) was plotted against the concentrations of trichloroethylene for three replicate determinations.

**Desorption efficiencies of trichloroethylene from charcoal tube**

The three known concentrations of trichloroethylene at 0.04971, 0.09942 and 0.1608 mg/ml were spiked onto the charcoal tube and left overnight to assure complete adsorption of chemical onto the charcoal. The trichloroethylene was extracted with one ml 0.005 % ethyl benzene in carbon disulfide. The solution was allowed to stand at least 30 min with occasional agitation. One μl of solution was injected into the GC/MS.

**Determination of trichloroethylene in air**

The front and back sorbent section of charcoal tubes were placed in separated vials. One milliliter of 0.005 % ethyl benzene in carbon disulfide was added to each vial. The vial was capped immediately. The vial was allowed to stand at least 30 min with occasional agitation. One μl of solution was injected into the GC/MS.

**Determination of trichloroacetic acid in urine**

The determination of trichloroacetic acid was carried out according to the published method [7] with modification. The 200-μl urine samples were pipetted into screw cap tubes (the clear portion of urine is used), 50 μl of internal standard solution (0.01 M butyric acid), 100 μl of methanol and 500 μl of the derivatizing reagent (H_2O: CH_3OH: CH_3COOH = 6:5:1) were added respectively. The solutions were heated at 70 °C for 10 min. After cooling to room temperature, 500 μl of hexane-dichloromethane (1:1) was added to the mixture and rotated for 15 min after that centrifugation at 600 g for 5 min. The 1 μl organic solution was injected into the GC/MS.

**Calibration curve of trichloroacetic acid in urine**

The working standard trichloroacetic acid at concentrations of 0.008015, 0.1253, 0.2505, 0.3758 and 0.5011 mg/ml urine were analyzed in the same manner as urine samples. The calculated relative peak area ratio of methyl ester of trichloroacetic acid and methyl ester of internal standard was plotted against the concentrations of trichloroacetic acid of three replicate determinations.

**Accuracy and precision of the method**

The known concentrations of trichloroacetic acid were prepared at concentrations of 0.05, 0.258 and 0.416 mg/ml urine. The solutions were analyzed in the same manner as urine samples. The percent recovery and coefficient of variations of three replicate determinations were calculated for between assays.

**Detection limit of the method**

Trichloroacetic acid standard solution were prepared at 1.603, 3.206, 4.809, 6.412 and 8.015 μg/ml urine and then analyzed. The detection limit of the method is the lowest trichloroacetic acid concentrations that can be detected [8].

**Determination of urinary creatinine**

Urinary creatinine concentrations were determined by the kinetic Jaffe'colorimetric method using picric acid [9].

**Health risk assessment**

The chronic daily intake [CDI] [10] of trichloroethylene through an inhalation route was calculated as follows:

\[
\text{CDI (mg/kg-day)} = \frac{CA \times IR \times ET \times EF \times ED}{(BW \times AT)}
\]

where:

- **CA** = Chemical concentration in air (mg/m³)
- **IR** = Inhalation rate (m³/hr)
- **ET** = Exposure time (hr / 24 hrs)
- **EF** = Exposure frequency (day / year)
- **ED** = Exposure duration (years)
- **BW** = Average body weight (kg)
- **AT** = Averaging time (period over which exposure is averaged in days)

The U.S. Environmental Protection Agency (EPA) classified trichloroethylene in group 2B/C: Probable human carcinogen.

**Statistical analysis**

The statistical analyses were performed with SPSS version 11.5. The relationship between qualitative variables and urinary trichloroacetic acid was illustrated by chi-square test. The relationships between quantitative variables and urinary trichloroacetic acid were illustrated by Spearman rank correlation.
RESULTS:

Calibration curve of trichloroethylene in air

The calibration curve of trichloroethylene ranging from 0.0205 to 0.204 mg/ml gave a linear relationship, $y = 7.736x - 0.0174$ with correlation coefficient of 0.9998. Where $y =$ peak area ratio of trichloroethylene and ethyl benzene (internal standard) plotted against the concentrations of trichloroethylene.

Desorption efficiency of trichloroethylene from charcoal

The desorption efficiency of spiked trichloroethylene at 49.71, 99.42 and 160.8 μg ($n=3$) gave trichloroethylene recovery at 93.7, 94.94 and 94.94 %, respectively.

Calibration curve of trichloroacetic acid in urine

The calibration curve of trichloroacetic acid ranging from 0.0080 to 0.5011 mg/ml urine displayed a linear relationship, $y = 3.1445x + 0.0288$. Where $y =$ peak area ratios of methyl ester of trichloroacetic acid/methyl ester of butyric acid (internal standard) and $x =$ trichloroacetic acid concentrations. The correlation coefficient of standard curve was 0.9994 ($n=3$). The method can detect trichloroacetic acid at 0.49 μg/ml urine.

Accuracy and precision of the method

The accuracy of the method for determining trichloroacetic acid at concentrations of 0.05, 0.258 and 0.416 mg/ml urine were 97.07 to 100.90% of recovery and the coefficients of variations between days were in the range of 1.75 to 3.35 %.

General characteristics of workers

Most workers were male (80.0 %) and their average age was 27.56 years old (Table 1). The average body mass index was 21.81 kg/m² and most of them (72.0 %) were in the range of normal body mass index. Eighty percent of workers drank alcohol and 68% were smoker. The average number of cigarette smoke per day was five. Eighteen workers worked in the cleaning section (72.0 %) and seven workers worked in the area close to the cleaning product area (28.0 %). The average working duration was 216.8 (SD=207.57) weeks. The results from the interview found that 24.0 % of workers used mask, 44.0 % used respirator, 72.0 % used cotton gloves, 68.0 % used rubber gloves and 84.0 % used safety glasses.

Health symptoms

Workers reported health symptoms during the week of sample collection. The first three highest symptoms reported were headache (32.0 %), dizziness (28.0 %) and sleepiness (24.0 %) (Table 2).

Concentrations of trichloroethylene in breathing zone of workers

The average trichloroethylene concentrations in breathing zone of workers in three factories are presented in Table 3. Workers in factory C had highest trichloroethylene exposure followed by factories B and A. The average trichloroethylene exposure concentration of workers in cleaning product areas in these three factories was 19.19 ppm or 103.62 mg/m³, whereas the average trichloroethylene exposure concentrations of workers in the neighboring areas were 6.99 ppm or 37.75 mg/m³. According to ACGIH, the recommended TLV-TWA is 10 ppm. The twelve workers exposed to trichloroethylene below the TLV-TWA ranging from 0.87 to 9.95 ppm with a mean of 7.02 ppm. Thirteen workers over exposed to trichloroethylene concentration ranging from 11.31 to 71.44 ppm with a mean of 19.28 ppm.

Concentration of trichloroacetic acid in urine of workers

The result showed that trichloroacetic acid was not detected in pre-shift urine of seventeen workers; eight workers had trichloroacetic acid ranging from 8.31 to 51.90 mg/g creatinine with an average of 20.64 mg/g creatinine. The trichloroacetic acid at the end of workweek was not detected in urine of fifteen workers; whereas ten workers had urinary trichloroacetic acid concentration ranging from 10.80 to 349.33 mg/g creatinine and the average concentration of 90.92 mg/g creatinine. Three out of ten workers had urinary trichloroacetic acid more than 100 mg/g creatinine, recommended Biological Exposure Indices (BEI) by ACGIH.

The relationship between risk factors and trichloroacetic acid in urine

The results showed that cigarette smoking was associated with urinary trichloroacetic acid with
a p-value of 0.028. The number of symptoms reported had relationship with urinary trichloroacetic acid with a p-value of 0.005. The relationship between personal protective device used and urinary trichloroacetic acid found that personal protective devices were not associated with urinary trichloroacetic acid (p-value >0.05). The Spearman rank correlation was used to determine relationship between concentrations of trichloroethylene at breathing zone and urinary trichloroacetic acid. The correlation coefficient of trichloroethylene at breathing zone and urinary trichloroacetic acid was 0.657 at p <0.01 (Figure 1).

Health risk assessment

The health risk assessment was calculated for two groups of workers; the first group worked in the cleaning product area and the other group worked in neighboring area.

Workers in the cleaning product area exposed to average trichloroethylene concentrations of 103.62 mg/m³ with an average working duration of 174.44 weeks. Cancer slope factor for inhalation of trichloroethylene was 4.0×10⁻¹ (mg/kg-day)⁻¹ and reference dose (RfD) for inhalation of trichloroethylene was 1.0×10⁻² mg/kg-day⁻¹.

\[
\text{CDI} = 103.62 \text{ mg/m}^3 \times 20 \text{ m}^3/24 \text{ hr} \times 8 \text{ hr} / \text{day} \times 6 \text{ days/week} \times 174.44 \text{ weeks} \\
= 0.404 \text{ mg/kg-day}
\]

\[
\text{Excess lifetime cancer risk} = \text{Chronic daily intake} \times \text{Cancer slope factor} \\
= 0.404 \text{ mg/kg-day} \times 4.0 \times 10^{-1} \text{ (mg/kg-day)}^{-1} \\
= 0.162
\]

Non cancer risk of workers worked in the cleaning product area:

\[
\text{CDI} = 103.62 \text{ mg/m}^3 \times 20 \text{ m}^3/24 \text{ hr} \times 8 \text{ hr} / \text{day} \times 6 \text{ days/week} \times 174.44 \text{ weeks} \\
= 0.45 \text{ mg/kg-day}
\]

\[
\text{Hazard quotient} = \text{CDI/RfD} = 0.45/1.0 \times 10^{-2} = 8.45
\]

The risk for workers in neighboring areas was also calculated based on the average exposure concentrations of 37.74 mg/m³ with an average working duration of 352.71 weeks.

\[
\text{CDI} = 37.74 \text{ mg/m}^3 \times 20 \text{ m}^3/24 \text{ hr} \times 8 \text{ hr} / \text{day} \times 6 \text{ days/week} \times 352.71 \text{ weeks} \\
= 0.27 \text{ mg/kg-day}
\]

\[
\text{Excess lifetime cancer risk} = 0.27 \text{ mg/kg-day} \times 4.0 \times 10^{-1} \text{ (mg/kg-day)}^{-1} \\
= 0.108
\]

Non cancer risk of workers in the neighboring area:

\[
\text{CDI} = 37.74 \text{ mg/m}^3 \times 20 \text{ m}^3/24 \text{ hr} \times 8 \text{ hr} / \text{day} \times 6 \text{ days/week} \times 352.71 \text{ weeks} \\
= 3.08 \text{ mg/kg-day}
\]

\[
\text{Hazard quotient} = 3.08/1.0 \times 10^{-2} = 308
\]

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<tr>
<th>Table 1</th>
<th>General characteristic of workers</th>
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<td>Variables</td>
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<td>Sex</td>
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<tr>
<td>Age (year)</td>
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<td>&lt;25</td>
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<tr>
<td>25-34</td>
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<td>≥35</td>
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<td>Body mass index (kg/m²)</td>
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<td>Working duration (week)</td>
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<th>Table 2</th>
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<td>Health symptom</td>
<td>Number of workers reported (%)</td>
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<tr>
<td>Headache</td>
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<td>Dizziness</td>
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<tr>
<td>Anorexia</td>
<td>20.0</td>
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<td>Sleepiness</td>
<td>24.0</td>
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<tr>
<td>Irritation</td>
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<tr>
<td>Finger tremor</td>
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<td>Heart palpitation</td>
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<td>Irritations of the nose</td>
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<td>Irritations of the throat</td>
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<th>Table 3</th>
<th>Trichloroethylene exposure of workers</th>
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<td>Factory</td>
<td>Trichloroethylene concentration (ppm)*</td>
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<tr>
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<td>cleaning product area</td>
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<tr>
<td>A</td>
<td>3.205 ± 3.404 (n=8)</td>
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<tr>
<td>B</td>
<td>23.35 ± 2.54 (n=6)</td>
</tr>
<tr>
<td>C</td>
<td>45.33 ± 27.46 (n=4)</td>
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*Mean ± SD

The excess cancer risk of workers in the cleaning product area was 0.162 and the hazard quotient was 845. Workers in the neighboring area also had high risk of developing cancer of 0.108 and the hazard quotient was 308.

DISCUSSION: Although factory A and B produce computers and use trichloroethylene for cleaning computer parts; the trichloroethylene exposure of workers in the two factories are different due to the duration of cleaning products used. Factory A used trichloroethylene for cleaning product...
approximately twenty minutes per day; whereas workers in factory B and C used trichloroethylene for cleaning products six to eight hours per day. Therefore, workers in factory B and C had higher exposure to trichloroethylene concentrations than those in factory A. The average trichloroethylene exposure of twenty five workers was 15.85 ppm ranging from 0.87 to 71.44 ppm. Thirteen workers were exposed to trichloroethylene above the TLV-TWA of 10 ppm. The trichloroethylene concentration in this study was slightly greater than the previous studies, 37.24 to 78.21 ppm in a degreasing operation\textsuperscript{12}, 0.56 to 26.82 ppm in vapor degreasing\textsuperscript{13} and 15.51 ppm in a special printing house on glass\textsuperscript{14}.

The trichloroacetic acid was detected in urine of ten workers. Three workers had trichloroacetic acid in urine greater than 100 mg/g creatinine, the BEI recommended by ACGIH. The three workers have never used respirators during working period and they worked in cleaning product area for 6 to 8 hours per day. When compared the results with other studies found that workers of a metal cleaning detected trichloroacetic acid in urine in the range of 6.07 to 87.02 mg/g creatinine\textsuperscript{15}. The workers in an electronic industry detected trichloroacetic acid in urine ranging from 1 to 386 mg/g creatinine\textsuperscript{16}. The non-detectable urinary trichloroacetic acid was found because those workers worked for few hours with trichloroethylene and some were in the neighboring area.

The personal protective used was not associated with the urinary trichloroacetic acid excretion of workers. It is because the mask and respirator used by workers may not effective enough to protect workers due to high concentration of trichloroethylene. The factory should install a local exhaust ventilation to reduce the trichloroethylene concentration at breathing zone of workers. The correlation coefficient of relationship between trichloroethylene concentration in breathing zone of workers and urinary trichloroacetic acid was 0.657 by spearman rank correlation (Figure 1). Imbriani et al. found that the correlation coefficient of this relationship was 0.32\textsuperscript{14}. They collected the urine samples on the first day of the week, but the current study collected urine samples at the end of work week following the ACGIH. Muller et al. found that trichloroacetic acid excretion was slow in the initial period and rose rapidly later. The elimination half-life of trichloroacetic acid in urine is 100 hours\textsuperscript{17}. Ulander et al. found that the correlation coefficient of trichloroethylene and trichloroacetic acid in urine was 0.51. They collected air sample on the last day of workweek and collected urine in the next morning after the air sample collection\textsuperscript{13}. The correlation coefficients of previous studies were different from the current study because of different technique of analysis and different periods of air and urine sample collection. Most studies have low number of subjects including this study.

Health risk assessment of workers exposed to trichloroethylene was calculated using US EPA model. The subjects exposed to different levels of trichloroethylene depending on their jobs, duration of exposure to trichloroethylene and the personal protective equipment used and the area of their work. The results found excess cancer risk of workers in the cleaning product area was 0.162. The estimated lifetime cancer risk of trichloroethylene was compared to the risk management range of $10^{-6}$ to $10^{-4}$ that is generally used by EPA; it was not acceptable. It means that every 1,000 workers exposed to trichloroethylene and 162 workers will have cancer in their lifetime. It is because they exposed to high level of trichloroethylene. For non cancer hazard, the risk of workers for systemic toxicity was also very high (Hazard quotient =845) and was not acceptable.

![Figure 1](https://example.com/figure1.png)

**Figure 1** Correlation between urinary trichloroacetic acid (mg/g creatinine) and trichloroethylene (ppm), correlation coefficient = 0.657
Workers may be at risk of liver and kidney diseases. Some workers had health symptoms because of high exposure to trichloroethylene. The workers in neighboring area also had high risk of cancer and organ toxicity. The EPA guideline of human health risk was used for exposure from environment such as emission of chemical into environment. The exposure among environmental exposed human populations tends to be lower than occupational exposed human population. This group of subjects had several health symptoms due to high exposure of trichloroethylene. The workers using trichloroethylene in cleaning product should learn about the hazards. The factory should install a locally exhaust ventilation at the cleaning product area to reduce trichloroethylene below the threshold limit. The workers need to protect themselves from trichloroethylene exposure by using personal protective device, such as respirator with cartridge during working. They should clean their body immediately when chemical splashes on them.

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