THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Bioscience)

DEGREE

Bioscience — **FIELD**

Science — **FACULTY**

TITLE: Evaluation of Cytotoxicity of Occupational Exposure to Trichloroethylene in Thailand

NAME: Miss Siriporn Singthong

THIS THESIS HAS BEEN ACCEPTED BY

THESIS ADVISOR

(Associate Professor Pannee Pakkong, M.S.)

GRADUATE COMMITTEE CHAIRMAN

(Associate Professor Kiattawee Choowongkomon, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON

DEAN

(Associate Professor Gunjana Theeragool, D.Agr.)

THESIS

EVALUATION OF CYTOTOXICITY OF OCCUPATIONAL EXPOSURE TO TRICHLOROETHYLENE IN THAILAND

SIRIPORN SINGTHONG

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Bioscience)
Graduate School, Kasetsart University
2014

Siriporn Singthong 2014: Evaluation of Cytotoxicity of Occupational Exposure to Trichloroethylene in Thailand. Doctor of Philosophy (Bioscience), Major Field: Bioscience, Faculty of Science. Thesis Advisor: Associate Professor Pannee Pakkong, M.S. 172 pages.

A cytotoxicity assessment of TCE exoposed to Thai workers were studied. This research were aimed to determine the level of TCE exposure rate in environment and the cytogenetic damage in peripheral blood by micronucleus (MN) assay test. The analyzed samples collected from 242 workers-and 66 controls. Everyone was informed about the scope of this study and individually interviewed. The TCE exposure rate was analyzed in airborne sampler. The cytogenetic toxicity were analyzed the level of TCE metabolites, Trichloroacetic acid (TCA) in urine and in the peripheral blood sample. The results showed that the mean value of TCE in environment at the first and second phase of the study were within the limit level of safety standards. However, the urinary TCA was 53.5 % of exposed workers were higher than standard limit values. Six months later after the participants were educated about universal precaution in the workplace was 11.13 mg/L. The results indicated that only 25 % of the exposed group had urinary TCA higher than standard values. The genotoxic test showed that 29.4% of workers exposed to TCE had an initial DNA damage. The frequencies of micronucleus in exposed workers and control were 5.778 and 1.339 /1000 MN, respectively. Individuals with high TCA level in urine showed a higher frequency of MN. The exposed group revealed a statistically significant increase in the level of DNA damage compared with the controls ($P <$ 0.01). This studied data had showed a significant correlation between TCA level in urine and MN frequency ($r=0.127$, $p<0.01$). In addition, there is a positive correlation between TCA level and duration of work per day, education level and individual age ($p<0.01$). In conclusion, this study had demonstrated an association between exposure to TCE and DNA damage (increased MN frequency). The cytogenetic damage in workers exposed to TCE was associated with occupational exposure time, concentration of TCE, mechanism of xenobiotic secretion and life style.

_____ _____ _____/_____/_____

Student's signature Thesis Advisor's signature

ACKNOWLEDGEMENTS

I am thankful and deeply indebted to Assoc. Prof. Pannee Pakkong my thesis advisor for advice, encouragement and valuable suggestion for the completion of this thesis.

I would like to offer my special thanks to the following: Dr. Kantima Choosang for her comments and suggestion; Ms.Netnapa Chingkittiand and Ms. Sirinya Wongsanit for their kind assistance; my friends and others in Reference Laboratory and Toxicology for their assistance and the laboratory support.

A special thanks is extended to the Interdisciplinary program of Bioscience, Faculty of Science, Kasetsart University.

To my family for their suggestion, encouragement and enthusiasm during my studies and research works.

Siriporn Singthong

March 2014

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

EVALUATION OF CYTOTOXICITY OF OCCUPATIONAL EXPOSURE TO TRICHLOROETHYLENE IN THAILAND

INTRODUCTION

Trichloroethylene (TCE), a volatile organic compound (VOC), is a common industrial solvent that has been used for dry-cleaning and metal-de-greasing of fabricated metal parts and as a lubricant (Kaneko *et al.*, 1997; Maull *et al.*, 1997). Consequently due to it used in many processes, most TCE is released into the environment as a result of use from both factories and laboratories. It has been reported as major industrial pollutant that identified as environmental contaminants of ground water, surface water and soil (Westrick *et al.*, 1984; Spencer *et al.*, 2006). TCE is a well-recognized animal carcinogen (Fisher and Allen 1993; Fahrig *et al.*, 1995) and known for many toxic effects such as cardiac effect, pulmonary toxicity, neurotoxicity and probably genotoxicity. TCE can cause both acute and chronic toxicity in humans. Although several studies have demonstrated the toxicities of TCE in a variety of systems, the genotoxic and carcinogenic effects occurred in human are still arguable (Kumar *et al.*, 2009; Kligerman *et al*., 1994). Common short-term health effects of TCE expo-sure include eye, nose, throat, and skin irritation. Headaches, nausea, dizziness, fatigue and shortness of breath may occur. Some epidemiological studies have linked it with increased incidence of urinary-tract tumors and lymphomas in TCE exposed workers and with childhood leukemia. In human, exposure to TCE induce adverse effects on the central nervous system, immune system, and endocrine (hormonal) system. The people who are exposed to TCE often reported symptoms of fatigue, sleepiness, headache, confusion, and blurred vision. One of the principal TCE metabolites, trichloroacetic acid (TCA), is a specific index for TCE absorption. Although TCA levels can not represent the severity of TCE induced disorder, it is helpful for making right and timely diagnosis of dermatitis induced by TCE. It should be noted that TCA has half time of excretion, which is about 2–5 day. Inhalation is the main route of exposure to TCE while, the less common routes are ingestion and dermal contact. In occupationally exposed workers involved in the manufacture or use

of TCE, increased incidences of chromosome abnormalities such as breaks, gap, deletion and hyperploidy have been observed in lymphocytes. (Lavin *et al.*, 2000). Other studies suggested the absence of sister chromatid exchanges when the subjects are exposed to TCE. In contrast, according to the study of Nagaya *et al.* (1989), there were no incidences of sister chromatid exchanges in TCE exposed workers. Cytogenetic biomarkers such as micronucleus assay is one of the preferred methods used to study the impact of environmental, occupational and medical factors on genomic stability and to evaluate end point in human bio monitoring. Analysis of lymphocytes according to CBMN method could provide evidence of many nuclear abnormalities such as binucleates (presence of two nuclei in a cell), karyorrhexis (nuclear fragmentation) (Infante*et al.*, 1990; Revazova *et al.*, 2001) chromosome breakage, chromosome loss, chromosome rearrangement (nucleoplasmic bridges), necrosis and apoptosis. This method has gained increased attention as a simple, sensitive and rapid assay for assessing chromosome damage in various cell types (Jenssen and Ramel, 1980; Sudha et al., 2011).

Micronucleus (MN) is the small nucleus that arises from acentric chromosome fragments or whole chromosomes which is not incorporated into one of the daughter cell during mitotic cellular division. MN test could be used as a powerful tool for detection and quantification of the genotoxic risk. This method is faster and easier than metaphase analysis and can be used either *in vitro* or *in vivo*. Frequency of micronucleus in cultured peripheral blood lymphocytes has also been shown as a reliable and sensitive cytogenetic biomarker for monitoring genetics instability in humans. The purpose of this study was to evaluate personal exposures to TCE and prevalence of work related symptoms. Furthermore, this study was also aimed at investigating the DNA damage in TCE exposed workers using the micronucleus cytochalasin-B test (CBMN). Age, smoking, and alcohol drinking which have been reported as the influential confounding factors of DNA damage were also analyzed.

OBJECTIVES

1. To study the Cytotoxicity of Trichloroethylene in exposed worker using TCA in urine combined with Micronucleus Assay.

2. To assess the health effects of occupational exposure to Trichloroethylene.

LITERATURE REVIEW

1. Trichloroethylene

Trichloroethylene (TCE) is a colorless volatile chlorinated solvent which typically large quantities as a dissolvent, metal degreaser, chemical intermediate, and a component of consumer products (Gist and Burg, 1995; ATSDR, 1997). It is liquid at room temperature with a boiling point of 189ºF and a vapor pressure of 58 mmHg. The molecular weight is 131.4 and the Chemical Abstract Registry number is 79-01-6. The structure of trichloroethylene is shown in Figure 1. The most important reactions of TCE are atmospheric oxidation and degradation by aluminum chloride. The auto oxidation is catalyzed by free radicals and is greatly accelerated by elevated temperature and exposure to light, especially ultraviolet radiation. The oxidation products, hydrogen chloride, carbon oxides, phosgene, and dichloroacetyl chloride are acidic, and corrosive. So far only the oxidative metabolism has been described with kinetic data. There, TCE is oxidized to chloral hydrate, followed by reduction to trichloroethanol, which is partly further oxidized to Trichloroacetic acid (TCA) (McNeill, 1979; Mertens, 1993; Greim, 1996).

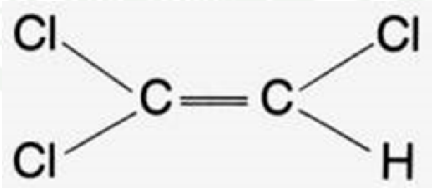


Figure 1 Structure of trichloroethylene.

1.1 Use of Trichloroethylene

TCE is now mainly used as a solvent to remove grease from metal parts. It is also used as a solvent in other ways and is used to make other chemicals. TCE can also be found in some household products, including typewriter correction fluid, paint

removers, adhesives, and spot removers. Most people can begin to smell TCE in air when there are around 100 parts of TCE per million parts of air (ATSDR, 1997).

Figure 2 TCE used as a solvent to remove grease from metal parts.

Figure 3 Trichloroethylene used as household products.

1.2 Metabolism of Trichloroethylene

TCE is a lipophilic compound that readily crosses biological membranes. Exposures may occur via the oral, dermal, and inhalation routes, with evidence for systemic availability from each route. TCE is absorbed well by the lungs during vapor inhalation, which is quickly absorbed into the blood stream and readily distributes to all compartments within the body. This process is mainly determined by the blood: tissue partition coefficients, which are largely determined by tissue lipid content. TCE attains high concentrations relative to blood in the brain, kidney, and liver all of which are important target organs of toxicity. In human, TCE metabolism occurs via conjugation with glutathione via glutathione S-transferase and oxidation via the cytochrome P450 enzymes (Lash *et al.*, 2000), the process occurs in the liver and kidney (Figure 4). TCE is eliminated from the body (Lash *et al.*, 2000; Merdink *et al.*, 1998), accounting for 50 to 99% of the absorbed dose. The major metabolic pathway in all species results in the production of trichloroethanol and trichloroacetic acid (TCA). Oxidation via P450 is considered the primary pathway of TCE metabolism, and metabolites formed via this pathway are implicated in the pulmonary and reproductive toxicities as well as the carcinogenicity of this chemical (IARC, 1995; DuTeaux *et al.*, 2003; Forkert *et al.*, 2006). TCE is cleared via metabolism mainly in three organs: the kidney, liver, and lungs. Metabolites of trichloroethylene are predominantly cleared in the urine with a small proportion eliminated in the bile and

feces. Most unmetabolized TCE is exhaled. The metabolism of TCE is an important determinant of its toxicity. Metabolites are generally thought to be responsible for toxicity-especially for the liver and kidney.

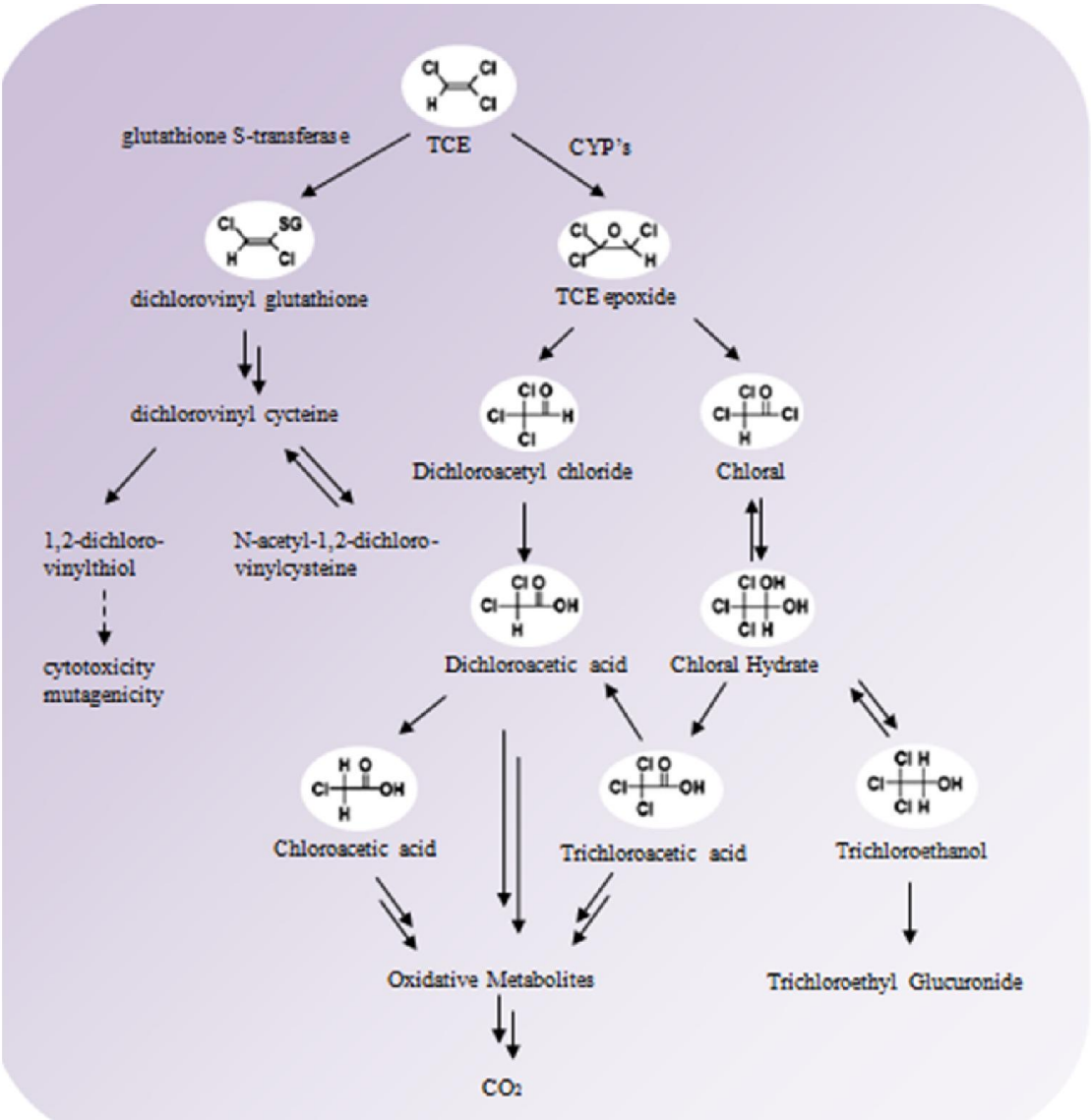


Figure 4 TCE metabolism process.

Source: Modify from Kim and Ghamayem (2006)

1.3 Toxicity and Exposure of Trichloroethylene

TCE affects many organs and systems of the body, consistent with its lipophilic nature and ability to distribute widely throughout the body. Based on the available human epidemiologic data and experimental and mechanistic studies, it is concluded that TCE poses a potential human health hazard for non-cancer toxicity to the CNS, kidney, liver, immune system, male reproductive system, and developing fetus.

1.3.1 Acute Toxicity

TCE is acutely toxic, primarily because it affects the central nervous system. The acute effects of TCE exposure are headache, dizziness, vertigo, tremors, nausea and vomiting, irregular heartbeat, fatigue, blurred vision, and intoxication similar to that of alcohol. Impairment of some central nervous system functions can be found in this exposure range, although some investigators have not been able to detect such adverse effects. Again, workers doing strenuous exercise or who have significant skin contact might be at risk even if they are exposed to "legal" levels of TCE. Acute exposure can also lead to dry throat, eye irritation and possibly liver damage. TCE exposure can also irritate skin leading to burns, rashes and dermatitis.

1.3.2 Chronic Toxicity

Chronic exposure to TCE may cause an enlarged liver, acute hepatitis, and effects in the respiratory system and the heart. Chronic, low-level exposure is known to cause decreased memory and impairment of the central nervous system. Epidemiologic studies have also demonstrated that TCE may be carcinogenic in humans. TCE addiction and peripheral neuropathy have been reported.

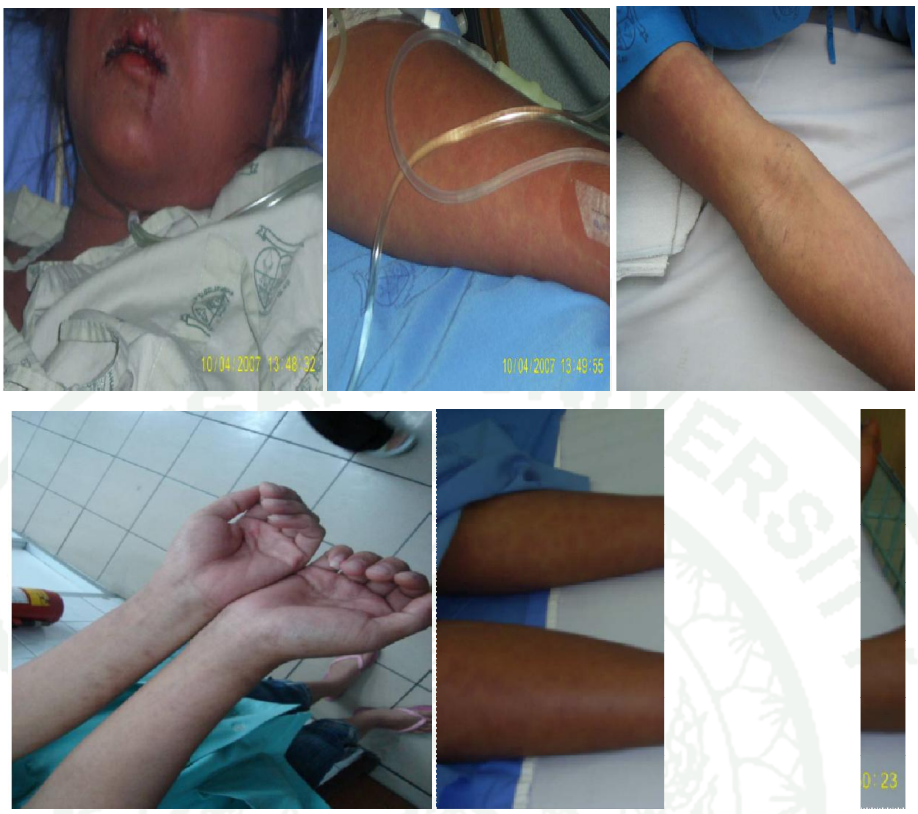


Figure 5 TCE produce contact dermatitis, rashes, and burns.

1.3.3 Respiratory Effects

The very few human data on TCE and pulmonary toxicity are too limited for drawing conclusions, but laboratory studies in mice and rats have shown toxicity in the bronchial epithelium, primarily in Clara cells, following acute exposures to TCE. Morphology of lung cells and P-450 activity in the lungs has been studied in rats and mice exposed to trichloroethylene. Acute pulmonary toxicity appears to be dependent on oxidative metabolism, although the particular active moiety is not known. While earlier studies implicated chloral produced in situ by CYP enzymes in respiratory tract tissue in toxicity, the evidence is inconsistent and several other possibilities are viable. Although humans appear to have lower overall capacity for enzymatic oxidation in the lung relative to mice, CYP enzymes do reside in human respiratory tract tissue, suggesting that, qualitatively, the respiratory tract toxicity observed in rodents is biologically plausible in humans. Therefore, overall,

data are suggestive of TCE causing respiratory tract toxicity, based primarily on short-term studies in mice and rats, with available human data too few and limited to add to the weight of evidence for pulmonary toxicity However, it has been reported that worker developed labored breathing and respiratory edema after welding stainless steel that had been washed in trichloroethylene (Sjogren *et al.*, 1991). Humans have died from breathing high concentrations of trichloroethylene fumes. Most of the reported deaths have been associated with accidental breathing of unusually high levels of trichloroethylene vapors in the workplace, often during its use in degreasing operations (Ford *et al.*, 1995; McCarthy and Jones, 1983) or dry-cleaning operations. These studies usually attributed death to ventricular fibrillation or central nervous system depression, since gross post-mortem abnormalities were not apparent. A number of the deaths occurred after the TCE exposure ended and involved physical exertion that may have contributed to the sudden deaths (Troutman, 1988). Death associated with liver damage has also been reported in persons occupationally exposed to TCE for intermediate and chronic durations, followed by a high acute-duration exposure. None of these cases provided adequate exposure level or duration data to define with accuracy the levels of inhalation exposure that cause human deaths.

Data on the lethality of longer-term exposure to TCE have been provided by studies of intermediate and chronic duration. Laboratory animals (rats, guinea pigs, monkeys, rabbits, and dogs) survived intermittent exposure to 700 ppm for 6 weeks or continuous exposure to 35 ppm for 90 days. There was no decrease in survival for rats and hamsters exposed to 500 ppm for 18 months, although a significant decrease in survival was seen for mice exposed to 100 ppm for the same amount of time (Henschler *et al.*, 1980).

1.3.4 Cardiovascular Effects

Windemuller and Ettema (1978) reported that exposure of 15 male volunteers to 200 ppm TCE for 2.5 hours had no effect on heart rate. Electrocardiograms of workers exposed to TCE in the range of 38-172 ppm for

periods ranging from less than 1 year to more than 5 years did not show any adverse effects (El Ghawabi *et al.*, 1973). A few case studies of persons who died following acute occupational exposure to trichloroethylene have revealed cardiac arrhythmias to be the apparent cause of death. Hypertension, enlarged heart, and arrhythmia were seen in some workers (number, sex, and exposure period unspecified) accidentally exposed to trichloroethylene at a level that was unspecified but at least 15 ppm (Sidorin *et al.*, 1992). Previous chronic exposure to TCE from using shoemaker's glue in an unventilated shop was implicated in a case of cardiac arrest and subsequent arrhythmia (Wemisch *et al.*, 1991). Inhalation of very high concentrations of TCE in incidents of poisonings or during its use as an anesthetic agent (Pembleton, 1974) has been reported to lead to cardiac arrhythmias. The mechanism is unclear, but high doses of hydrocarbons such as TCE could act upon the heart to cause cardiac sensitization to catecholamines. This is supported by animal studies. For example, dogs (Reinhardt *et al.*, 1973) and rabbits (White and Carlson, 1982) exposed to very high concentrations of TCE (5,000 or 10,000 ppm, and 3,000 ppm, respectively) for ≤1 hour showed increased arrhythmias when injected intravenously with epinephrine. In animals, TCE itself, rather than its metabolites, is apparently responsible for the cardiac sensitization because chemicals that inhibit the metabolism of trichloroethylene increase its potency, while chemicals that enhance the metabolism of TCE decrease its potency (White and Carlson, 1981). Histopathological changes were not detected in the hearts of rats exposed to 600 ppm TCE 7 hours/day, 5 days/week for 104 weeks (Maltoni *et al.*, 1988).

1.3.5 Liver Effects

Liver toxicity has also been associated with TCE exposure in both human and animal studies. This is not unexpected, as most metabolism of TCE occurs in the liver. Several studies have reported TCE can induce hepatotoxicity in humans, the results demonstrated by significant changes in serum liver function tests, widely used in clinical settings to identify patients with liver disease, or changes in serum cholesterol or serum bile acids (Nagaya *et al.*, 1993; Driscoll *et al.*, 1992). The evidence for TCE induced liver toxicity from many reports suggesting an association

between TCE exposure and liver disorders. In addition, case reports of liver toxicity including hepatitis accompanying immune-related generalized skin diseases, jaundice, hepatomegaly, hepatosplenomegaly, and liver failure in TCE-exposed workers have been discussed by several researchers. Alterations of plasma bile acids have been noted in workers at inhalation exposures of less than 5 ppm (Driscoll *et al.*, 1992; Neghab *et al.*, 1997). Elevations of serum bile acids, possibly an early sign of liver dysfunction, have also been seen in exposed rats (Wang and Stacey, 1990; Hamdan and Stacey, 1993). Alterations of cholesterol metabolism have been observed in workers with low-level chronic TCE exposure, effects that persisted after 2 years (Nagaya *et al.*, 1993). TCE exposure also can alter insulin and endocrine profiles in occupationally exposed individuals (mean, 30 ppm) (Chia *et al.*, 1997; Goh *et al.*, 1998), effects that may be important to responses of the liver. Increased liver weight, primarily resulting from cytomegaly, is one of the most frequently reported effects in animals dosed chronically with TCE by either the inhalation or oral route. Barton and Clewell (2000) summarize several studies in which orally administered TCE changed the liver-weight-to-body-weight ratio in rats and mice. These effects have been observed at gavages doses as low as 50 mg/kg-d for 14 days (Berman *et al.*, 1995). TCE was reported to increase cell replication but not cytotoxicity or reparative hyperplasia. TCE metabolites can also stimulate cell replication; TCA, DCA, and CH have all been associated with increased liver size (Bull, 2000). However, TCE exposure induce hepatotoxicity was not occurred in all cases, for example, in rats and mice, TCE exposure causes hepatomegaly without concurrent cytotoxicity. Like humans, laboratory animals exposed to TCE have been observed to have increased serum bile acids, although the toxicological importance of this effect is unclear. Other effects in the rodent liver include small transient increases in DNA synthesis, cytomegaly in the form of enlarged hepatocytes. Available data also suggest that TCE likely through its oxidative metabolites clearly leads to liver toxicity in laboratory animals, with mice appearing to be more sensitive than other laboratory animal species, but there is only limited epidemiologic evidence of hepatotoxicity being associated with TCE exposure.

1.3.6 Kidney Effects

Kidney, appear to be the most sensitive organs of TCE toxicity, as discussed both in vitro and in vivo. A range of kidney toxicity following human exposure to TCE has been reported in several studies (ATSDR, 1997; Lash *et al.*, 2000b). Pharmacokinetic data indicate substantially more production of GSH-conjugates thought to mediate TCE kidney effects in humans relative to rats and mice. Due to the role of GSH conjugation metabolites in TCE-induced nephrotoxicity, depend on the amount of DCVC bio activated in the kidney. Several studies are considered reliable for developing candidate reference values for these endpoints. Tubular damage, as assessed by altered excretion of urinary proteins, has been noted in occupationally exposed individuals (ATSDR, 1997), as well as in renal cell carcinoma patients with high-level occupational exposure to trichloroethylene (Brüning *et al.*, 1999a, b). TCE causes dose-related nephrotoxicity in male and female rats and mice (NTP, 1990; Maltoni *et al.*, 1988). The lesions are not the chronic interstitial nephrosis usually observed in aged rats, but consist of cytomegaly, kayomegaly, and toxic nephrosis of tubular epithelial cells in the inner renal cortex (Lash *et al.*, 2000b). For histopathological study, the data demonstrated that TCE can change the kidney-weight-to-body-weight ratio in rats and mice (Barton and Clewell, 2000; Maltoni *et al.*, 1988; Woolhiser *et al.*, 2006; Kjellstrand *et al.*, 1983a).

1.3.7 Immunological Effects

TCE induced immune system alteration has been noted in TCE exposed individuals (ATSDR, 1997). The ability of TCE to alter immune responses in the form of hypersensitivity has been observed in mice exposed to TCE via drinking water or inhalation (Khan *et al.*, 1995; Aranyi *et al.*, 1986; Hobara *et al.*, 1984; Parks *et al.*, 1993). Mice exposed to TCE in drinking water at 0.1 mg/mL and higher showed inhibition of humoral and cell-mediated immunity, as well as effects on macrophage function and monocyte-granulocyte progenitor cells (Sanders *et al.*, 1982). Numerous studies have demonstrated accelerated autoimmune responses in autoimmune-prone mice, including changes in cytokine levels similar to those

reported in human studies, with more severe effects, including autoimmune hepatitis, inflammatory skin lesions and alopecia, manifesting at longer exposure periods. Evidence of localized immune suppression has also been reported in mice and rats.

In human, a relationship between systemic autoimmune diseases, such as scleroderma (or systemic sclerosis, is a chronic connective tissue disease generally classified as one of the autoimmune rheumatic diseases), fascitis, and systemic lupus erythematosus (SLE) (Niertert *et al.*, 1998; Bovenzi *et al.*, 1995; Goldman, 1996; Schaeverbeke *et al.*, 1995; Waller *et al.*, 1994). The occupational exposure to TCE has been reported in several recent studies, especially, a meta-analysis of scleroderma studies resulted in a statistically significant increase symptoms compared to the non exposed group. Females showed a greater susceptibility than males to these immunotoxic effects of TCE (Sanders *et al.*, 1982; Barton and Clewell, 2000). TCE and one of its metabolites have also been shown to affect bone marrow function or components. Sanders *et al.* (1982) observed an inhibition of stem cell colonization in both male and female mice. Lock *et al.* (1996) reported that DCVC causes renal toxicity and fatal aplastic anemia in calves at a single dose of 4 mg/kg, a dose much larger than that expected from TCE metabolism, and that toxicity may be related to the metabolic pathway (Anderson and Schultze, 1965; Bhattacharya and Schultze, 1967).

Additional human evidence for the immunological effects of TCE includes studies reporting TCE-associated changes in levels of inflammatory cytokines in occupationally-exposed workers and infants exposed via indoor air at air concentrations typical of such exposure scenarios, a large number of case reports of a severe hypersensitivity skin disorder, distinct from contact dermatitis and often accompanied by hepatitis; and a reported association between increased history of infections and exposure to TCE contaminated drinking water. Overall, the human and animal studies of TCE and immune-related effects provide strong evidence for a role of TCE in autoimmune disease and in a specific type of generalized hypersensitivity syndrome, while there are less data pertaining to immunosuppressive effects.

1.3.8 Genotoxic Effects

TCE and its metabolites such as CH, DCA, and TCA have been evaluated in a large number of genotoxic assays. On the basis of the available data for TCE genotoxicity investigations definitive conclusions in humans have not been conclusive but are suggestive of clastogenic effects. In vivo, there is some evidence that TCE or its metabolites bind to DNA and can induce single-strand DNA breaks in both hepatic and kidney cells. However, the dose required to cause these DNA breaks was very high (4-10 mM of TCE/kg body weight) and the response was very low. More recent data show TCE to affect DNA methylation in whole-liver preparations at lower exposures with increased expression of the proto-oncogenes such as c-myc (Tao *et al.*, 1999). A study of chromosomal aberrations among trichloroethylene-exposed workers detected an increase in hypodiploid cells but found no evidence of chromosomal breaks in lymphocytes (Konietzko *et al.*, 1978). Another study showed an increase in sister chromatid exchange for workers exposed to trichloroethylene (Gu *et al.*, 1981). In a more recent study, men using TCE as a degreasing agent were tested for lymphocyte chromosomal abnormalities- specifically, breaks, gaps, deletions, inversions, translocations, and hyperdiploidy. The same study also investigated the rate of nondisjunction for the Y chromosome in sperm. Positive results were observed for chromosomal aberrations and hyperdiploid cells, but the results were negative for chromosomal nondisjunction (Rasmussen *et al.*, 1988). The frequency of sister chromatid exchange in the peripheral lymphocytes of TCE exposed workers was the focus of another investigation (Seiji *et al.*, 1990). Smokers and nonsmokers were included in this study. The only positive result obtained was for smokers who were also exposed to TCE. This general comparison between smokers and nonsmokers showed no significant differences in the rate of sister chromatid exchange. Therefore, the study authors suggest that smoking and TCE exposure may act together to produce increased sister chromatid exchange frequencies (Seiji *et al.*, 1990). In addition, it was unclear whether exposure to other solvents also occurred. Finally, other researchers have found no significant increase in the rate of sister chromatid exchange among either smoking or nonsmoking workers exposed to TCE (Nagaya *et al.*, 1989a).

In a dominant lethal study, male mice were exposed to TCE concentrations ranging from 50 to 450 ppm for 24 hours and mated to unexposed females; the results were negative (Slacik-Erben *et al.*, 1980). The splenocytes of mice exposed to up to 5,000 ppm trichloroethylene for 6 hours exhibited no aberrations in sister chromatid exchange or cell cycle progression and no increase in the number of micronuclei in cytochalasin B-blocked binucleated cells or bone marrow polynucleated erythrocytes (Kligerman *et al.*, 1994). In the same study, however, rats under the same exposure regime showed a dose-related increase in bone marrow micronuclei, as well as a reduction in polychromatic erythrocytes at 5,000 ppm, indicating the possibility of aneuploidy. These results are contrary to those expected since mice are generally more susceptible to tumor induction by trichloroethylene than rats. A possible explanation is that chloral hydrate, a metabolite of trichloroethylene, is known to induce aneuploidy in the predominant pathways in rats, whereas in mice the chloral hydrate pathway becomes saturated.

Many studies, both human epidemiological studies and animal laboratory studies, have examined the associations between TCE exposure and cancer. Some studies have shown cancer associated with TCE exposure and some studies have not shown a link between cancer and TCE. Of the human studies that do show a link, they often have a small number of participants and are confounded by exposure to other solvents. The strongest evidence of a relation exists for liver cancer, kidney cancer and non-Hodgkin's lymphoma. Relations have also been shown for prostate cancer, lymphatic cancer, bladder cancer, esophageal cancer, cervical cancer and multiple myeloma (Lemen, 2001; National Toxicology Program, 2005). Several research and regulatory agencies have given their current conclusions on the carcinogenicity of TCE. Many of the new studies have more sophisticated exposure assessment and thus allow for more accurate classification of TCE exposed workers (Scott and Chiu, 2006). Meta-analyses can be useful for evaluating risks for rare or uncommon cancers. Wartenberg *et al.* (2000) conducted a comprehensive review of over 80 studies and evaluated the evidence for over 20 cancer sites. The review categorized the cohort studies into tiers based on the quality of the exposure assessments. Average risks were calculated for multiple cancer sites for each tier as

well as for the case-control studies. In addition, meta-analyses have been published for liver cancer (Alexander *et al.*, 2007), pancreatic cancer (Ojajarvi *et al.*, 2001), NHL (Mandel *et al.*, 2007), and multiple myeloma and leukemia (Alexander *et al.*, 2006). However, there are limitations in these meta-analyses. Scott and Chiu (2006) updated the literature since the Wartenberg et al. review for kidney, liver and NHL. Overall, the body of literature provides convincing evidence of a causal association between TCE exposure in humans and site-specific cancers, particularly in the kidney. Recent studies have also found statistically significant associations between high TCE exposure and breast cancer (Sung *et al.*, 2007), and prostate cancer (Krishnadasan *et al.*, 2007).

1.4 Trichloroethylene in Workplace and Environment

Most TCE produced today is used for metal degreasing, in a number of industries (Bakke *et al.*, 2007). The highest environmental releases are to the air. Ambient air monitoring data suggests that levels have remained fairly constant since 1999 at about 0.3 μg/m^3. Indoor levels are commonly 3 or more times higher than outdoors due to releases from building materials and consumer products. TCE is one of the most common groundwater contaminants and the median level based on a large study by the U.S. Geological Survey is 0.15 μg/L (USGS, 2006). It has also been detected in a wide variety of foods in the 1-100 μg/kg range. None of the environmental sampling has been done using statistically based national surveys. However, a substantial amount of air and groundwater data has been collected allowing reasonably well supported estimates of typical daily intakes by the general U.S. population: inhalation - 13 μg/day and water ingestion - 0.2 μg/day. The limited food data suggests an intake of about 5 μg/day, but this must be considered preliminary (U.S. EPA, 2009a) High exposures have occurred to various occupational groups. Bakke *et al.* (2007) reviewed occupational exposure to TCE and reported that the arithmetic mean (AM) of the measurements across all industries and decades was 38.2 ppm. The highest personal and area air levels were reported in vapor degreasing (AM of 44.6 ppm). Occupational exposures have likely decreased in recent years due to better release controls and improvements in worker protection. However, some of

that protection relies on personal protective equipment, not always consistently used, rather than engineering controls. Exposure to a variety of TCE-related compounds, which include metabolites of TCE and other parent compounds that produce similar metabolites, can alter or enhance TCE metabolism and toxicity by generating higher internal metabolite concentrations than would result from TCE exposure by itself. Available estimates suggest that exposures to most of these TCE-related compounds are comparable to or greater than that to TCE itself. The maximum concentration in the workplace air of a chemical substance that generally has no known adverse effects on the health of employees and causes no unreasonable annoyance, even when the person is repeatedly exposed during long periods, given a 40-hour working week. The maximum concentration is given as a time-weighted average concentration over an 8-hr working period (TWA) (DFG, 2001). The American Conference of Governmental and Industrial Hygienists (ACGIH) recommend 100 ppm in air as so exposure limit for an 8-hour workday. NIOSH recommends that peak exposure never exceed 150 ppm. It is important to note that these values are based upon information derived from acute, high-level exposure incidents, not long-term, low-level exposures. Lauwerys has suggested a "Tentative Maximum Permissible Level" for TCA in the plasma of 50 ppm, designed to keep total uptake of TCE below levels found to be safe in resting volunteers.

1.5 Biological Monitoring

Biological monitoring is the measurement and assessment of chemicals or their metabolites in exposed workers. These measurements are made on samples of breath, urine or blood, or any combination of these. Biological monitoring measurements reflect the total uptake of a chemical by an individual by all routes (inhalation, ingestion, through the skin or by a combination of these routes). Thus it differs from environmental monitoring which measures an individual’s exposure.

During the manufacture of TCE and its use in chemical synthesis, workers may be exposed by the inhalation and dermal routes. Inhalation exposure to the vapor is likely where operators breach the closed plant or as a result of spillages. Dermal

exposure may occur where workers come into contact with surfaces contaminated by splashes or condensed vapor or as a result of direct splashes on to the skin. During the use of TCE, workers may again be exposed by the inhalation and dermal routes. Inhalation exposure to the vapor will occur during activities such as metal cleaning and the use of adhesives. As in TCE manufacture, dermal exposure may also occur where workers come into contact with surfaces contaminated by splashes or condensed vapor or as a result of direct splashes on to the skin. This may be particularly evident where operators handle degreased components or directly handle adhesives. The number of workers exposed to TCE throughout the EU is estimated to be in excess of 60,000, with about 10,000 of these in the UK. Reasonable worst-case exposure levels for the different occupational scenarios are identified in Table 1. A further complicating factor is that there is some evidence from studies in Japanese workers that some persons have a limited capacity to metabolize TCE to TCA. Exposure levels nearing 100 ppm in air appear to saturate their metabolism (Ikeda, 1977). Thus, in such persons, exposure over 100 ppm may not give rise to higher levels of TCA than would be found in exposure under 100 ppm. Measuring TCA in urine at the end of shift of the workweek, suggest that the level of TCA in the urine not exceed 100 mg/L. Although Droz (1978) has argued that TCA in urine is a fairly insensitive index of overexposure to TCE, adoption of this BEI for TCA in urine would likely require that concentrations of TCE in air be kept well below 100 ppm. Biological monitoring for chemical exposure contributes to the aim of preventing unacceptable health risks by providing information on the control of occupational exposure. It can give an indication of absorption by all routes of exposure; consequently, it is often used to complement personal air monitoring (which measures the concentration of a chemical in the air in a person's breathing zone). Therefore biological monitoring may be particularly useful for those chemicals which are easily absorbed through the skin or taken in by ingestion, or where exposure is controlled by personal protective equipment.

Table 1 Reasonable worst-case exposure levels for the dermal and inhalation routes.

Exposure scenario	Exposure level via inhalation route	Exposure level via dermal exposure
Manufacture and recycling	8-hour TWA[1)] – 10 ppm	0.1 mg/cm^2/day
Metal degreasing	8-hour TWA – 50 ppm	1.0 mg/cm^2/day
Adhesives (manufacture)	8-hour TWA – 10 – 20 ppm (with LEV[2)]) and 100 – 140 ppm (without LEV)	1.0 mg/cm^2/day
Adhesives (use)	8-hour TWA – Wide use, controls uncertain – not quantifiable	1.0 mg/cm^2/day
Use as an intermediate	8-hour TWA – 11.5 ppm	0.1 mg/cm^2/day

1) TWA: time weighted average
2) LEV: local exhaust ventilation

Source: Institute for Health and Consumer Protection (2014)

2. Cytotoxicity

Cytotoxicity is a well established and easily accessible endpoint to get first information on the general or acute toxic potential of a test substance. In order to further investigate the possible involvement of metabolism and the mechanism of toxicity different test systems and endpoints should be investigated. The reaction of an organism to exposure to a toxic substance is called the response. The observed response can be any measurable physiological change such as nausea, blindness, sterility, birth defects, or death. In animal studies, the easiest response to measure is the number of deaths in a population of organisms. Because no two individuals of the same species respond to a given dosage identically, the dose-response function is expressed in statistical terms. The comparative potency of different substances is often expressed as “lethal dose fifty” (LD50), the dose at which death is observed in

50 percent of the experimental organisms in question. A similar term, “lethal concentration fifty” (LC50), is used when the toxicants are gases, vapors, or particulates.

The severity and type of a toxin’s effect also depends on how rapidly the dose is received (duration) and how often the dose is received (frequency). A chemical may produce no toxic effect if the dose is received slowly enough that the rate of detoxification keeps pace with its intake. The same chemical could produce a toxic effect if received in a rapid dose. Dose, then, is usually specified as the amount (or concentration) of a chemical and the length of time over which it interacts with the organism. In animal or human studies, the toxic dose of a chemical also usually depends on the size of the organism exposed. Thus, dose is often defined in relative terms rather than absolute quantities. For substances administered orally, dose is measured as quantity per unit mass of the organism and is usually expressed in milligrams per kilogram of body weight (mg/kg).

In environmental or occupational toxicology, dose is usually synonymous with exposure. An important corollary to the idea that all substances are poisons is that virtually any chemical can be used safely if exposure to the chemical is kept below tolerable limits. Of course, tolerable exposure is extremely low for some highly toxic chemicals. Risk is directly related to the combination of toxicity and exposure.

3. Biomarkers of Toxicity

Biomarkers are the indicators of variation in cellular or biochemical components or processes, structure or function that are measurable in biologic systems (NRC, 1989). The biomarkers may represent signals in correlation between exposure and resultant disease (Schulte, 2005).Biomarkers can be classified into three main types: biomarkers of exposure, biomarkers of effect and biomarkers of susceptibility as shown in Figure 6. For any given toxicant exposure, there may therefore be several biomarkers with different levels of sensitivity and specificity that

can be measured in different body fluids and tissues (Timbrell, 1998; Schulte, 2005; Moore and Harrington-Brock, 2004).

3.1 Biomarkers of Exposure

These are the xenobiotic (may be called toxic) substance of a metabolite(s) or product of its interaction with a biological molecule or cell, that are measured in a compartment within an organism (NRC, 1989). They can be divided into two types: markers of internal dose or markers of effective dose. While external exposure is the level of xenobiotic substances an organism is exposed to, the internal dose is the amount of the xenobiotic compound that is actually absorbed into the organism and effective dose is the amount of the xenobiotic compound that interacts with critical subcellular, cellular and tissue targets (NRC, 1989). Biomarkers of internal dose therefore indicate that exposure to a xenobiotic compound has taken place by measuring the compound or its metabolite(s) in body fluids. Biomarkers of effective dose indicate that exposure to a xenobiotic compound has resulted in the compound or its metabolite(s) reaching a toxicologically significant target (Timbrell, 1998). Examples of biomarkers of exposure in the occupational health setting include urinary cadmium as a long term marker of cadmium exposure, TCA in urine as a marker of TCE exposure and the DNA adduct Styrene oxide-guanine as a marker of styrene exposure (Waterfield and Timbrell, 2000).

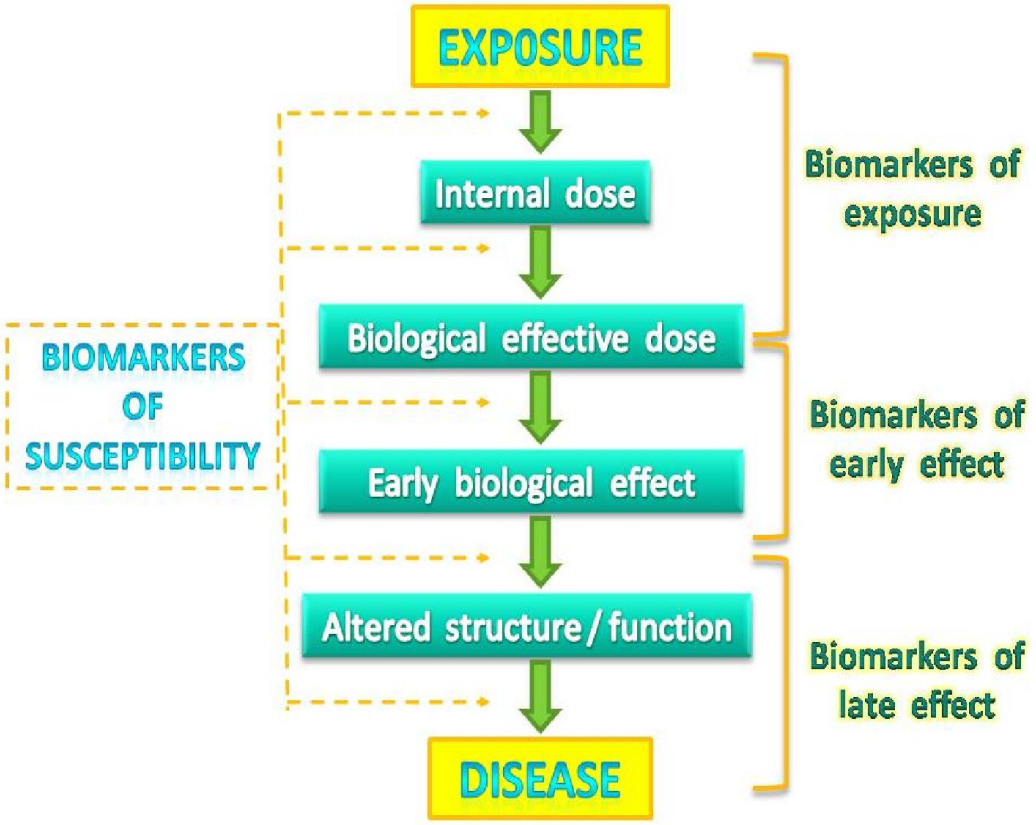


Figure 6 Flowchart of the position of different types of biomarkers in the pathway from exposure to disease. Solid arrows indicate progression. The pathway is a temporal sequence where the higher event generally precedes the lower event. Biomarkers in the first three blocks can be considered biomarkers of exposure and biomarkers in the lower four blocks can be considered biomarkers of effect. Biomarkers of susceptibility can occur at any stage in the pathway.

Source: Modify from NRC (1987)

3.2 Biomarkers of Effect

These are measurable physiologic, biochemical, or other changes within an organism that can be recognized as a potential health defect or disease (NRC, 1989). Biomarkers of effect defined as a recognized disease, an early precursor of a disease process, or an event that is nonessential to any disease process but is correlated with development of the disease. A biological marker of effect can be mentioned as any change that is quantitatively or qualitatively predictive of a disease associated with exposure (NRC, 1989). As far as exposure is concerned, biomarkers of effect are, in theory, non-specific. However, in occupational situations it is often possible to exclude other factors affecting the biomarker levels and therefore a relative specificity is possible (Aitio, 1999). Biomarkers of effect range from simple, for example body weight, to complex, for example, determination of specific isoenzymes. In addition, biomarkers of effect can be classified as either early or late biomarkers. Biomarkers of effect are perhaps best regarded as indicators of early changes that could later lead to clinical disease (Mutti, 1999). An ideal biomarker of effect would be able to indicate early reversible events. Examples of biomarkers of effect include the enzyme aspartate aminotransferase (AST) as a measure of myocardial damage, induction of cytochrome P-450 isoenzymes as a marker of exposure to polycyclic aromatic hydrocarbons (PAHs) and organochlorine compounds, and increased metallothionein levels as a marker of heavy metal exposure (such as cadmium) (Waterfield and Timbrell, 2000).In addition, biomarkers of effect also give measures of the alterations on important genetic targets like DNA, causing DNA-breaks, chromosome aberrations and micronucleus. Biomarkers of biochemical effect provide information about oxidative damage in DNA and proteins, alterations in a wide range of enzymes like DNA-repair enzymes, and metal-binding proteins, among others (Frenzilli *et al*. 2009; Rojas, 2009).

3.3 Biomarkers of Susceptibility

These indicate an acquired or inherent limitation in the ability of an organism to respond to the exposure to specific xenobiotic substance (NRC, 1989). Therefore, any variation in the response of an individual to identical exposures could indicate a difference in susceptibility due to either the genetic make-up of the individual or to factors or environmental influences such as diet or the uptake and absorption of the xenobiotic substance. However, the most likely source of variability is due to the metabolism of the substance by the organism, which may be genetically determined. (Waterfield and Timbrell, 2000). Biomarkers of susceptibility include, among others, polymorphisms in genes responsible for DNA repair, genomic stability and chemical activation or detoxification. There are however numerous ethical, legal and social issues surrounding the use of biomarkers of susceptibility (Schulte, 1991).

4. Cytogenetic Detection

Cytogenetics is the study of the structure and properties of chromosomes, chromosomal Cytogenetic is the study of the structure and properties of chromosomes, chromosomal behavior during somatic cell division in growth and development (mitosis) and germ cell division in reproduction (meiosis), chromosomal influence on the phenotype and the factors that cause chromosomal changes (Singh, 1988). The most commonly used methods for the detection of DNA damage, related to physical activity, are the single cell gel electrophoresis (SCGE or comet) assays, the micronucleus (MN) assay, and its further developed version, the cytokinesis block micronucleus (CBMN) assay. Furthermore, there are many methods used to detect changes in DNA, DNA damage and chromosome damage as shown in this section.

4.1 DNA Single (SSB) and Double Strand Breaks (DSB)

Another approach for evaluating the possible consequences of environmental metal pollution involves the assessment of genotoxic damage measured as DNA-breaks. Most metals interact indirectly with DNA, via generation

of ROS, causing single and double strand breaks (Mussali-Galante *et al*., 2005; Frenzilli *et al*., 2009).

4.2 Chromosome Aberrations (CA)

CA are used as a biomarker of effect, it is a valuable tool for studying environmental hazards and have been used as cytogenetic biomarkers in the past decades. The use of valid biomarkers of risk in populations exposed to genotoxic agents is the most suitable and well-established approach for analyzing many modern exposures (Tucker and Preston, 1996; Bonassi *et al*., 2005). CA is induced by agents that damage chromosomal DNA. A large amount of evidence demonstrates that DNA-DSB is the principal lesions in the process of CA formation (Pfeiffer *et al*., 2000). DSB arise spontaneously at high frequencies through a variety of cellular processes (Bonassi *et al*., 2005). However, the majority of chemical mutagens are not able to induce DSB directly but lead to other lesions in chromosomal DNA which, during repair or DNA synthesis, may give rise to DSB and eventually to CA (Tucker and Preston, 1996; Obe *et al*. 2002).

The purpose of the *in vitro* chromosomal aberration test is to identify agents that cause structural chromosome aberrations in cultured mammalian cells. Structural aberrations may be of two types, chromosome or chromatid. With the majority of chemical mutagens, induced aberrations are of the chromatid type, but chromosome type aberrations also occur. An increase in polyploidy may indicate that a chemical has the potential to induce numerical aberrations. However, this method is not designed to measure numerical aberrations and is not routinely used for that purpose. Chromosome mutations and related events are the cause of many human genetic diseases and there is substantial evidence that chromosome mutations and related events causing alterations in oncogenes and tumor suppressor genes of somatic cells are involved in cancer induction in humans and experimental animals. This test is used to screen for possible mammalian mutagens and carcinogens. Many compounds that are positive in this test are mammalian carcinogens; however, there is not a perfect correlation between this test and carcinogenicity. Correlation is

dependent on chemical class and there is increasing evidence that there are carcinogens that are not detected by this test because they appear to act through mechanisms other than direct DNA damage.

4.3 Sister Chromatid Exchange (SCE)

This assay is a well-known cytogenetic technique that has been used extensively to assess DNA damage at the chromosomal level (Hagmar *et al*., 1994). The sister chromatid exchange (SCE) assay is a short-term test for the detection of reciprocal exchanges of DNA between two sister chromatids of a duplicating chromosome. They represent the interchange of DNA replication products at apparently homologous loci which involve DNA breakage and rejoin (Gauthier *et al*., 1999; Wilson and Thompson 2007). During the S-phase of the cell cycle, DNA is replicated, and each chromosome becomes duplicated into two closely associated daughter chromatids that are linked tightly at the centromere. Sister chromatids are visible cytologically in late prophase and early metaphase of mitosis before chromosome segregation occurs (Kaina, 2004). Detection of SCEs requires some means of differentially labeling sister chromatids, which can be achieved e.g. by incorporation of bromodeoxyuridine (BrdU) into chromosomal DNA for two cell cycles. SCEs can also be measured in mammals and in non-mammalian systems.

4.4 Fluorescent *in situ* Hybridization (FISH)

FISH is a well known molecular cytogenetic technique that is widely used in genetic toxicology to detect chromosome aberrations which induced *in vivo* and *in vitro* by physical and chemical agents (Eastmond *et al.*, 1995). Various probes have been used to detect chromosomal alterations, among these probes, the ones which have been used to detect the origin of micronuclei by labeling the centromeric region (Becker *et al.*, 1990; Eastmond *et al.*, 1995).The most important steps to perform FISH are (1) denaturation of the nativeDNA to a single-strand DNA, which can bond to form DNA double strands and (2) incubate the DNA with specific-labeled DNA (of the probe), which lead to complementary strand tight binding (hybridization)

(Wolman, 1994). DNA probes for FISH can be labeled directly with fluorochromes, or indirectly by modifications introducing reporter groups such as biotin in the probe molecules. In the second type, the detection of the probe is achieved via reporter binding molecules conjugated with fluorochromes, enzymes or with metallic compounds (Joos *et al.*, 1994).

4.5 Single cell gel electrophoresis assay (Comet assay)

Comet assay is one of the standard methods for assessing DNA damage, with applications in fundamental research in DNA damage and repair (Collins, 2004). It is a rapid, simple, and sensitive technique to measure the DNA damage that results from negatively charged loops and fragments drawn to anode and form the tail of the comet (Müller, 2007). The name "Comet assay" is derived from the shape of nuclear DNA which will be like a comet, after some experimental steps. Comet assay is also called the single cell gel electrophoresis (SCGE), single cell gel assay (SCG) or micro gel electrophoresis (MGE) (Fairbairn *et al.*, 1995; Müller, 2007; Singh *et al*., 1988; Tice *et al*., 2000; Mussali-Galante *et al*., 2005; Rojas, 2009). In principle, it is suitable for all cells, tissues and organs and small cell samples are needed. As a result, the comet assay has become one of the major tools for environmental bio monitoring studies. In the initial version of the comet assay, Ostling and Johanson (1984) applied a micro gel electrophorese technique, where electrophoresis was carried out under neutral conditions, which enabled to detect DSBs. Subsequently, Singh *et al.* (1988) and Tice *et al.* (2000) adopted protocols, where electrophoresis is carried out under alkaline conditions (pH > 13), in order to detect SSBs, DSBs and apurinic sites. The comet assay under alkaline conditions (standard version; pH > 13) is suitable for monitors DNA strand breaks and alkali labile sites (Collins *et al.*, 2008; Tice *et al.*, 2000). The key principle of the method is based on the migration of the damaged DNA in an electric field, forming comet shaped images (Dusinska and Collins, 2008). The amount of DNA in the tail represents the frequency of breaks (Collins *et al.*, 2008). It is important to mention that even when the Comet assay is sensitive to detect strand breaks; it is a nonspecific chemical biomarker of genotoxicity (Dhawan *et al*., 2009). In the literature, the results of comet assays are inconsistently reported as %

DNA in tail, tail moment and/or tail length. However, the tail moment does not have a standard unit and there are several possibilities how to calculate this unit, which complicates the interpretation of results. Indeed, tail length is informative when the levels of DNA damage are low, otherwise it soon becomes maximal. Thus using consistently % DNA in tail is strongly recommended (Collins *et al.*, 2008).

4.6 Micronucleus and Cytokinesis-block micronucleus assay (CBMN)

Chromosomal mutation is an important event in carcinogenesis. Therefore, studying DNA damage at the chromosome level is an essential part of genetic toxicology. A micronucleus (MN) is formed during the metaphase/anaphase transition of mitosis (cell division). It may arise from a whole lagging chromosome (a eugenic event leading to chromosome loss) or an acentric chromosome fragment detaching from a chromosome after breakage (clastogenic event) which do not integrate in the daughter nuclei. Hence, micronuclei may be a consequence of either chromosomal breakage or dysfunction of the spindle mechanism (Lindberg *et al*., 2007). These types of micronuclei can be distinguished (Boei and Natarajan, 1995), and there is evidence that genotoxic agents can be differentiated by whether they induce chromosomal breakage or loss (Chen *et al*., 1994; Fenech and Crott, 2002) and/or centromeric modifi-cations (Fenech *et al.*, 1999). They have been studied for many years, in experimental research as well as in environmental monitoring. In the last decade, MN assay has gained a lot of attention because it offers several advantages: a) MN can be observed in almost any eukaryotic cell type, b) Rapidity, simplicity and low cost of the analysis, and c) the non-requirement for metaphase cells. Thus, MN analyses can be employed in studies with different experimental conditions, in a wide variety of animal species (Bonassi *et al*., 2005). Scoring of micronuclei can be performed relatively easily and on different cell types suitable for human biomonitoring for example lymphocytes, fibroblasts and exfoliated epithelial cells, without extra in vitro cultivation step.

The most important steps to perform an *in vitro* analysis of lymphocytes are the adding of cytochalasin-B (added 44-47 hours after the start of cultivation), an inhibitor of actins, which allows distinguishing easily between mononucleated cells and binucleated cells which completed nuclear division during in vitro culture. This method is called the cytokinesis-block micronucleus assay which is one of the preferred methods for chromosome damage assessing (Fenech, 2000). In cytokinesis-block method, micronuclei are scored only in the cells which have been inhibited from undergoing cytokinesis, hence called cytokinesis blocked cells (Figure 8, 9 and 10). Furthermore, this endpoint method has been reported to detect DNA damage caused by dietary, environmental and lifestyle factors and a causal link between MN and the risk of cancer has been described in a recent cohort study (Bonassi *et al.*, 2007; Fenech *et al.,* 2011). In addition to micronucleus, the CBMN assay enables the detection of nucleoplasmic bridges and nuclear buds. The frequency of micronucleated binucleated cells is calculated from the cell counts. At least 1000 binucleated cells should be scored for individual cultivation. To strengthen the sensitivity of the test, about 2000 cells were scored in our experiments. As a measure of cytotoxicity the proportion of binucleated cells of total viable cells was calculated (Fenech, 2000). It is important to reach at least 50% cytotoxicity at the highest concentration tested to ensure that the proper range of concentrations are used (Kirsch-Volders *et al.*, 2000).

Mechanisms of micronuclei formation: Micronuclei which contain chromosomal fragments are formed from direct double-strand DNA breakage, conversion of single strand breaks into double strand breaks after cell replication, or inhibition of DNA synthesis (Mateuca *et al.,* 2006).While, micronuclei which contain whole chromosomes are formed from failure of the mitotic spindle, kinetochore, or other parts of the mitotic apparatus or by damage to chromosomal substructures. In addition, defects in the chromosome segregation machinery lead to micronuclei harboring whole chromosomes (Albertini *et al.,* 2000). Micronuclei can also arise by gene amplification via breakage-fusion-bridge (BFB) cycles when amplified DNA is selectively localized to specific sites at the periphery of the nucleus and eliminated via nuclear budding during the S-phase of the cell cycle (Fenech, 2002). Briefly,

Chromosome breakage (clastogenesis) and spindle disruption (aneuploidogenesis) are two major mechanisms for micronuclei formation (Tuckerand Preston, 1996). In a recent study, based on live cell imaging, Rao *et al.* (2008) have proposed a novel mechanisms of MN formation, these are: 1) MN inherited from mother cells, 2) Micronuclei originated from the nuclear fragments that appeared during mitosis, 3) MN originated from chromosomes that were extruded from a mitotic cell and 4) MN appeared after chromosomes de-condensed to form interphase nuclei following a normal mitosis (Figure 7-9).

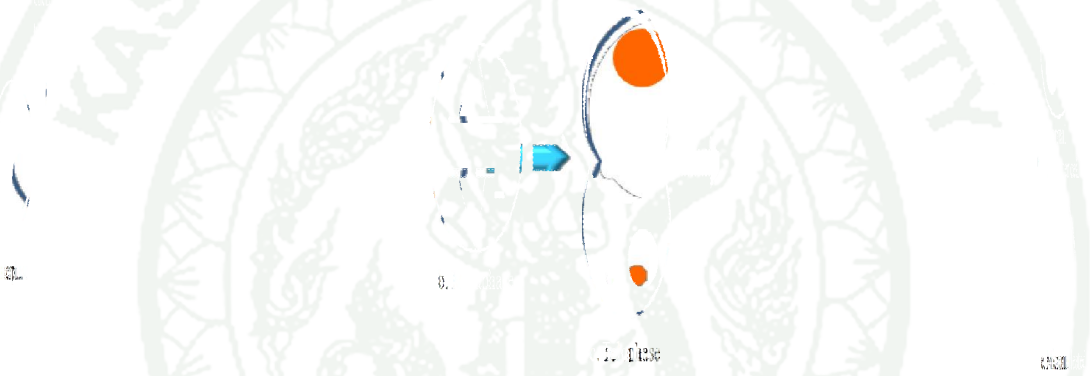


Figure 7 Micronucleus formation.

Source: Modify from Aardema and Volders (2001)

Figure 8 Micronucleus can arise from whole chromosomes/chromatids that lag behind in anaphase due to misattachment of tubulin fibers on kinetochore.

Source: Modify from Mateuca *et al.* (2006)

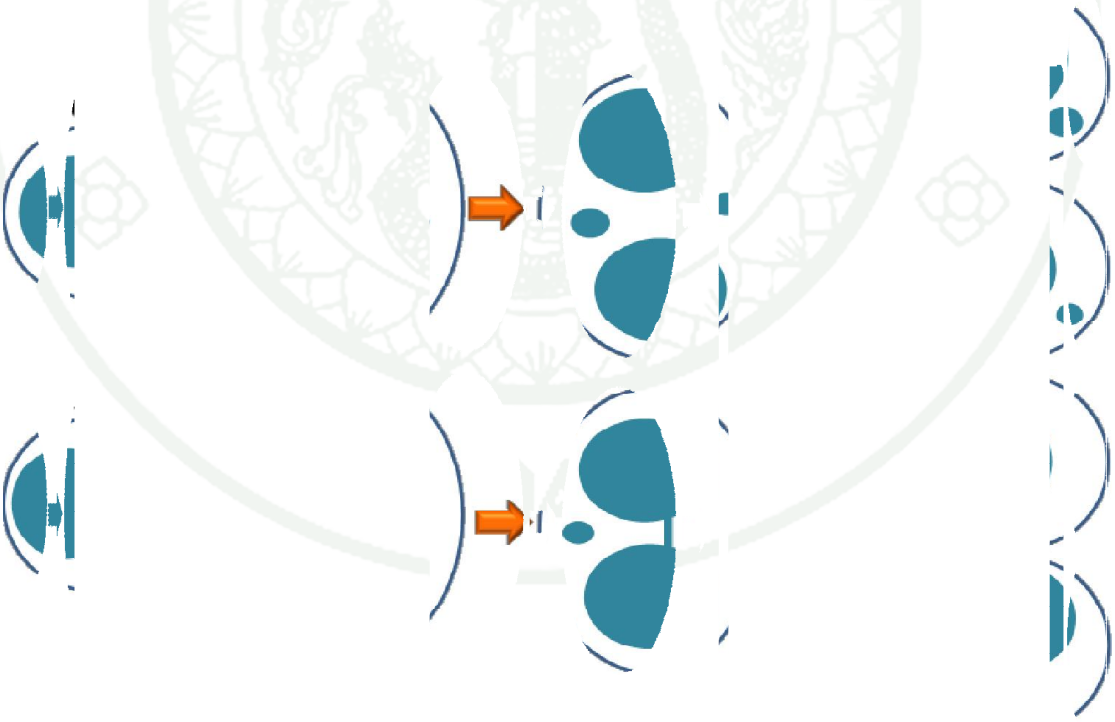


Figure 9 Micronucleus can arise from acentric chromosome/chromatid fragments.

Source: Modify from Mateuca *et al.*(2006)

5. Applications of the CBMN assay in population monitoring and genotoxicity testing

As mention above, human micronucleus analysis has been used to investigate the genotoxic effects of new chemicals *in vitro* and also the DNA damaging effect of *in vivo* exposure (Fenech *et al.*, 1999). MN assay has been successfully used to study the genetic damage in the people living in regions contaminated with complex mixture of pesticides were reported (Pastor *et al*., 2001; Ergene *et al.*, 2007). MN assay has been successfully used for genotoxicity testing of environmental pollutants (Klobučar *et al.*, 2003). In addition, the genotoxic effect of cigarettes smoking has been investigated by using CBMN assay (Bonassi *et al.*, 2003), in many regions and by several working teams from different countries (da Cruz *et al.*, 1994; Holmén *et al.*, 1995; Cheng *et al.*, 1996). This method has many applications in nutrigenomics and toxicogenomics and the combination of both of them (Fenech, 2007). CBMN assay has been used also to evaluate the genotoxicity of cytostatic drugs in hospital and pharmacy employees(Hessel *et al.*, 2001). In addition, this method has been used to study the genotoxic effect of skin medicines (e.g. Acitretin) (Stephanou *et al.*, 2004), hypertension medicines (Andrianopoulos *et al.*, 2006), anticancer drugs (Efthimiou *et al.*, 2007) and new designed anticancer drugs (Efthimiou *et al.*, 2010). The Human Micronucleus (HUMN) project has completed a study involving atotal of 6,718 subjects from ten countries, screened in twenty laboratories for MN frequency between 1980 and 2002; interestingly, a significant increase of all cancer incidence was found for subjects in the groups and high MN frequency (Bonassi *et al.*, 2007).

6. Human lymphocyte culture

Culturing blood cells has many advantages including: (1) excellent growth potential of cells after mitogen stimulation (2) blood is one of the easiest tissues to study and (3) rapid results can be obtained, after 2-3 days culturing (Gosden *et al.*, 1992). In lymphocyte culture, it is necessary to stimulate quiescent T cells to divide, hence many agents are used to stimulate their division (e.g. phytohaemagglutinin), which cause blood cells to become mitotically active in cell culture. These agents are

mitogens for white cells (Gosden *et al.*, 1992). Serum also acts as a nutrient supplement for the growing cells in culture (Victor *et al.*, 2002). The commonly used types include fetal bovine serum and fetal calf serum. Furthermore, there are many types of media which are commonly used in cytogenetics laboratory (Victor *et al.*, 2002). The different banding techniques allow precise identification of each chromosome as well as to detect structural chromosomal rearrangements. A combination of several banding techniques also help in obtaining the information necessary for chromosomal analysis.

MATERIALS AND METHODS

Materials

1. Chemicals

1.1 Acetic acid
1.2 Air zero
1.3 Antibiotics (100 UI/mL penicillin and 100 µg/mL streptomycin)
1.4 Creatinine reagents
1.5 Cytochalasin-B (Cyt-B)
1.6 Deionized water
1.7 Dimethyl Sulfoxide (DMSO)
1.8 Ethyl Alcohol
1.9 Fetal bovine serum (FBS)
1.10 Formaldehyde
1.11 Giemsa
1.12 Helium gas
1.13 Heparin
1.14 Hydrogen gas
1.15 L-glutamine
1.16 Methanol
1.17 Nitrogen gas
1.18 Phytohemagglutinin (PHA)
1.19 Potassium chloride (KCl)
1.20 RPMI 1640 medium (Gibco)
1.21 Trichloroacetic acid

2. Instrumentations

2.1 Headspace Vials, 10 mL

2.2 Analytical Balance 4 Digit, Mettler Toledo ModelAG 204, USA

2.3 Auto Pipette Tip, 1,000-5,000 µL

2.4 Auto Pipette Tip,10-100 µL

2.5 Auto Pipette Tip, 200-1,000 µL

2.6 Auto pipette, 1,000-5,000 µL

2.7 Auto pipette,10-100 µL

2.8 Auto pipette, 200-1,000 µL

2.9 Autoclave

2.10 Bigger sizes 50, 100, 250, 500 mL, Pyrex, England

2.11 Blood Collection Tube (Heparin Tubes, BD Vacutainer)

2.12 Capillary GC column, GsBP-FFAP, 50 m x 0.32 mm x 0.50 µm

2.13 Centrifuge

2.14 CO_2 Incubator

2.15 Conical centrifuge tube 15 mL

2.16 Cylinder, 100 mL

2.17 Dry Oven

2.18 Freezer -20ºC

2.19 Headspace Gas Chromatography
(GC, Model 6890; Hewlett Packard, USA)

2.20 Laminar Flow

2.21 Microscope

2.22 Needle, 18G x 1.5

2.23 Syringe, 3 mL

2.24 Test tube, Pyrex, England

2.25 Vortex Mixer ModelGenie 2, USA

Methods

1. Ethical Issue

The study was approved by the Ethics Committee of Department of Disease Control, Ministry of Public Health. Participation was voluntary, and all participants received detailed information concerning the aims of the research work. Informed consent was obtained from all of them prior to begin of the study.

2. Study Population

This study carried out a cross-sectional study among workers with occupational exposure to TCE. The study design was selected the establishment that has been used TCE in production process. The study included 52 men and 190 women from the factory in Bangkok, who were occupationally exposed to TCE study or exposed group. Sixty six healthy volunteers were used as control group, who work in other departments that are not exposed to TCE or who have a residential area near the factory.

The study was divided into two phases.

Phase 1 of the study collected the urine sample to assess the situation of TCE exposure, determination of TCA in urine. Evaluation and presentation to the board of directors of the plant to provide workers with the knowledge and behavior change.

Phase 2 of the study occurred after six months ago. The urine sample and blood sample were collected in this phase, to analyst both of TCE exposure and DNA damage.

3. Questionnaire

All subjects were interviewed face to face using a pretested, standardized questionnaire, administered by specially trained interviewers. The questionnaire included: basic demographic variables and residence characteristic; habits of tobacco smoking (age, sex, typical number of cigarettes per day; history of alcohol drinking, duration of consumption, history of disease presence, opportunity of dairy exposure to toxicant and TCE) See questionnaire in Appendix A.

4. Environmental Samples

The analytical methods for detecting trichloroethylene in workplace are used as the standard methods. This methods used for air samples are the methods approved by the National Institute for Occupational Safety and Health (NIOSH); NIOSH 1022. The methods of analyzing for trichloroethylene in air are Gas Chromatography (GC) combined with flame-ionization detector (FID). Air samples are usually pumped through a sample collection column, coconut charcoal, the most common adsorbents. Trichloroethylene is absorbed by coconut charcoal in charcoal tube. Desorbed trichloroethylene from the coconut charcoal by carbon disulfide . Allow to stand 30 min with occasional agitation, after that inject sample aliquot with auto sampler. To measure peak area and calculation (see analytical NIOSH 1022 method in Appendix C).

5. Urine Samples

Urine samples were collected to determine the amount of exposure to TCE in the form of TCA. The urine samples were collected in 15 mL of conical centrifuge tube and labeled with subject identification number, date, time. The samples were transported in a cooler. The samples were divided into several small volume aliquots and stored at -20 ºC to minimize the effect of freeze-thaw on the stability of specimens.

6. Method Validation and Determination of TCA in Urine Sample

A method for TCA in urine analysis was developed by collaborators in our laboratory, in-house method. This method have to confirm by method validation process. Method validation is used to confirm that the analytical procedure employed for a specific test is suitable for its intended use and is required for complete compliance with cGMP and GLP regulations, US FDA and international regulatory guidelines. Specific method validation needs, typical method validation parameters include: Specificity, Selectivity, Precision, Reproducibility, Accuracy, Trueness, Bias, Linearity, Range, Limit of detection, Limit of quantitation, Robustness, Ruggedness. (Figure 10) When tested to confirm the reliability characteristics of the method (method validation) then we have been participating in a proficiency testing program by comparing results between laboratories (Proficiency Testing: PT) and certified results. Therefore, the method developed can be applied to operations.

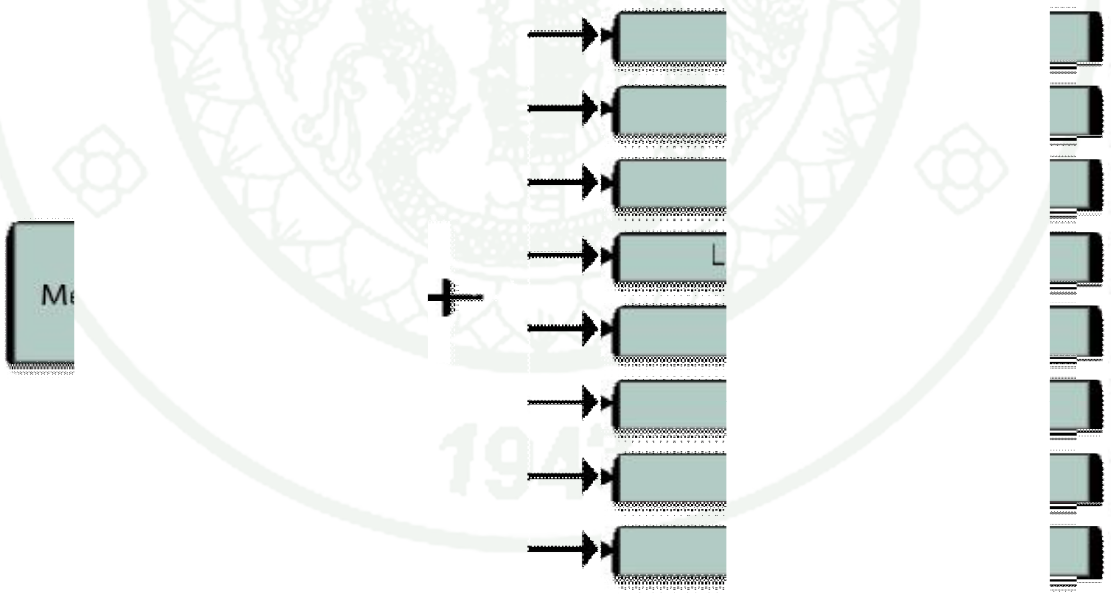


Figure 10 Reliability Characteristics/Method validation parameters.

6.1 The reliability characteristics of the method (method validation)

6.1.1 Accuracy : The accuracy of the analytical method and measured values are close to the true value is given by the percentage recovery.

6.1.2 Precision : The precision of the analysis is repeated several times, the difference in analytical results obtained from the analysis repeated. Obtained from the standard deviation (Standard Deviation, SD) or coefficient of variance (Coefficient of Variation, CV).

6.1.3 Selectivity : The ability of the method with the specificity to analyze the substances

6.1.4 Sensitivity : The ability to analyze the substance in very small quantities. Or a method that can separate different concentrations less accurate.

6.1.5 Limit of Detection and Limit of Quantitation (LOD, LOQ) : Calculated by the formula : Limit of Detection = X + 3SD and Limit of Quantitation = X + 10SD

6.1.6 Linearity : The ability of analytical methods to the analysis that is proportional to the concentration of the substance in the concentration specified. Which can be obtained by analyzing samples at different concentrations. From least to most to be measured and then used to calculate Correlation coefficient.

6.1.7 Range: The range of concentrations to be measured from the lowest concentration to the highest concentration measured with accuracy, precision, and linearity is acceptable according to the specifications. Standards are generally measured at concentrations of at least 6 points.

6.1.8 Ruggedness / Robustness: The persistence of test method even if it is done in different laboratories gave results with little deviation.

6.2 Determination of TCA in Urine Sample

The well mixed urine samples were aliquot 1 mL onto 10 mL headspace vials and crimp seal with septa. The samples in headspace vials were analyzed using a HP 6890 GC coupled with an electron capture detector (ECD), a HP 7649 headspace auto-sampler and a HP 6890 injector. The system was equipped with split-splitless injection inlet and 1 µL of the sample was injected in split mode at 250 ºC. The capillary column (GsBP-FFAP, 50 m x 0.32 mm, df 0.50 µm) was used with nitrogen as carrier gas at a constant flow (1.5 mL /min). The GC oven was operated with the following temperature program: initial temperature 120 ºC held for 1 min, ramped at 10 ºC/min to 240 ºC not held, followed by a ramp of 3 ºC/min to 240 ºC. Temperature of the ECD detector was at 300 ºC. The total run time was 12 min and Agilent ChemStation chromatography data system was used for instrument control and data analysis. Quantification of the urine metabolite was by peak area using the external standard method. Principle diagram analysis by headspace sampler techniques and the instrument used in the analysis shown in Figure 11-12.

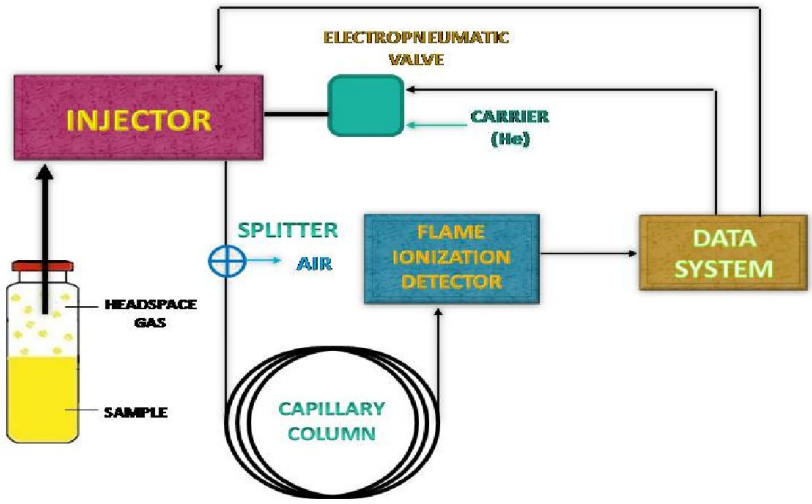


Figure 11 Principle diagram analysis by headspace sampler techniques.

Figure 12 Gas chromatography- Electron Capture Detector with Headspace sampler (GC-ECD-HS).

7. Blood Sample and Lymphocytes Cultures

Blood samples were taken by venipuncture (approximately 5 mL) and drawn from each subject into heparinized tubes. Samples were coded and processed within 3 hr. Lymphocytes were isolated and washed. DNA damaged were evaluated by MN assays. Peripheral blood samples were obtained and cultured together with the second urine collection in phase 2

Heparinized blood samples (0.5 mL) were incubated for 72 hours in CO_2 incubator at 37 ºC in 4.5 mL of the RPMI 1640 medium that was supplemented with 10% heat-inactivated fetal bovine serum (FBS), 3% Phytohemagglutinin (PHA), 1% antibiotics (100 UI/mL penicillin and 100µg/mL streptomycin) and 2% of 2 M L-glutamine. Two parallel cultures of each person were treated for 44 hours with 600 µg/mL Cytochalasin-B (Cyt-B). The Cyt-B was dissolved in Dimethyl sulfoxide. Blood collection and lymphocyte culture shown in Figure 13.

Figure 13 Blood collection and lymphocyte culture.

8. Micronucleus assay

According to the method of Fenech and Morley (1985), after 44 hours of incubation, Cyt-B was added to cultures to give a final concentration of 600 µg/mL. The cultures were stopped at 72 hours. The lymphocytes were collected by centrifugation at 800 x g at room temperature for 10 min and treated with 10 mL of hypotonic solution (0.075 M KCl). Pellet the cells by centrifugation. After removing the supernatant, the pellet cells were fixed by add 5 mL of 3:1 methanol-glacial acetic acid with 1% formaldehyde and centrifuged at 800 x g at room temperature for 10 min and fixed in two changes of 3:1 methanol-glacial acetic acid without 1% formaldehyde (Donmez-Altuntas *et. al.,* 2003). The fixed cells were spread onto glass slides and stained with 3% Giemsa for 10 min. The entire slide were coded and read blind. In order to determine intra individual differences, two parallel cultures of each person were made for each group and the different slides of two parallel cultures were prepared. Cells with two macronuclei surrounded by cytoplasm and a cell membrane were scored for the presence of micronuclei. Micronucleus assay procedure shown in Figure 14.

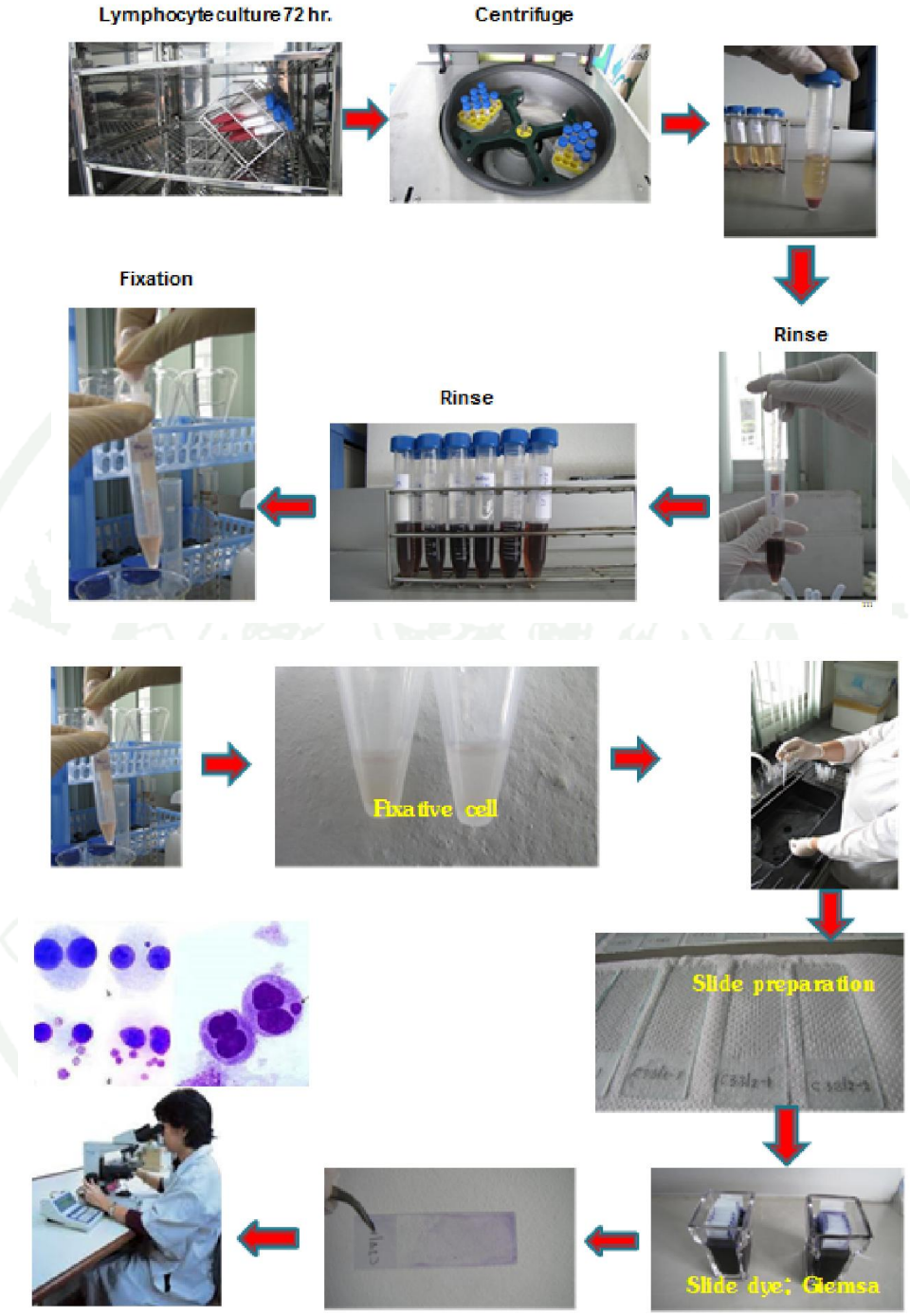


Figure 14 Micronucleus assay Procedure.

9. Scoring criteria

9.1 Criteria for selecting binucleated cells which can be scored for the presence of micronuclei and nucleoplasmic bridges. The cytokinesis-blocked cells that scored for MN frequency should have the following characteristics:

9.1.1 The cells should be binucleated.

9.1.2 The two nuclei in a binucleated cell should have intact nuclear membranes and be situated within the same cytoplasmic boundary.

9.1.3 The two nuclei in a binucleated cell should be approximately equal in size, staining pattern and staining intensity.

9.1.5 The two nuclei within a BN cell may be attached by a fine nucleoplasmic bridge which is no wider than one-fourth of the largest nuclear diameter.

9.1.6 The two main nuclei in a BN cell may touch but ideally should not overlap each other. A cell with two overlapping nuclei can be scored only if the nuclear boundaries of each nucleus are distinguishable.

9.1.7 The cytoplasmic boundary or membrane of a binucleated cell should be intact and clearly distinguishable from the cytoplasmic boundary of adjacent cells.

9.2 Criteria for scoring micronuclei (MN)

MN are morphologically identical to but smaller than the main nuclei. They also have the following characteristics:

9.2.1 The diameter of MN in human lymphocytes usually varies between 1/16 and 1/3 of the mean diameter of the main nuclei which corresponds to 1/256 and 1/9 of the area of one of the main nuclei in a BN cell, respectively.

9.2.2 MN are round or oval in shape.

9.2.3 MN are non-refractile and they can therefore be readily distinguished from artefacts such as staining particles.

9.2.4 MN are not linked or connected to the main nuclei.

9.2.5 MN may touch but not overlap the main nuclei and the micronuclear boundary should be distinguishable from the nuclear boundary.

9.2.6 MN usually has the same staining intensity as the main nuclei but occasionally staining may be more intense.

9.3 Exclusion Criteria. The cell do not score were : shown in figure 15

9.3.1 Trinucleated, quadranucleated, or multinucleated cells

9.3.2 Cells where main nuclei are undergoing apoptosis (because MN may be gone already or may be caused by apoptotic process)

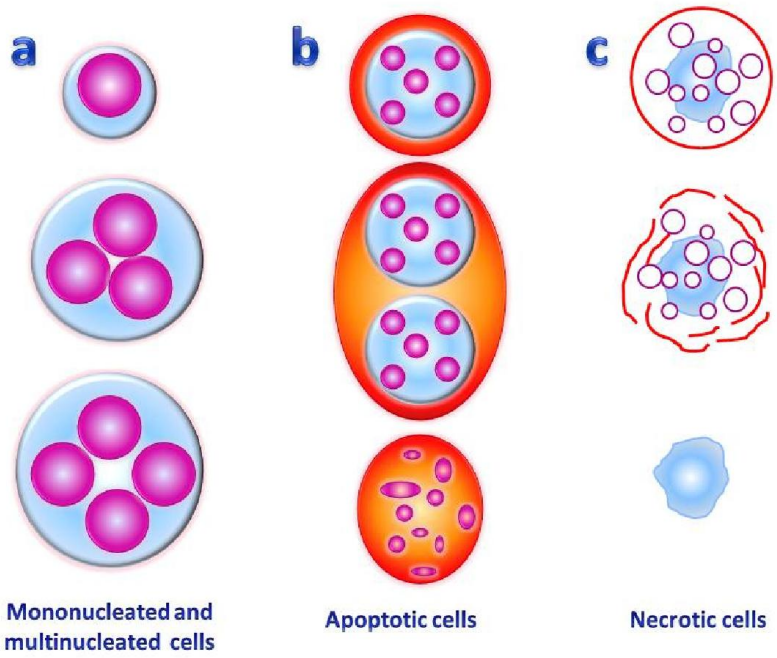


Figure 15 The Characteristics of cells do not score.

10. Statistical analysis

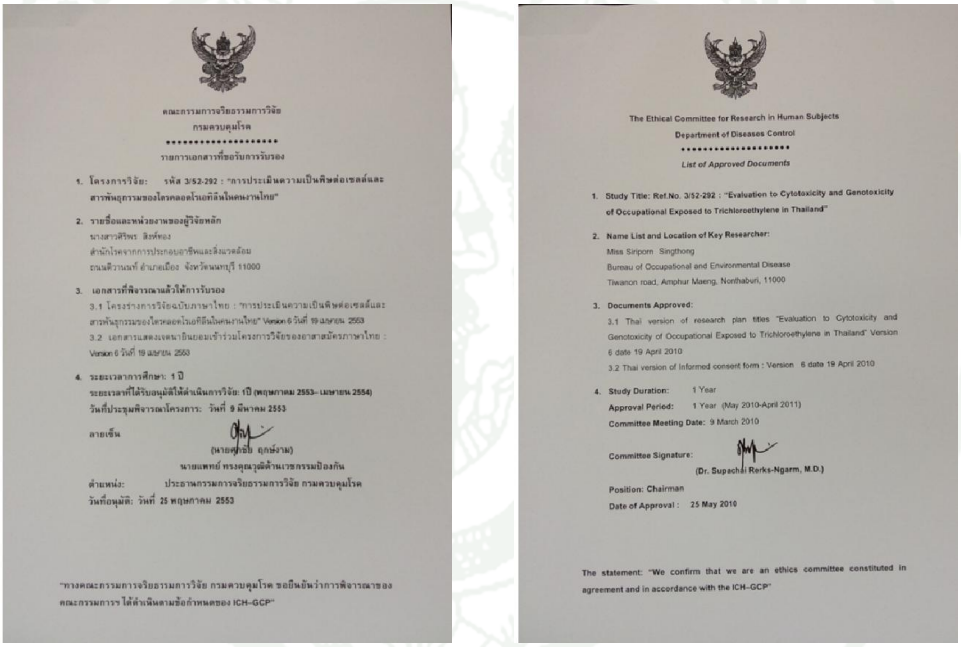
The descriptive statistics such as median, standard deviation and percentage were used to describe the general information of the subjects. The inferential statistics such as paired sample t-test was used to compare TCA in urine and the level of DNA damage in blood of the exposed and control groups. In addition, Multiple regression Analysis was used to predict the in fluent factors such as concentration of chemical exposure, work duration, job duty, health risk factors, domicile, sex, age, weight, health history, work history, life style genetics and stress.

RESULTS AND DISCUSSION

Results

1. Ethical issue

The study was approved by the Ethics Committee of Department of Disease Control, Ministry of Public Health, Thailand. (Figure 16)



คณะกรรมการจริยธรรมการวิจัย
กรมควบคุมโรค
••••••••••••••••••••
รายการเอกสารที่ขอรับการรับรอง

1. โครงการวิจัย: รหัส 3/52-292 : "การประเมินความเป็นพิษต่อเซลล์และสารพันธุกรรมของโครคลอโรเอทธีลีนในคนงานไทย"
2. รายชื่อและหน่วยงานของผู้วิจัยหลัก
นางสาวศิริพร สิงห์ทอง
สำนักโรคจากการประกอบอาชีพและสิ่งแวดล้อม
ถนนติวานนท์ อำเภอเมือง จังหวัดนนทบุรี 11000
3. เอกสารที่พิจารณาเพื่อให้การรับรอง
3.1 โครงร่างการวิจัยฉบับภาษาไทย : "การประเมินความเป็นพิษต่อเซลล์และสารพันธุกรรมของโครคลอโรเอทธีลีนในคนงานไทย" Version 6 วันที่ 19 เมษายน 2553
3.2 เอกสารแสดงเจตนายินยอมเข้าร่วมโครงการวิจัยของอาสาสมัครภาษาไทย : Version 6 วันที่ 19 เมษายน 2553
4. ระยะเวลาการศึกษา: 1 ปี
ระยะเวลาที่ได้รับอนุมัติให้ดำเนินการวิจัย: 1ปี (พฤษภาคม 2553- เมษายน 2554)
วันที่ประชุมพิจารณาโครงการ: วันที่ 9 มีนาคม 2553
ลายเซ็น
(นายศุภชัย ยุกษ์งาม)
นายแพทย์ ทรงคุณวุฒิด้านเวชกรรมป้องกัน
ตำแหน่ง: ประธานกรรมการจริยธรรมการวิจัย กรมควบคุมโรค
วันที่อนุมัติ: วันที่ 25 พฤษภาคม 2553

"ทางคณะกรรมการจริยธรรมการวิจัย กรมควบคุมโรค ขอยืนยันว่าการพิจารณาของคณะกรรมการฯ ได้ดำเนินตามข้อกำหนดของ ICH-GCP"

The Ethical Committee for Research in Human Subjects
Department of Diseases Control
••••••••••••••••••••
List of Approved Documents

1. Study Title: Ref.No. 3/52-292 : "Evaluation to Cytotoxicity and Genotoxicity of Occupational Exposed to Trichloroethylene in Thailand"
2. Name List and Location of Key Researcher:
Miss Siriporn Singthong
Bureau of Occupational and Environmental Disease
Tiwanon road, Amphur Maeng, Nonthaburi, 11000
3. Documents Approved:
3.1 Thai version of research plan titles "Evaluation to Cytotoxicity and Genotoxicity of Occupational Exposed to Trichloroethylene in Thailand" Version 6 date 19 April 2010
3.2 Thai version of Informed consent form : Version 6 date 19 April 2010
4. Study Duration: 1 Year
Approval Period: 1 Year (May 2010-April 2011)
Committee Meeting Date: 9 March 2010
Committee Signature:
(Dr. Supachai Rorks-Ngarm, M.D.)
Position: Chairman
Date of Approval : 25 May 2010

The statement: "We confirm that we are an ethics committee constituted in agreement and in accordance with the ICH-GCP"

Figure 16 The study was approved by the Ethics Committee of Department of Disease Control, Ministry of Public Health, Thailand.

2. Study population

The cytogenetic monitoring was studied in Thai workers who worked in the factory that used TCE as the solvent.(Figure 17) The non-exposed healthy persons of the same communities who had no occupational contact with TCE, were used as a control group. The purpose of this study was to assess the toxicity of TCE to the

lymphocyte cells. All of the participants, recruited as described in Appendix A, had answered the questionnaires.

Figure 17 Factory used TCE as the solvent and product characteristics.

3. Questionnaire

The primary purpose of the questionnaire was to screen the workers for exposure to known and suspected genotoxins and to quantify any such exposure. The four parts of questionnaire were personal data, occupational chemical exposure, persona habits, and medical history. Occupational genotoxins covered exposure to heavy metals, solvents, pesticides and radiation. Personal habits included smoking, alcohol consumption, caffeine consumption, and use of artificial sweeteners. The medical section covered chronic health problems, recent infections, cancer history (including cancer in family), medications, vaccinations, and x-ray exposure. The personal part of the questionnaire covered sex, age, marital status, income, and education. These data were used to make a demographic comparison with the control group.

The descriptive statistics such as median, standard deviation and percentage were used to describe the general information of the subjects. The inferential statistics such as paired sample t-test was used to compare TCA in urine and the level of DNA damage in blood of the exposed and control groups. In addition, Multiple regression analysis was used to predict the influent factors such as concentration of chemical exposure, work duration, job duty, health risk factors, domicile, sex, age, weight, health history, work history, life style genetics and stress.

4. Description of the Study Group

After the questionnaires were completed, the characteristics of the study group in the criteria included sex, age, marital status, education level, domicile, duration of employ, year of living, working hour, work history, health history, family health history and life style are presented in Appendix Table 1. The data also show a raw number and percentage of each characteristics.

The general characteristics of study group. (Figure 19) The participants in study group were 52 men (21.5%) and 190 women (78.5%), the most were in the age range 21 - 30 years which was 38.8% (Figure 18). Sixty-two percent of the study group were married at the time of survey (Figure 20). A majority of the participants had completed primary school (36.4%) and junior high school (34.3%) (Figure21). Approximately one-third (33.4%) of study group had stayed in Bangkok about 2 – 5 years (Figure 22). Almost one-half (48.8%) of participants resided in Northeast and only 0.8% were from west region of Thailand (Figure 23).

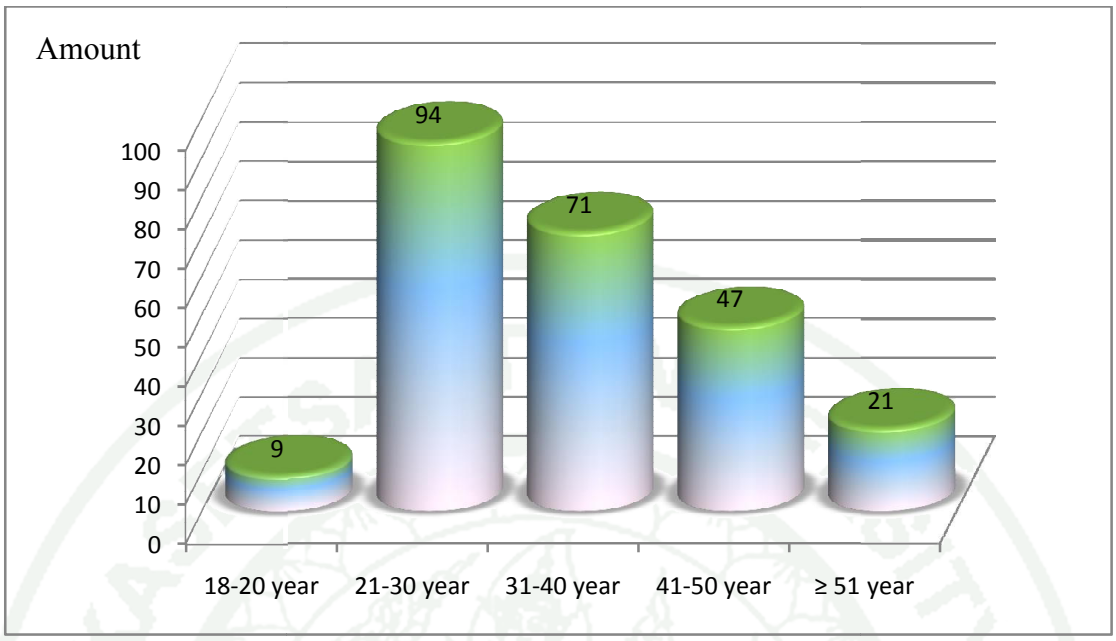


Figure 18 Demographic statistics of study group (Age).

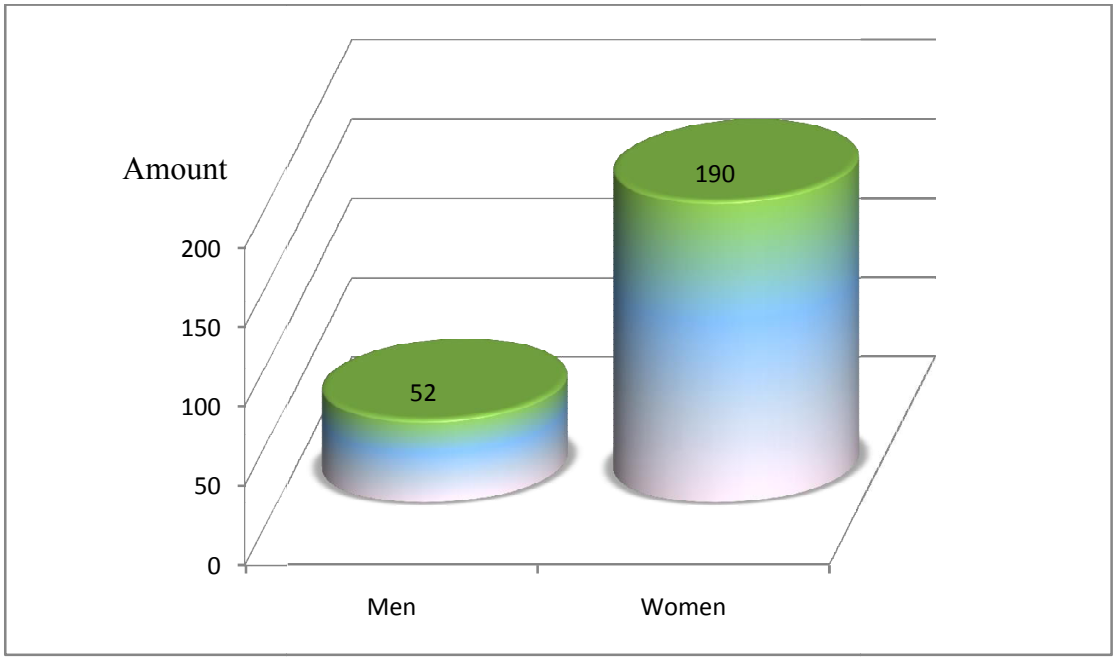


Figure 19 Demographic statistics of study group (Gender).

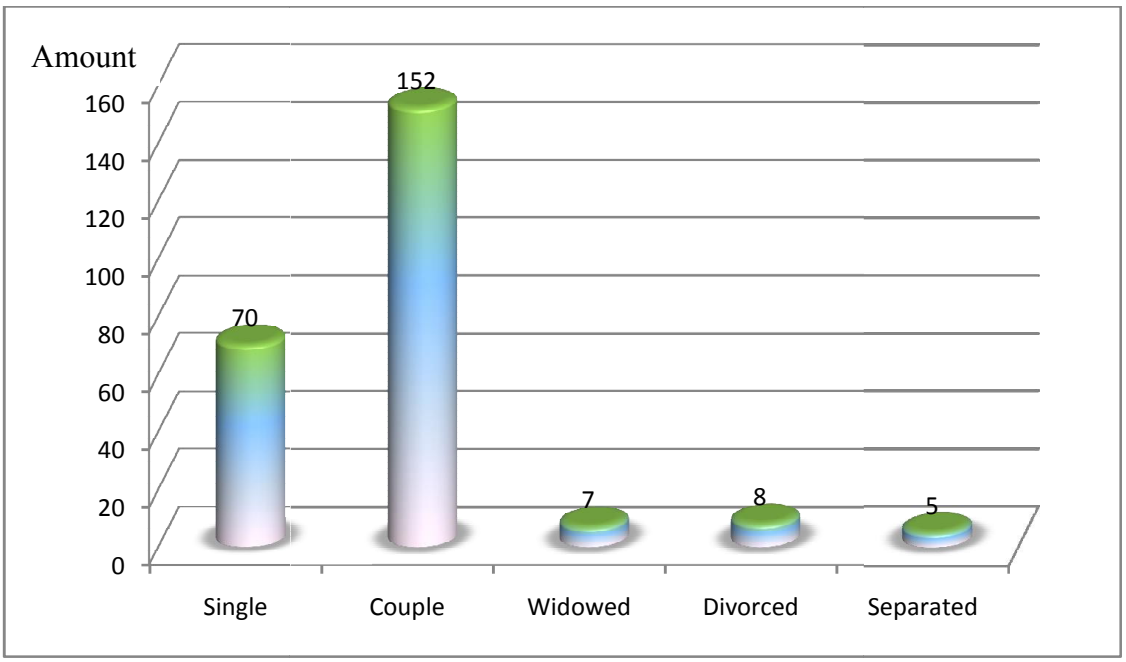


Figure 20 Demographic statistics of study group (Marital status).

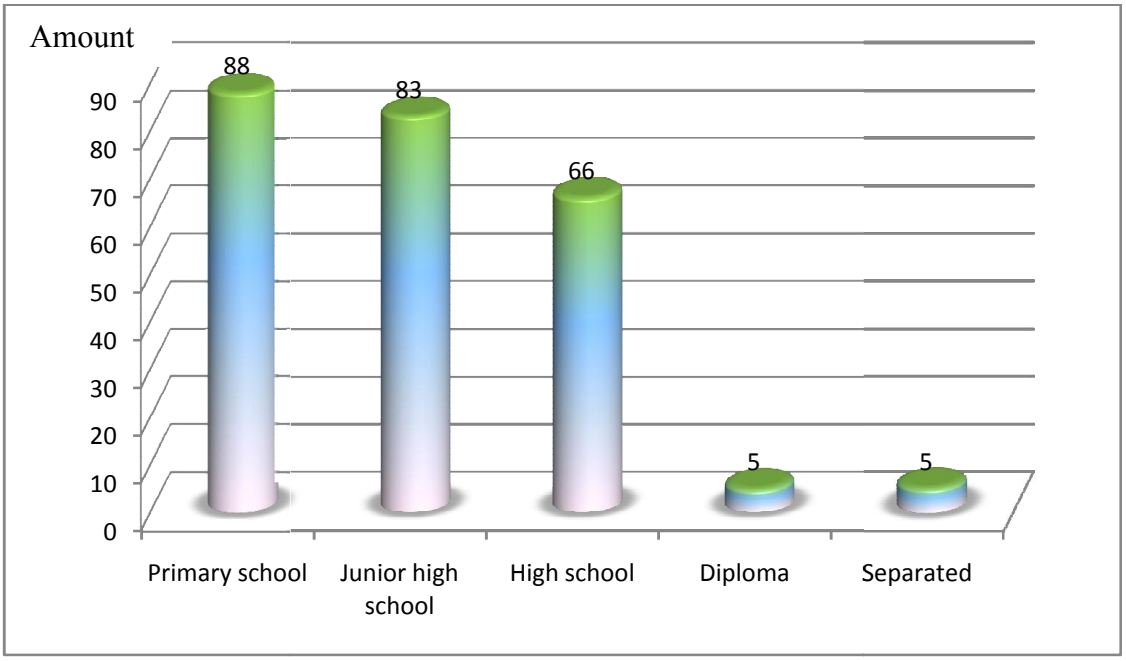


Figure 21 Demographic statistics of study group (Education level).

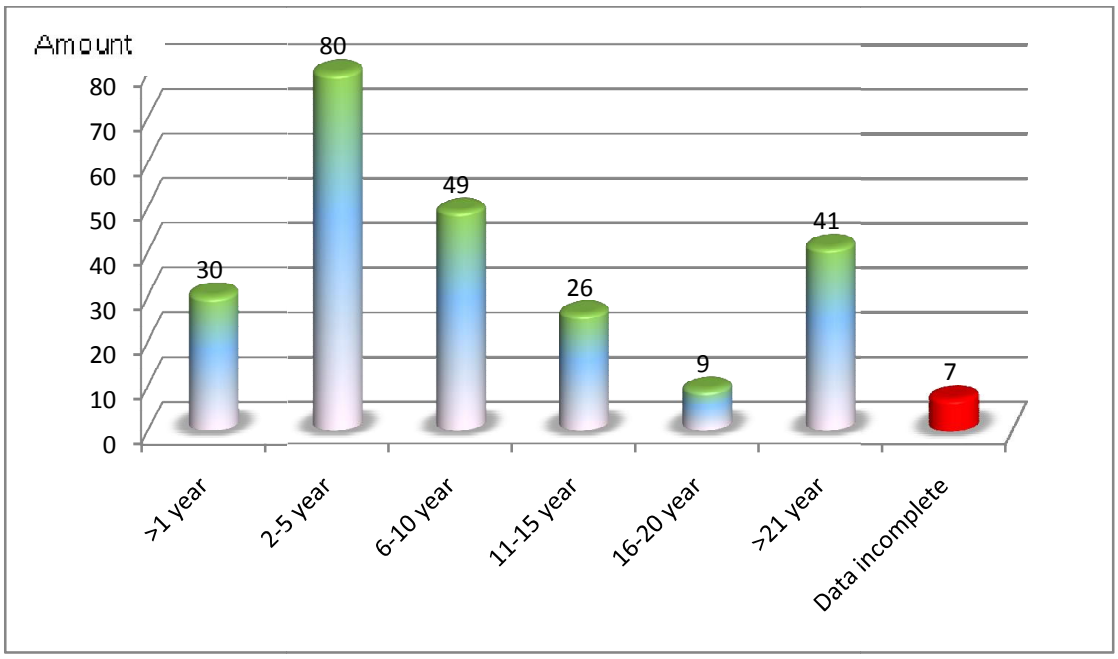


Figure 22 Demographic statistics of study group (Length of stay).

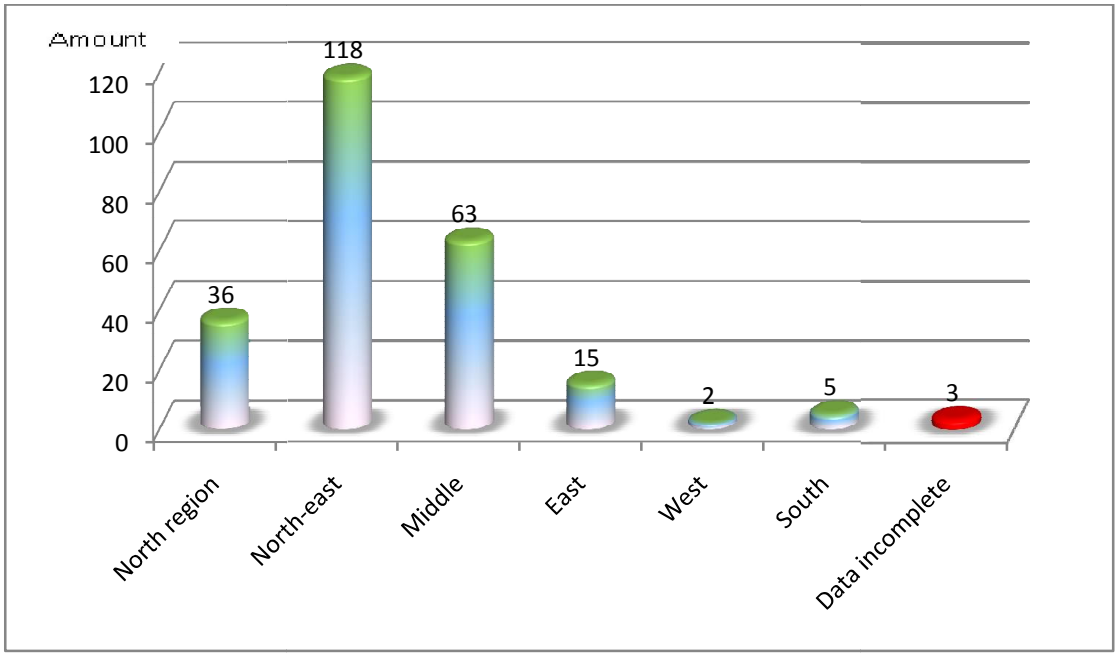


Figure 23 Demographic statistics of study group (Domicile).

The analyses of occupational history are shown in Figure 24-27. Participants were specifically asked about prior employment in chemical factory or other places where they may have been exposed to genotoxic substances. Thirty eight percent of participants, who had worked at least 1 factory, reported experience of chemical exposures based on their job duties. Considering for the job position, seventy percent of participants worked in the position of unexposed. The majority of exposed group (33.5%) reported the duration of work between 2-5 years. Nearly 60% of health risk factors were chemical substances. The majority personal protective equipment was masks (31.0%) or gloves (3.7%), however only 0.8% used both of masks and gloves at the same time.

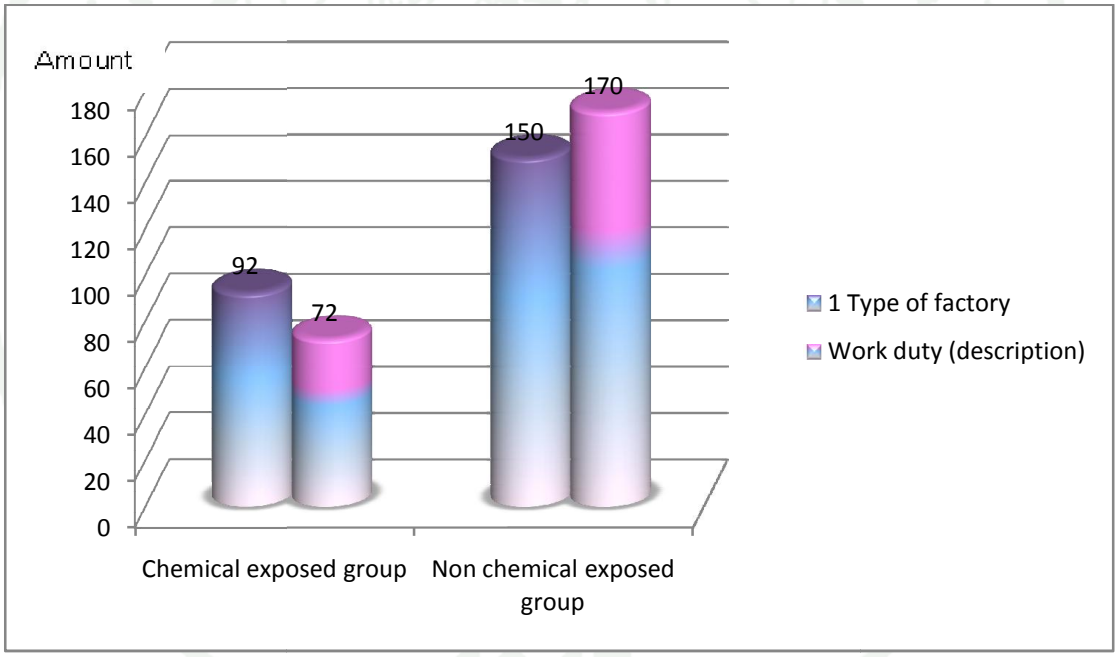


Figure 24 Study group differentiated by occupational history : Benchmark between type of factory 1 and work duty (description).

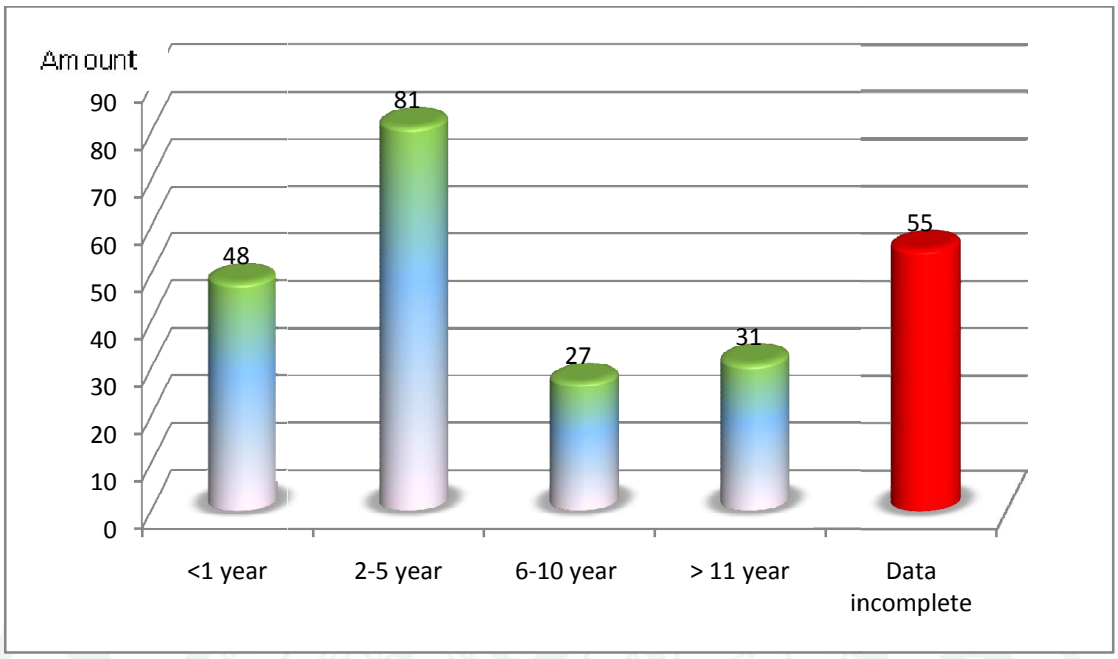


Figure 25 Study group differentiated by occupational history : Duration of employ.

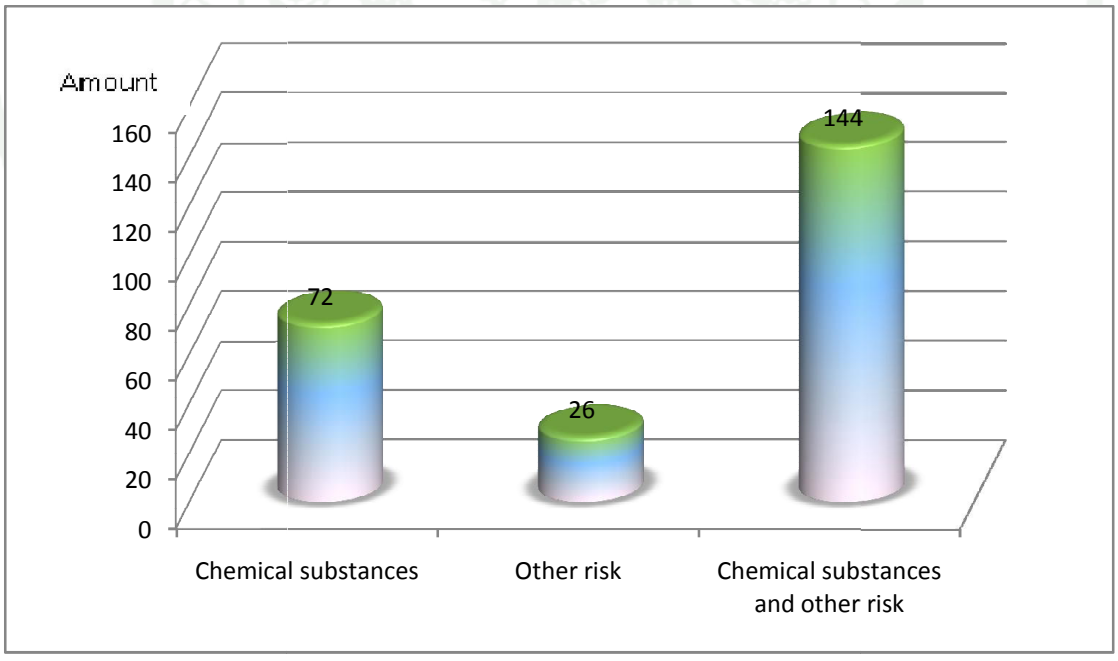


Figure 26 Study group differentiated by occupational history : Health risk factor.

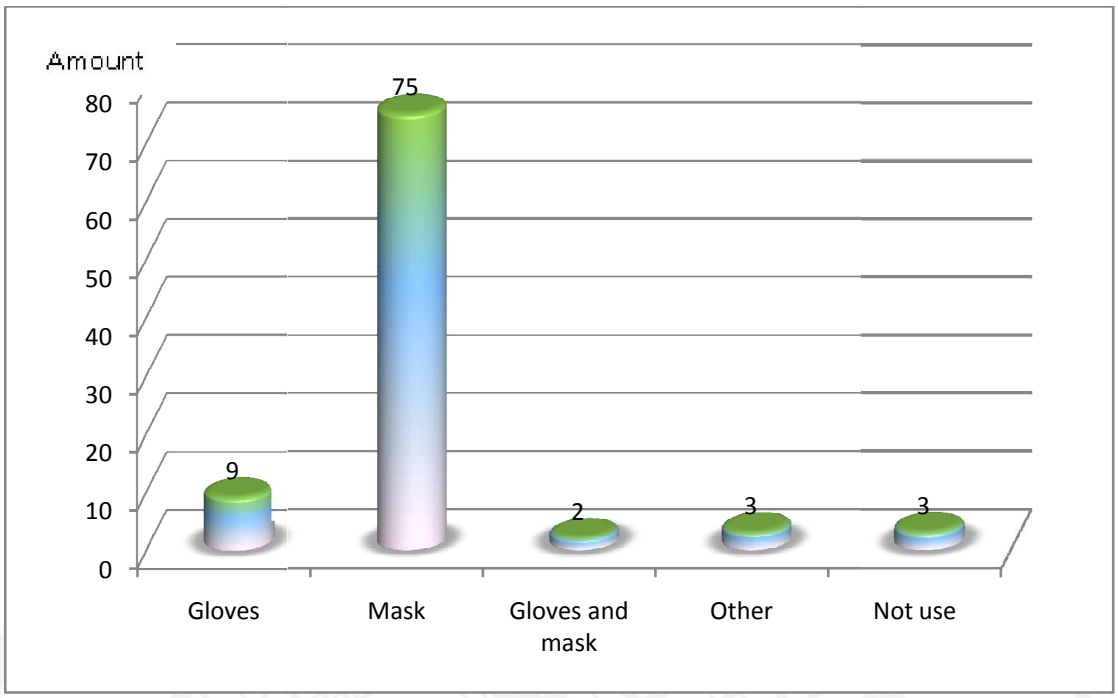


Figure 27 Study group differentiated by occupational history : Personal protective equipments**.**

Approximately thirty four percent of participants, who used to work in 2 factories, reported experience of chemical exposures based on their job duties. Related to the job category, it was shown that 33.5% of participants reported particularly work-related chemical exposure (Figure 28). The majority of exposed group (16.1%) had the duration of work between 2-5 years (Figure 29). Eighteen percent of exposed group reported that health risk factors were trichloroethylene (Figure 30).

Approximately 3.7% of participants, who used to work at least 3 factories, reported chemical exposures based on their job duties. Considering for the job position, 2.5% of participants worked as polishing and 1.2% worked in the position of line puncture (Figure 31). About 1.2% of exposed group reported the duration of work between 2-5 years and 6-10 years (Figure 32). Less than one percent (0.8%) of exposed group reported that health risk factor was trichloroethylene and 1.7% reported exposed to polishing substances and other chemical substances(Figure 33).

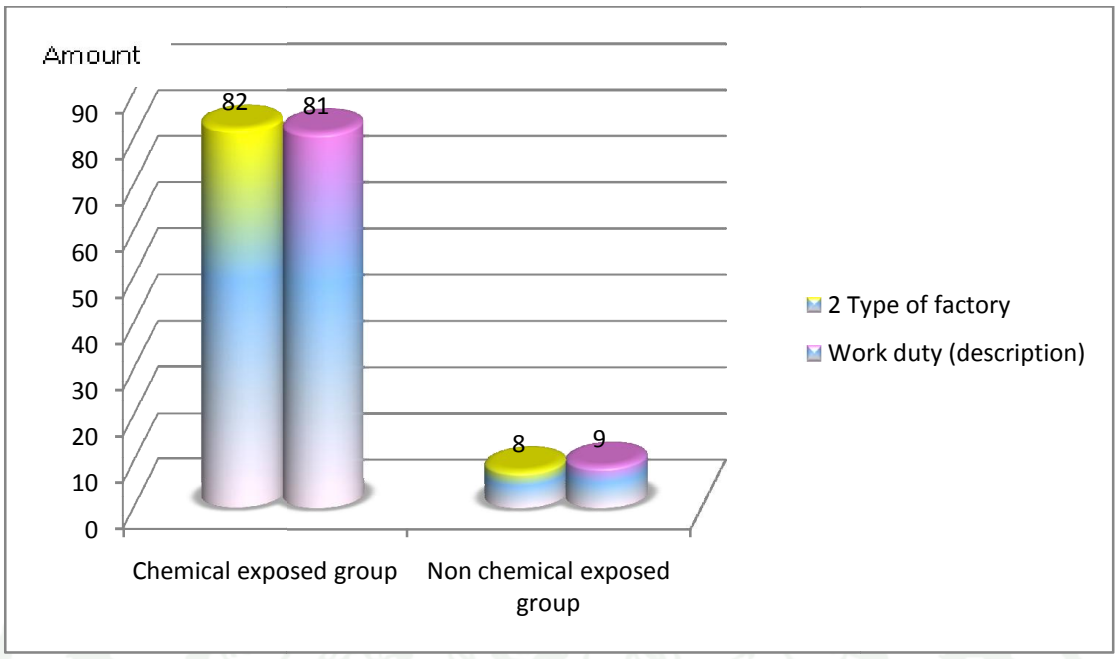


Figure 28 Study group differentiated by occupational history : Benchmark between type of factory 2 and work duty (description).

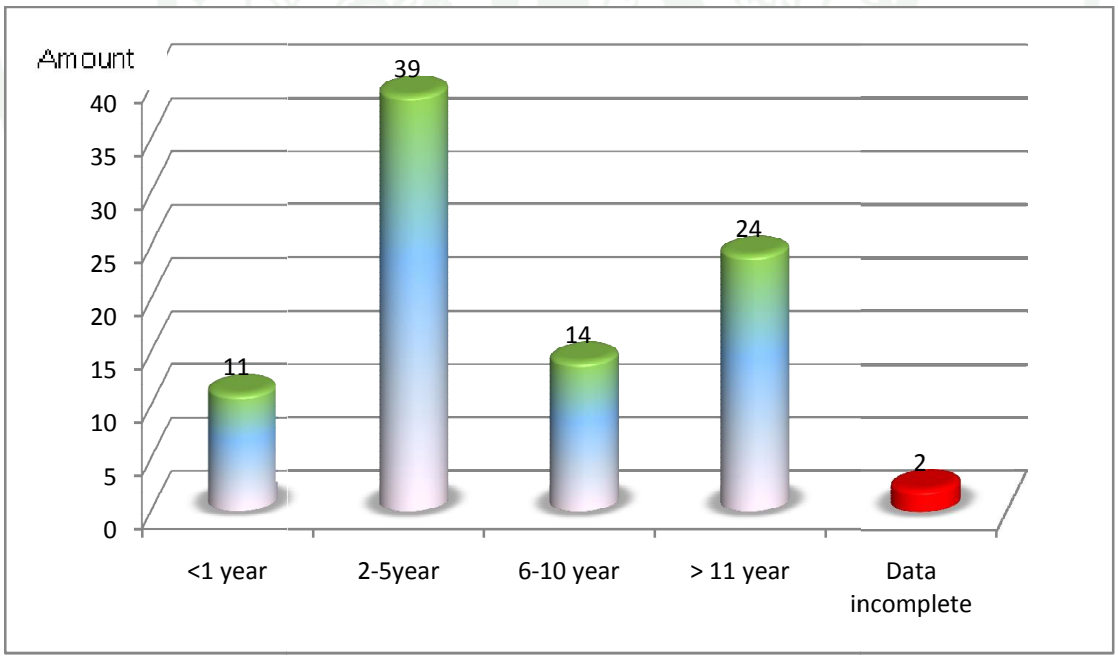


Figure 29 Study group differentiated by occupational history : Duration of employ**.**

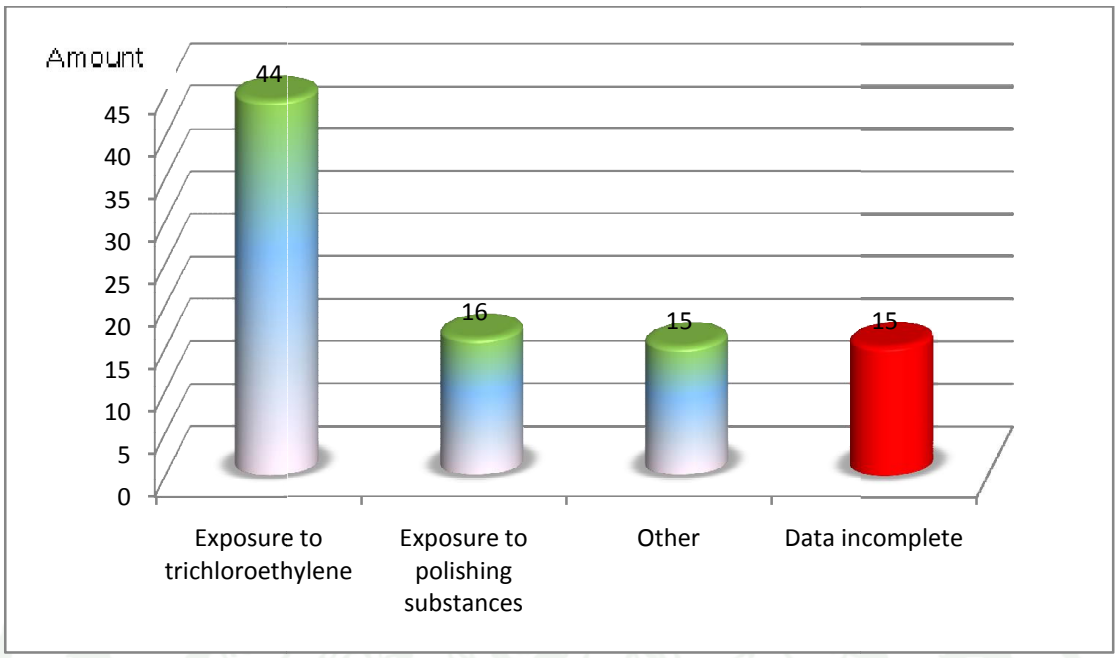


Figure 30 Study group differentiated by occupational history : Health risk factors.

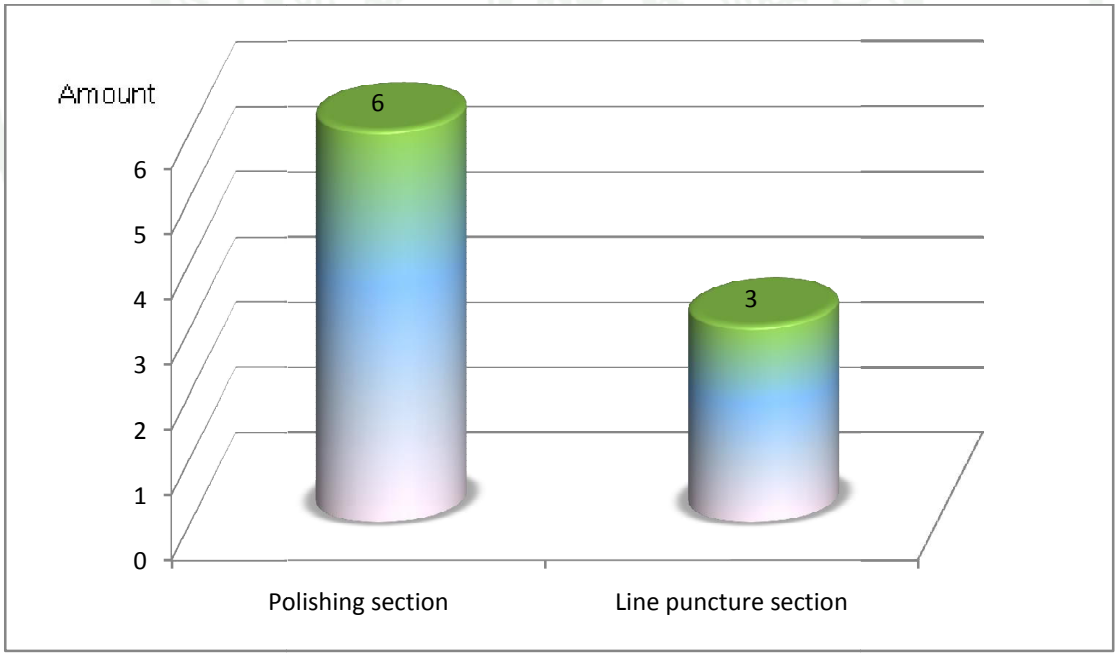


Figure 31 Study group differentiated by occupational history : factory 3.

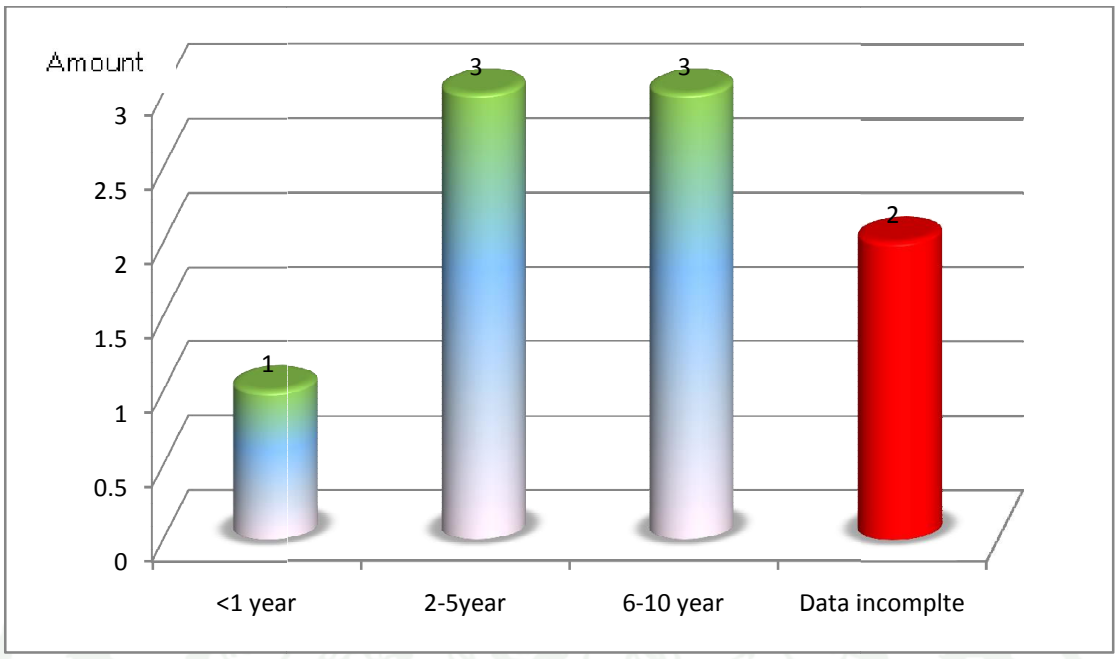


Figure 32 Study group differentiated by occupational history : Duration of employ.

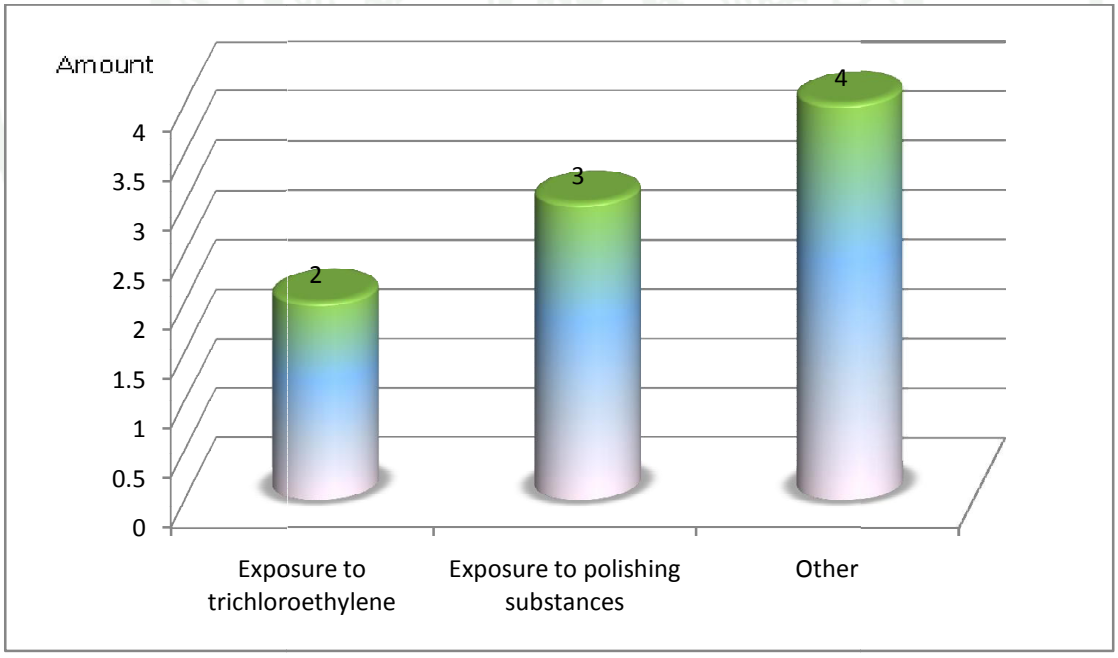


Figure 33 Study group differentiated by occupational history : Health risk factors.

Figure 34-36 shows the overview data of health history of participants. The majority of this group was generally healthy. Approximately, 85.5% of participants reported no evidence of cancer; however 14.5% were diagnosed cancer. Respiratory diseases and skin diseases were reported by 3.7% of participants surprisingly, 81.8 % reported no evidence of respiratory diseases. Chronic health problems were reported such as diseases of nervous system (3.7%), allergic (14.5%), high blood pressure (4.1%) and anemia (1.7%). In addition, 7.9% of participants, who reported the health problem, not indicated the specific diseases.

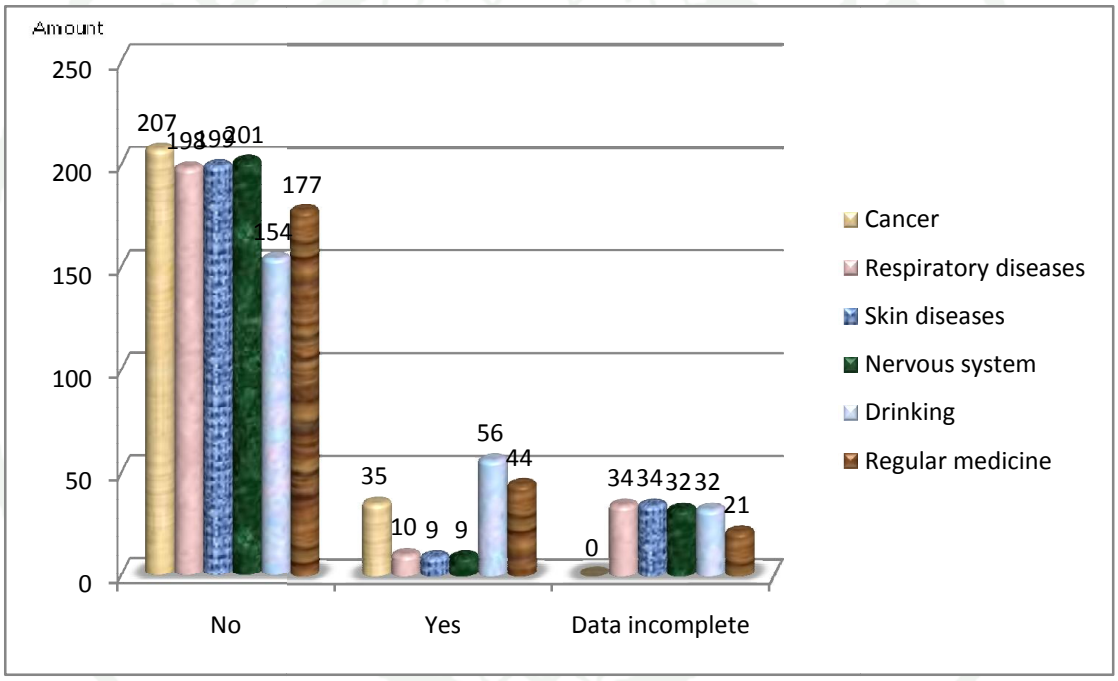


Figure 34 Study group differentiated by healthy history : Healthy history.

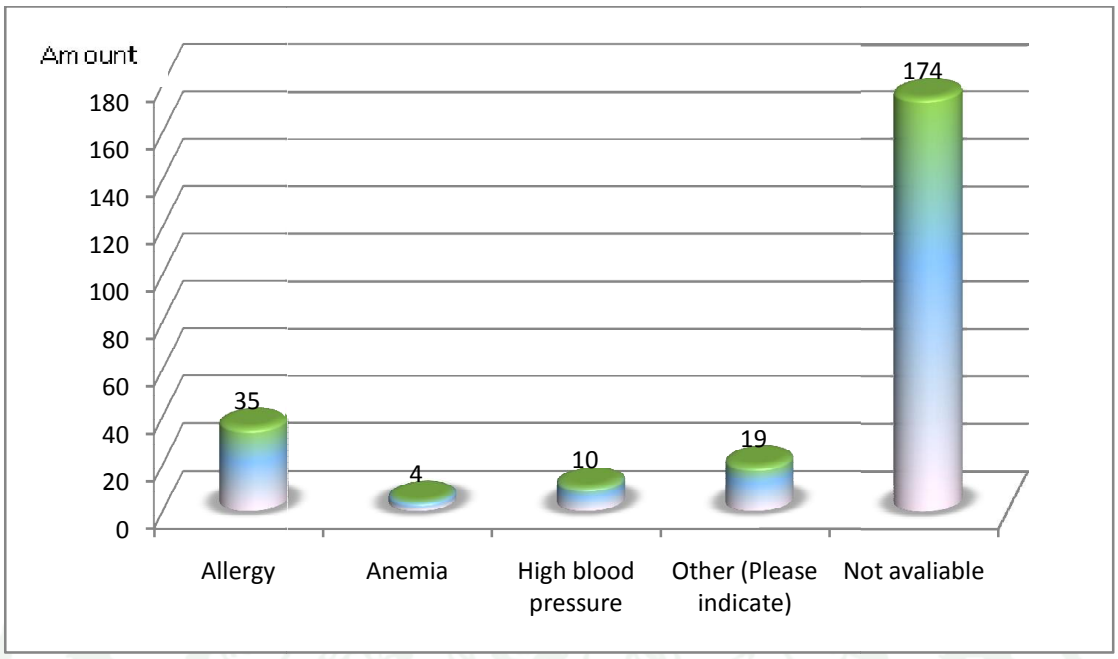


Figure 35 Study group differentiated by healthy history : Chronologically ill**.**

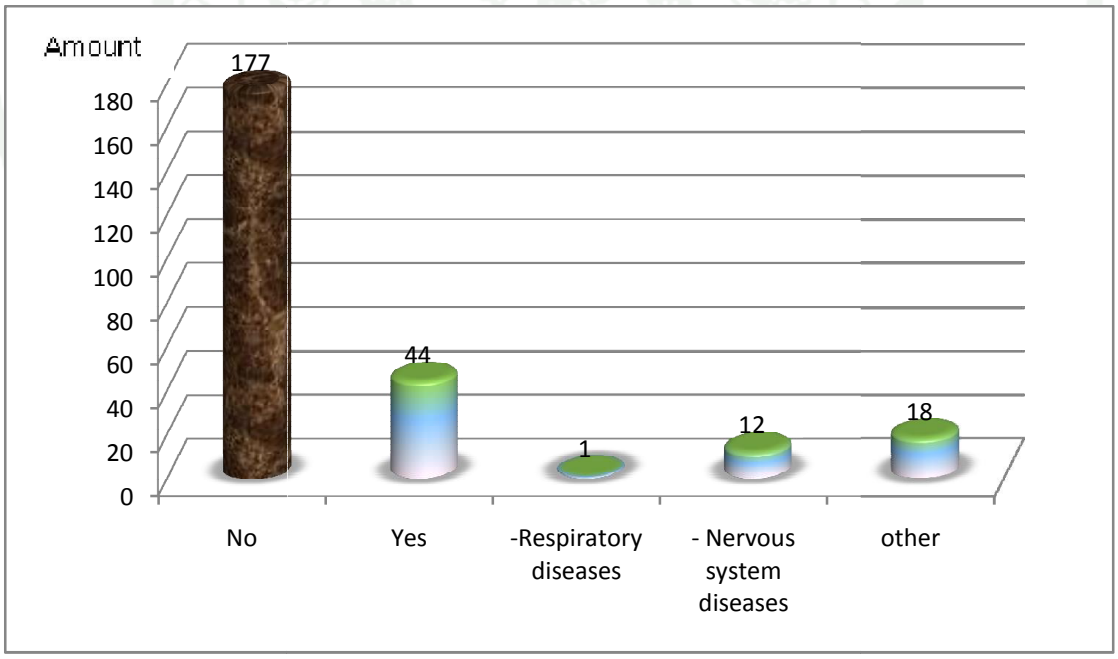


Figure 36 Study group differentiated by healthy history : Regular medicine.

The analyses of family health history of study group are shown in Figure 37-38. Among participants, 14.5% reported a family history of cancer, 21.1 % reported a family history of high blood pressure, 7.9% reported a family history of asthma, and 4.1% reported a family history of allergic. In addition, about 2.1 % reported tuberculosis (TB) in some family member. Abnormal of gastrointestinal system, cardiovascular diseases and hormone disorder were reported in 21.1% of participants.

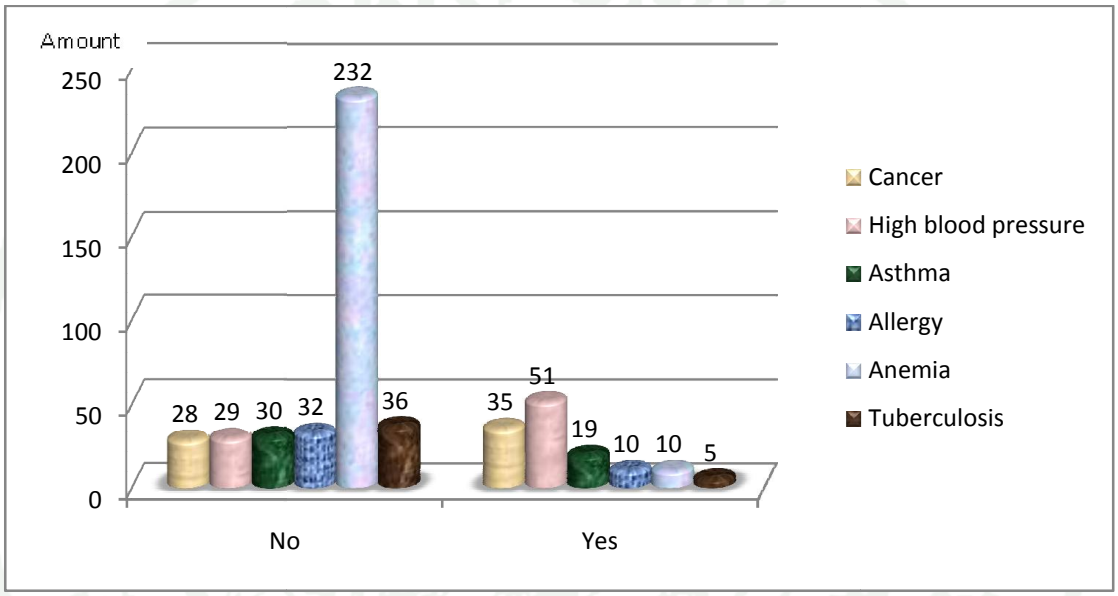


Figure 37 Study group differentiated by family healthy history : Family healthy history.

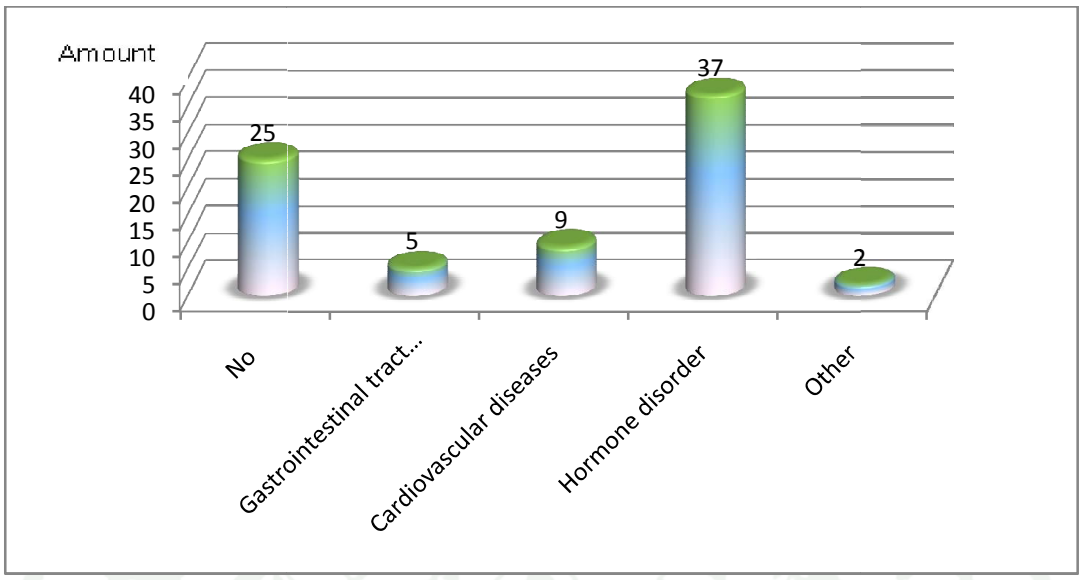


Figure 38 Study group differentiated by family healthy history : Other disease.

The analyses of current health of study group are shown in Figure 39-40. Participants reported a high prevalence of symptoms which occurred more than three times during the past year or which lasted more than 1 week. Symptom prevalence was lower, but still notable, when defined as symptoms which had occurred at least once a month or lasted more than a week in the past year. Respiratory tract symptoms were experienced more frequently such as cough (8.3%), suffer from sneeze and produce sputum were reported by 9.1% of population, 4.10% reported tightness in the chest, 3.7% reported wheezing in the chest, 5.4% reported shortness of breath, 2.5% reported bronchitis while impairment of smell were reported by only 0.4% of participants. Of the individual skin symptoms included in our questionnaire, the more prevalent symptoms reported were red hands or fingers with fissures (2.1%) and skin itch (6.2%). The other skin symptom such as white scar was reported less prevalence by 4.1% of participants.

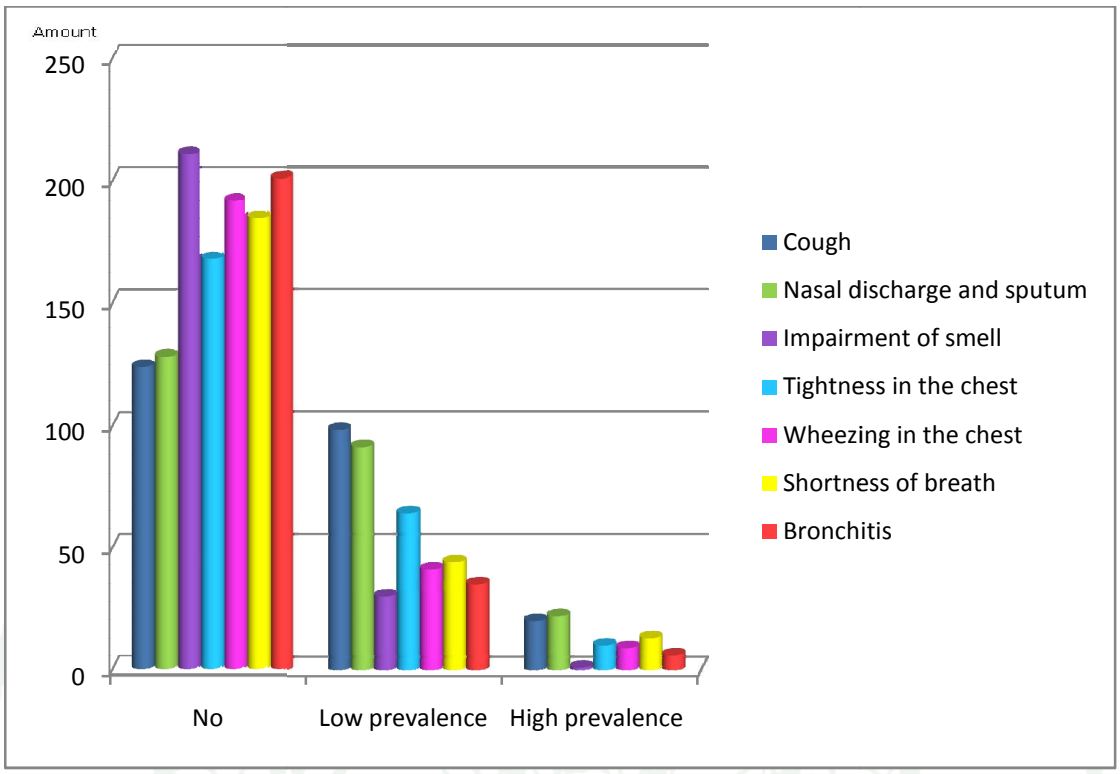


Figure 39 Current health status and disease : Health status and disease.

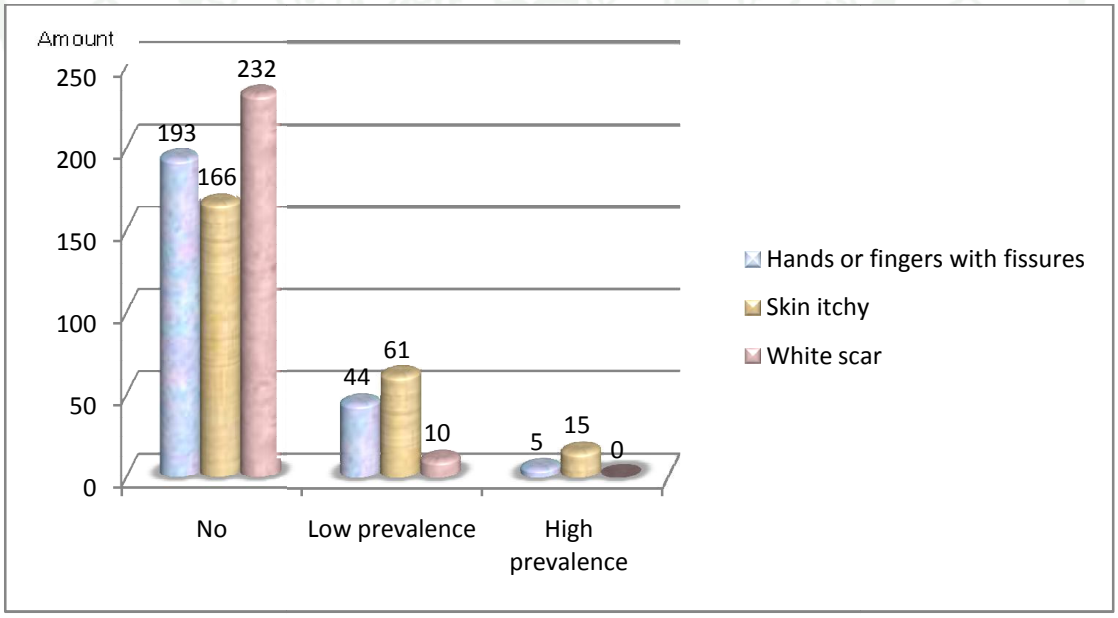


Figure 40 Current health status and disease : Skin symptom.

The most common complaint reported by participants about nervous system was headache (14.5%) followed by Dizziness (12%), sleepless (7.96%), poor vision (7.4%), Memory loss (7.4%), Limb numbness (7%), muscle weakness (7%), Fatigue (6.2%), Symptoms from the eyes (running) (5.0%), and other types of nervous symptom were reported less than 5% as shown in Figure 41.

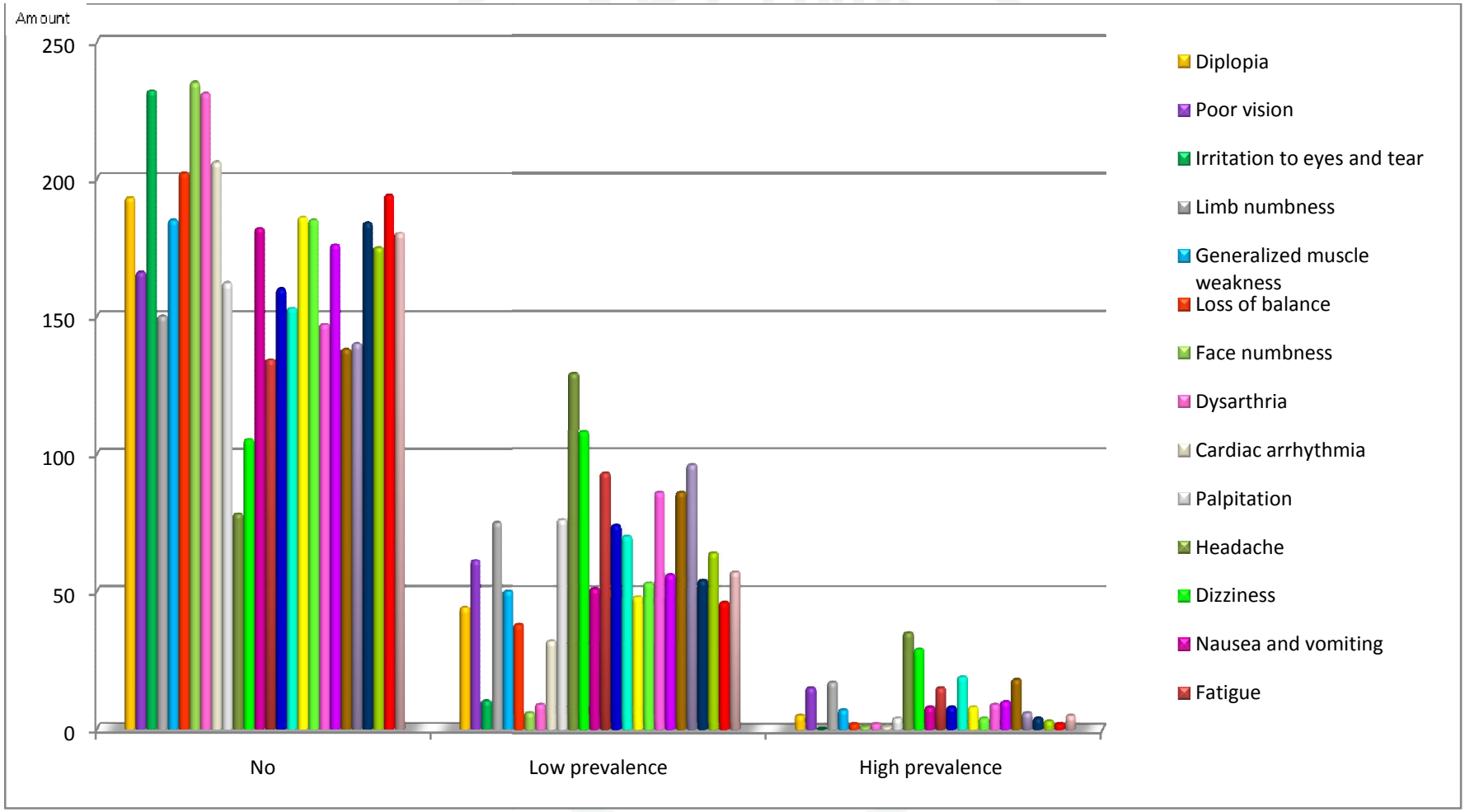


Figure 41 Current health status and disease : Nervous system symptom.

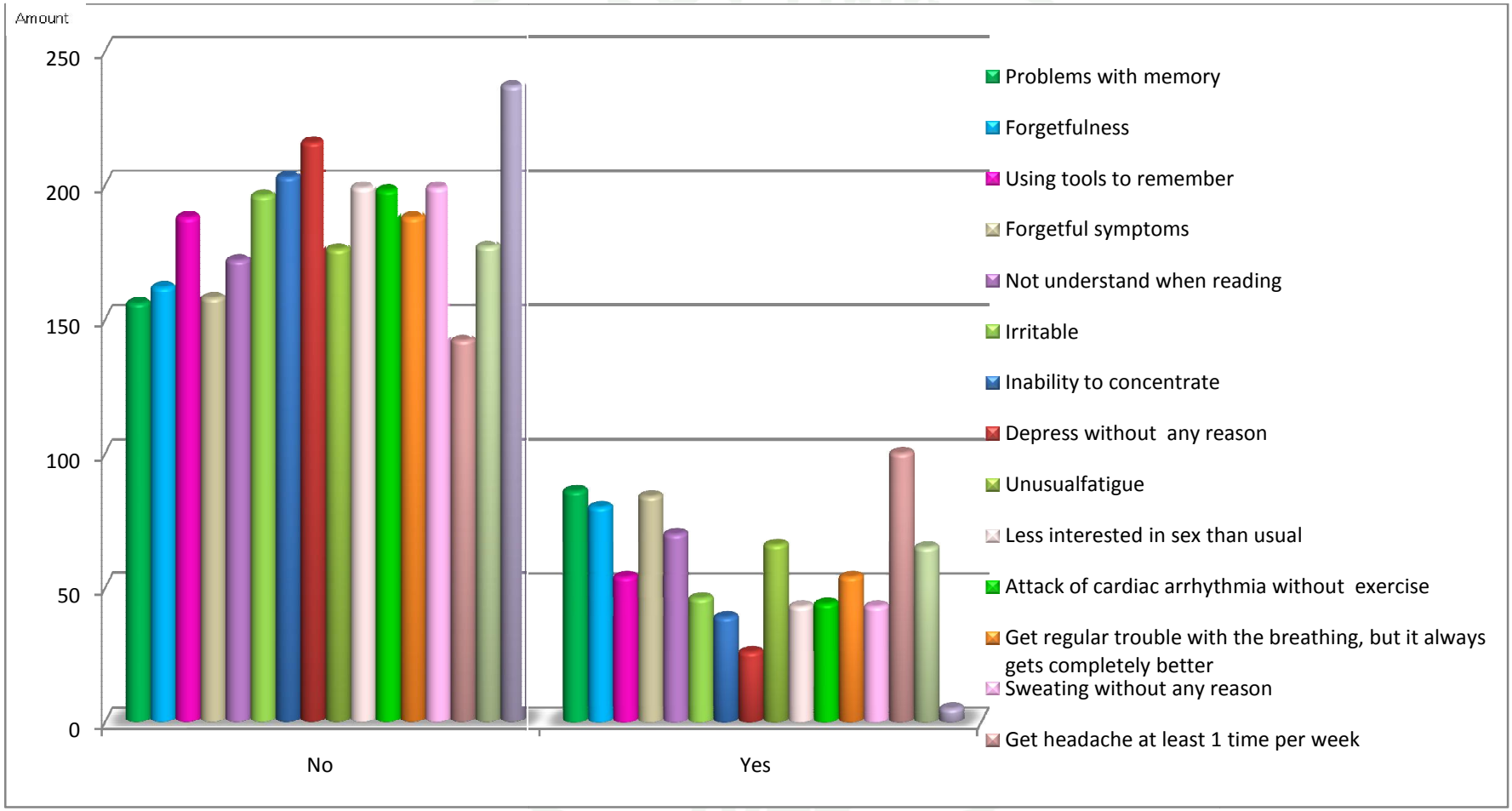


Figure 42 Participants differentiated by lifestyle.

The analyses of life style of study group are shown in Figure42. Approximately 35.5% reported that they had problems with memory, 33.1% reported forgetfulness, 22.3% reported using tools to remember**,** and 34.7% reported having repetition. Of the individual emotional symptoms included in our questionnaire, 28.9% reported having trouble with reading, while 16.1% reported inability to concentrate, 19% reported that their emotion is irritable, 10.7%. When asked about sexuality, 17.8% reported less interested than usual. Looking at cardiac related symptom, 18.2% reported arrhythmia without any activity, 22.3% got regular trouble with the breathing, 17.8% reported sweating without any reason, 41.3% had suffered from headache at least 1 time per week and 26.9% reported muscle pain.

Regarding the years at work, 36.0% of the participants worked up to 11 years, 33.9% worked between 2-5 years. 72.3% of the sample-studied worked in the position of polishing and 45.9% reported exposure to TCA all time of work day (Figure 43-46).

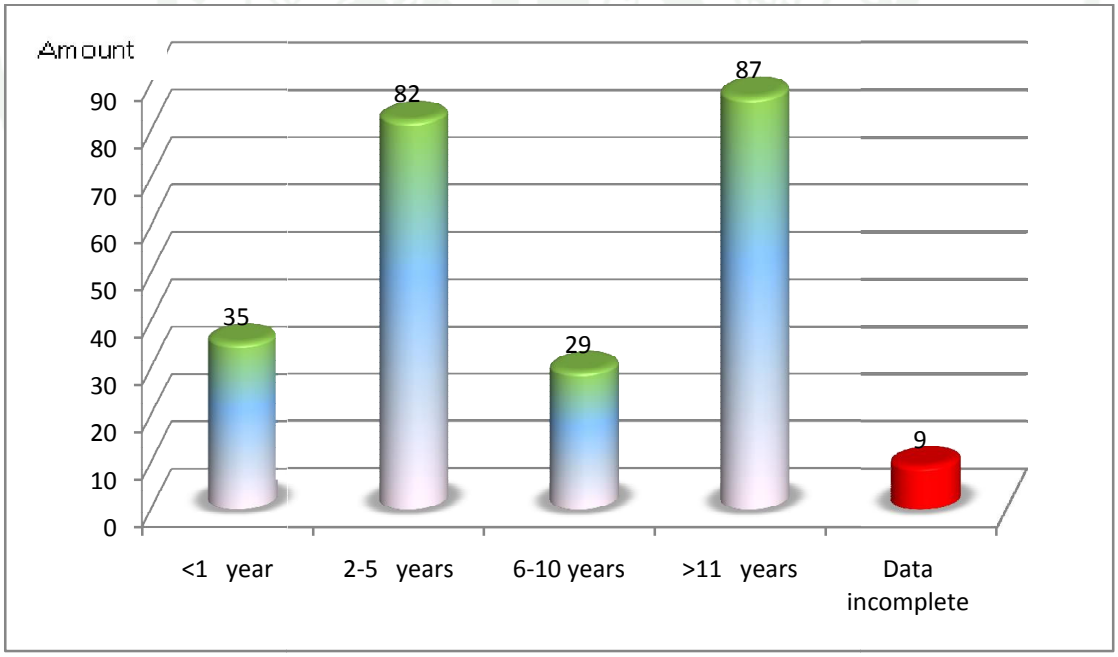


Figure 43 Health history of TCE exposed participants : Duration of work.

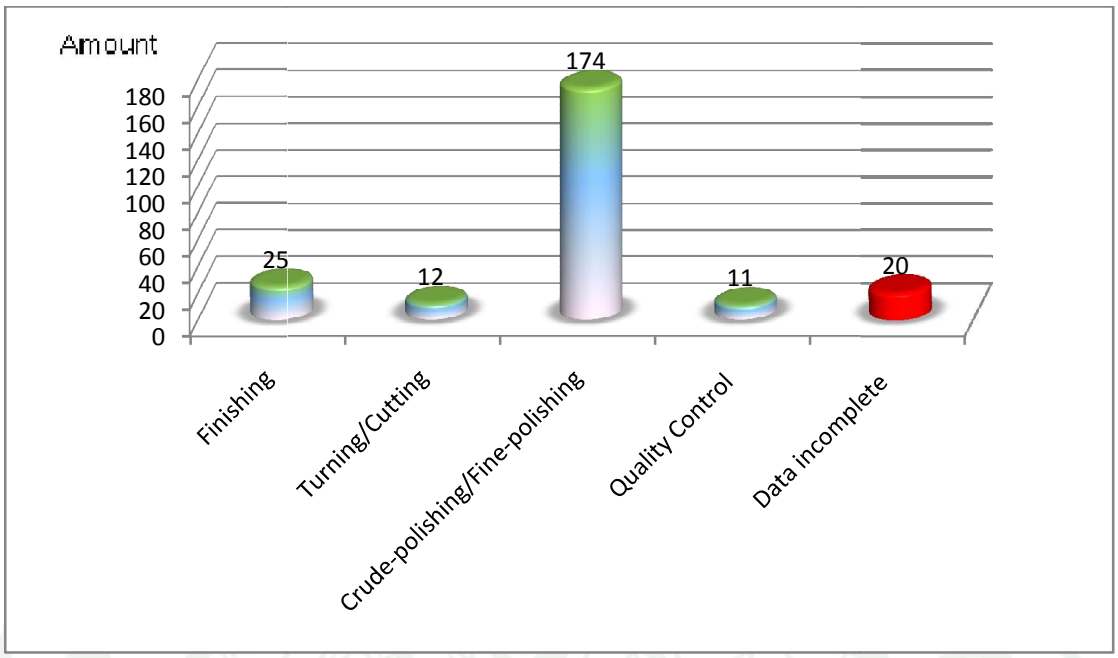


Figure 44 Health history of TCE exposed participants : Department.

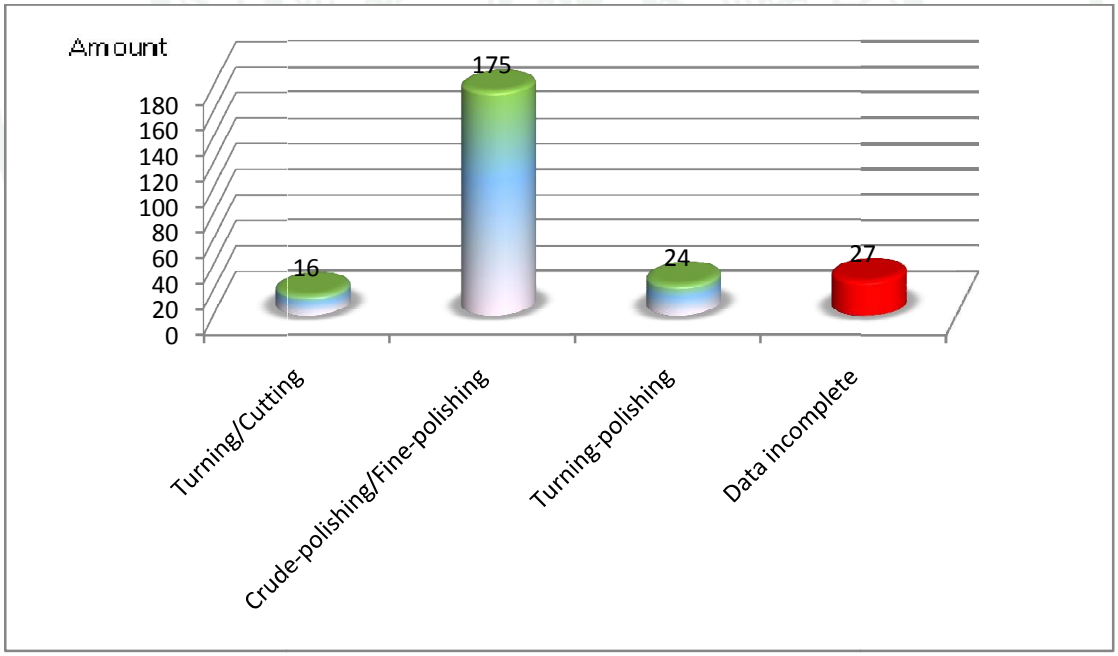


Figure 45 Health history of TCE exposed participants : Job description.

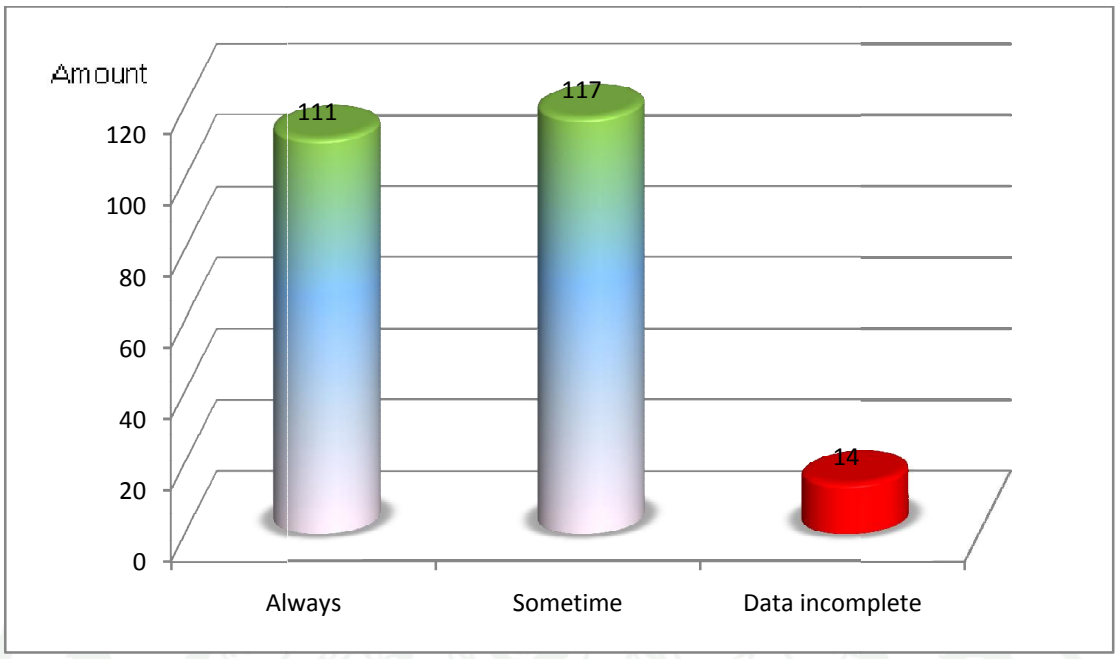


Figure 46 Health history of TCE exposed participants : Chance to be exposure to TCE.

About 14.9% of participants reported exposure to TCE in high concentration and 12.8% reported exposure to TCE at medium concentration, 1.7% and 0.8% (Figure 47), experienced exposure for 8 hours per day and 6 hours per day, respectively. Regarding occupational exposures history (Figure 48), 15.7% were exposed to chemicals, 7.4% reported high prevalence of TCE exposure during work, 3.3% reported medium prevalence of exposure, most of them (85.1%) worked in shifts. Moreover, 41.3% of these participants had holidays and 64 % had a holiday per week. (Figure 49).

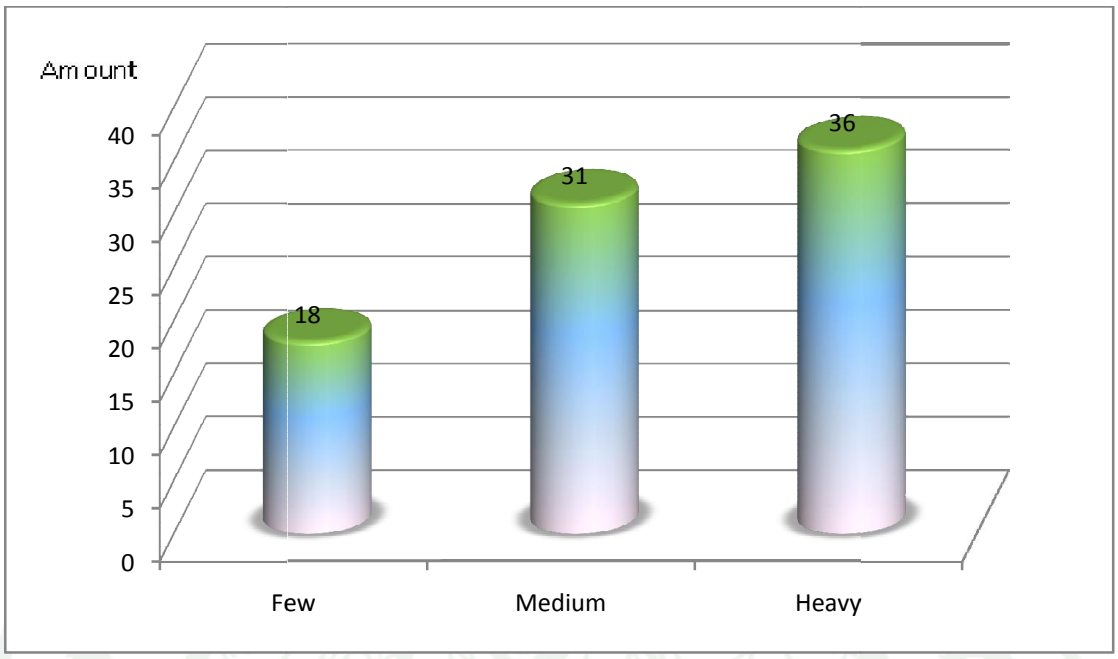


Figure 47 Health history of TCE exposed participants : Exposure to TCE.

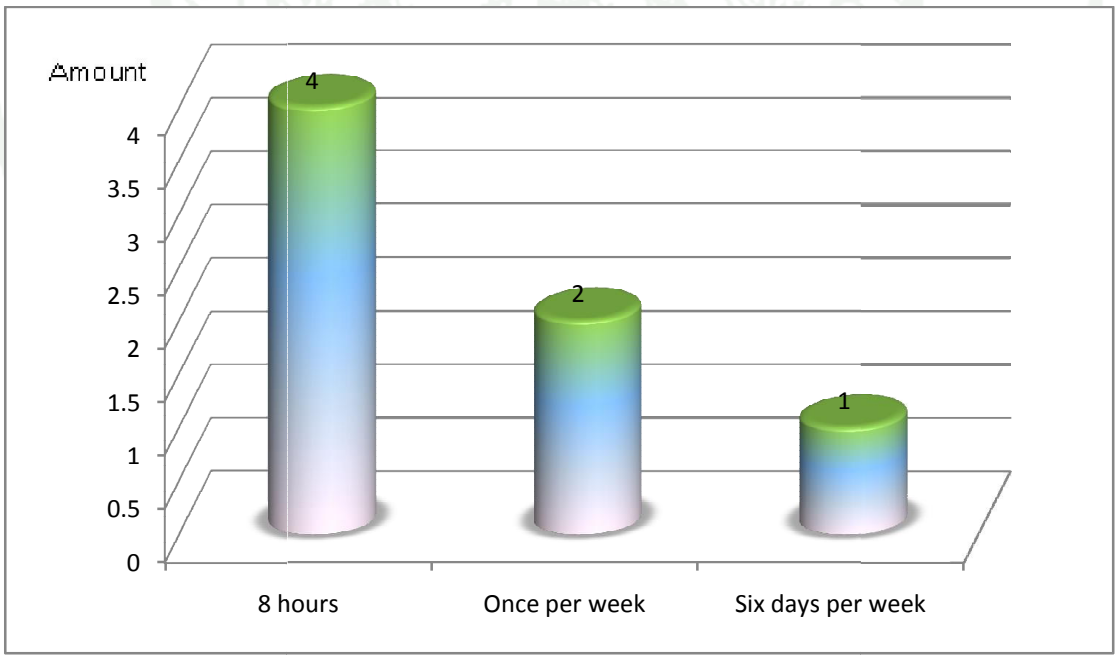


Figure 48 Health history of TCE exposed participants : Duration of TCE exposure.

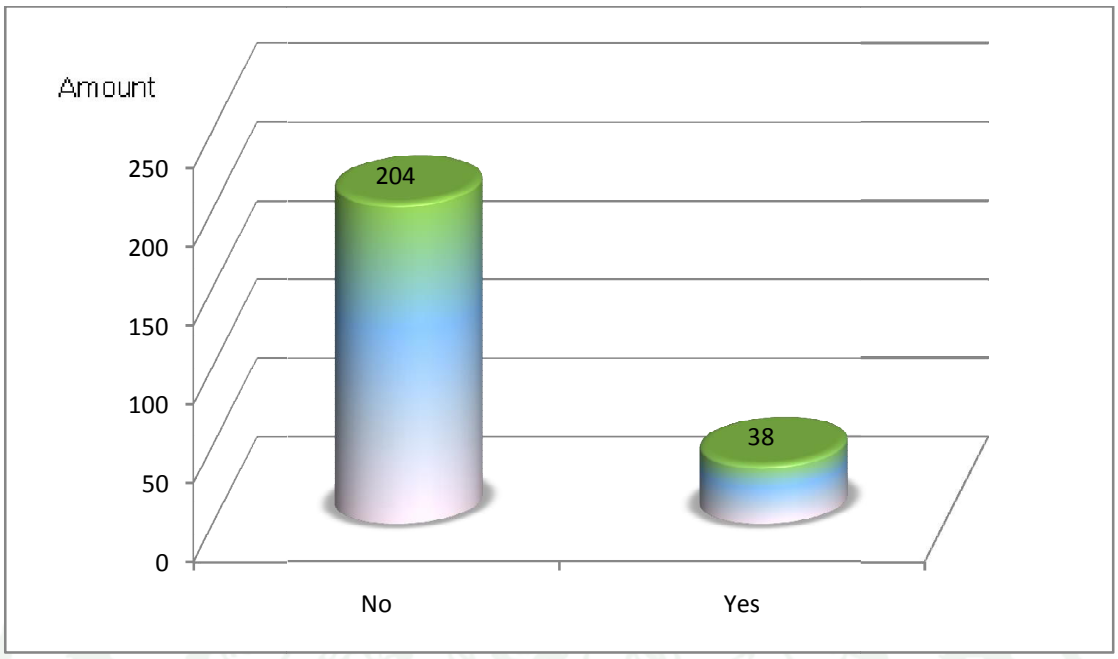


Figure 49 Health history of TCE exposed participants : Used to work with TCE.

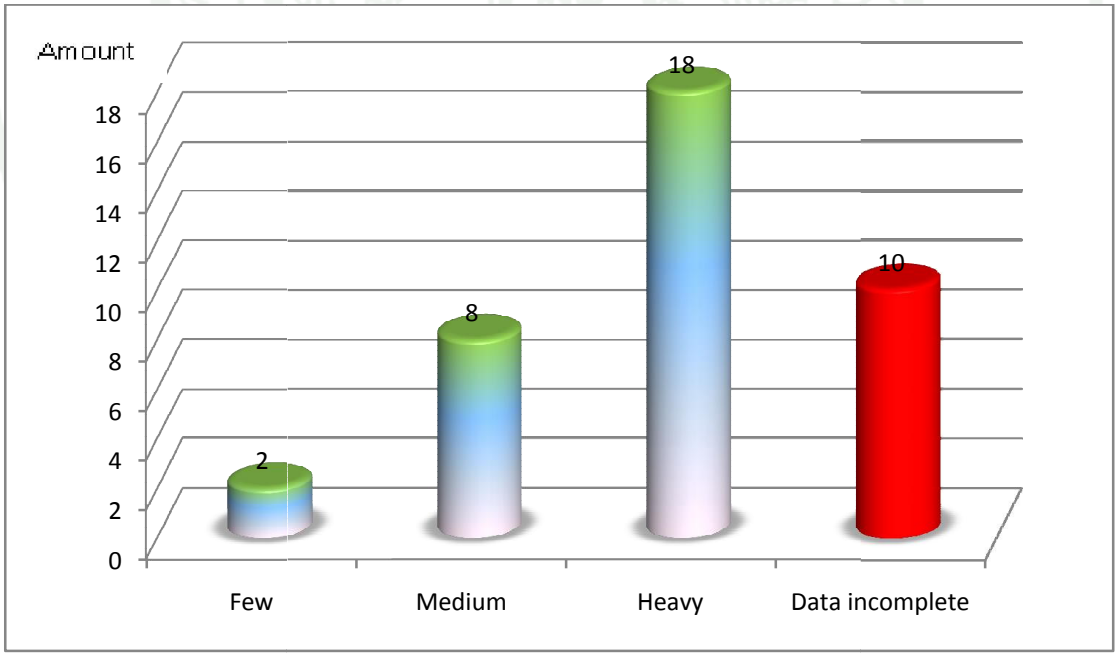


Figure 50 Health history of TCE exposed participants : Used to expose to TCE.

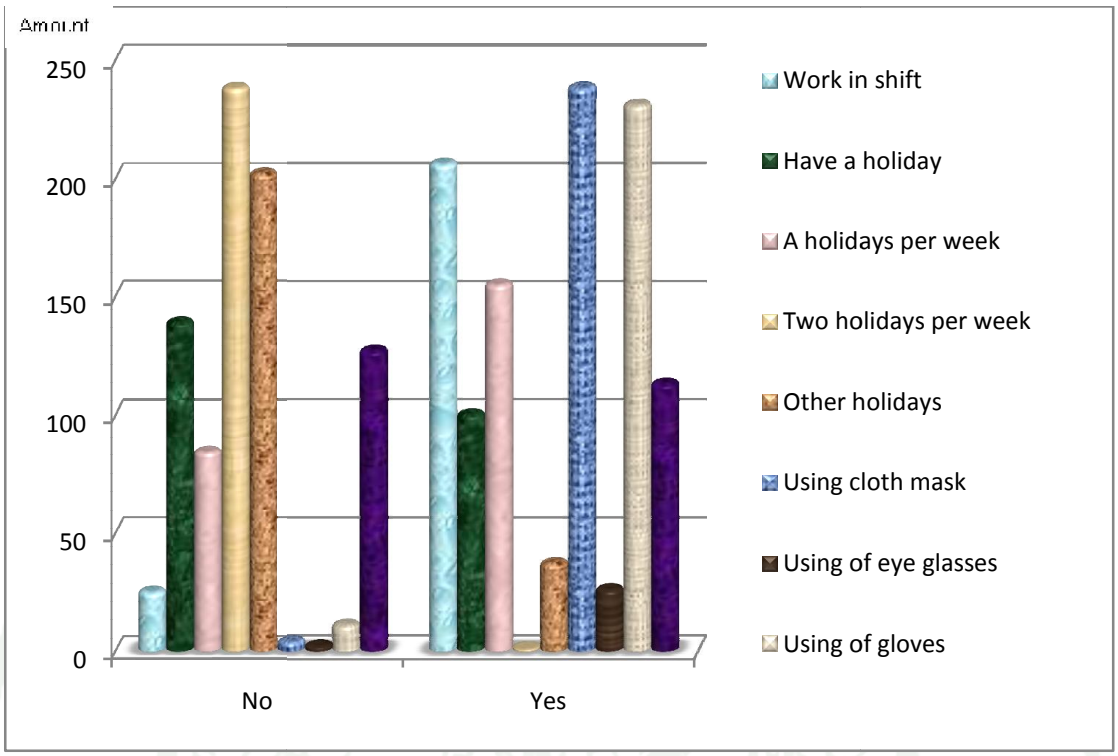


Figure 51 Health history of TCE exposed participants : Behavior.

Figure 51 shows personal protective equipment (PPE) used during work hour. The majority of the respondents (98.3%) used cloth mask during the process of working and 86.4% wore mask all time, 10.7 % wore eye glasses at least sometimes while 5.8% used goggles at least sometimes. Ninety five percent of workers used gloves during their work, 80.2% always wore gloves. In addition, 26.9% reported that they always used chemical protective clothing.

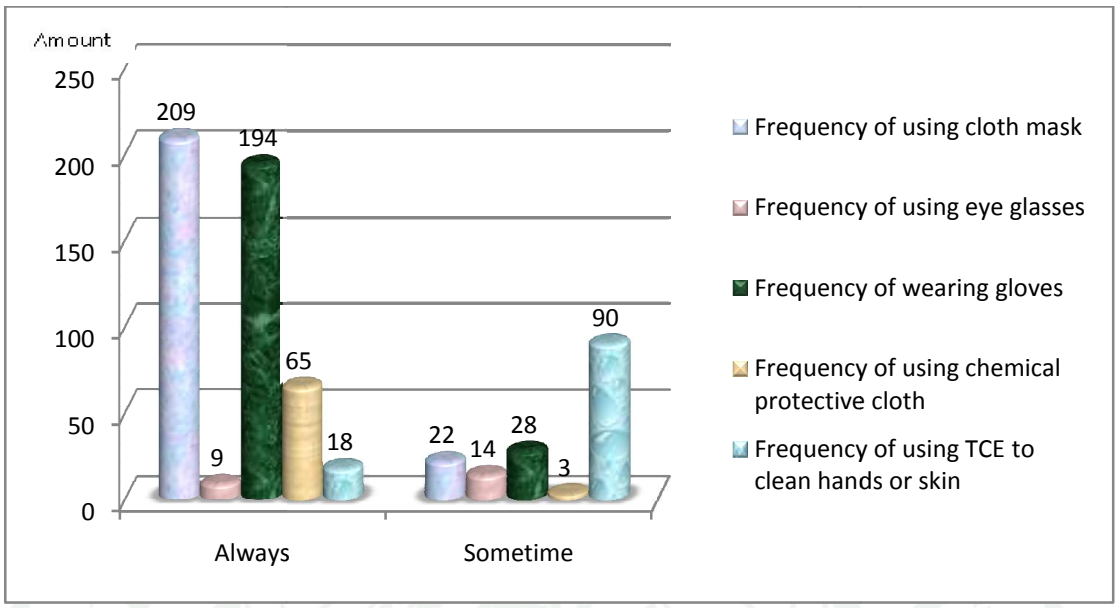


Figure 52 Health history of TCE exposed participants : Frequency of behavior.

Forty six percent of participants washed their hands and skin after using TCE, 37.2% were exposed to TCE at least someday, 63.2% reported strong smelling TCE during their work but 20.2% reported low TCE smelling (Figure 52-53).

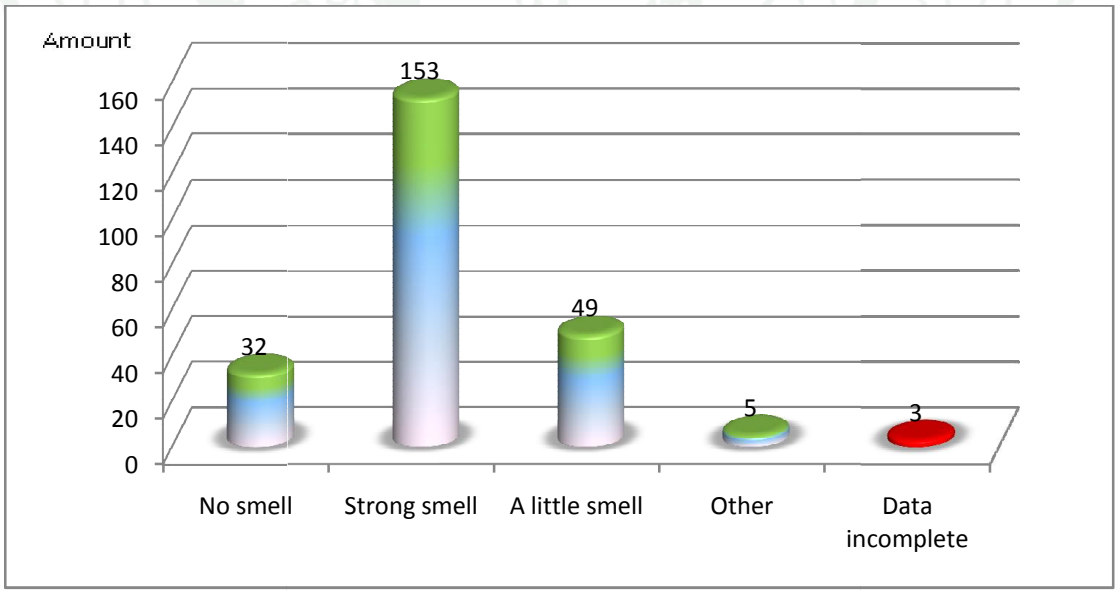


Figure 53 Health history of TCE exposed participants : Smelling of TCE during work period.

Figure 54 shows that in the last six months, 47.5% of workers have suffered from headache, 31.0% experienced dizziness, 13.2% nausea, 9.5 % vomiting and 39.3% reported feeling fatigue. It also shows that 21.1% of participants had suffered from sleepless, 18.2% forgetfulness, 28.5% itchy eyes, 10.3% poor vision. 39.3% suffered from lower back pain and 20.2% reported limb numbness. The skin problems such as itchy hands and legs were reported (48.6%). In addition, the main problems of upper respiratory tract were reported such as easy to catch a cold (14.0%), amsonia (1.2%) and allergic rhinitis (16.1%).

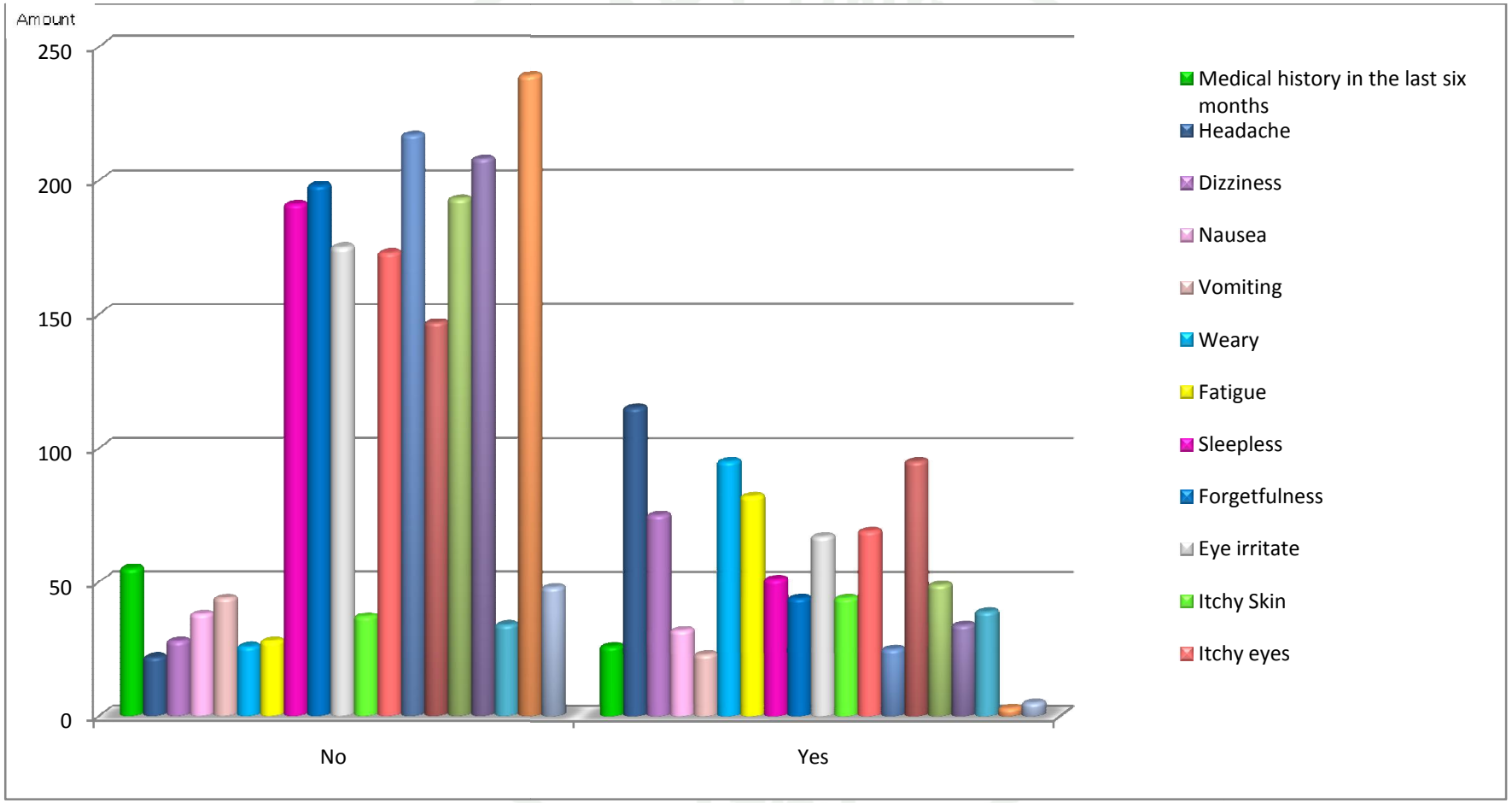


Figure 54 Health history of TCE exposed participants : Health history.

Table 2 shows the frequency of doctor-diagnosed health problems. In the assessment of allergic symptom, 1.7% of participants had asthma and 1.2% suffered from allergy.

Table 2 The frequency of doctor-diagnosed health problems.

Diseases	Number	Percentage (%)
HCP.B	1	0.4
Gastro-Esophageal Reflux Disease	1	0.4
Gastritis	1	0.4
Hypertension	1	0.4
Style	1	0.4
Thyroid	1	0.4
Diabetes	1	0.4
Headache	1	0.4
Catch a cold	1	0.4
Dry skin	1	0.4
Allergy	4	1.7
Migraine	2	0.8
Rheumatoid	1	0.4
Gout	1	0.4
Hyperventilation	1	0.4
Irritable Bowel Syndrome	1	0.4
Anemia	1	0.4
Asthma	3	1.2
Not answer	214	88.4
Total	242	100.0

Ninety percent of participants were generally healthy. In the first day of week, only 1.7% had itchy. In the assessment of symptom by observer, 9.1% of participants had headache, 7.4% expressed confusion, 3.7% had nausea and vomiting, 14.9% had muscle ache, 12.0% had lower back pain and 16.9% had fatigue. (Figure 55-56).

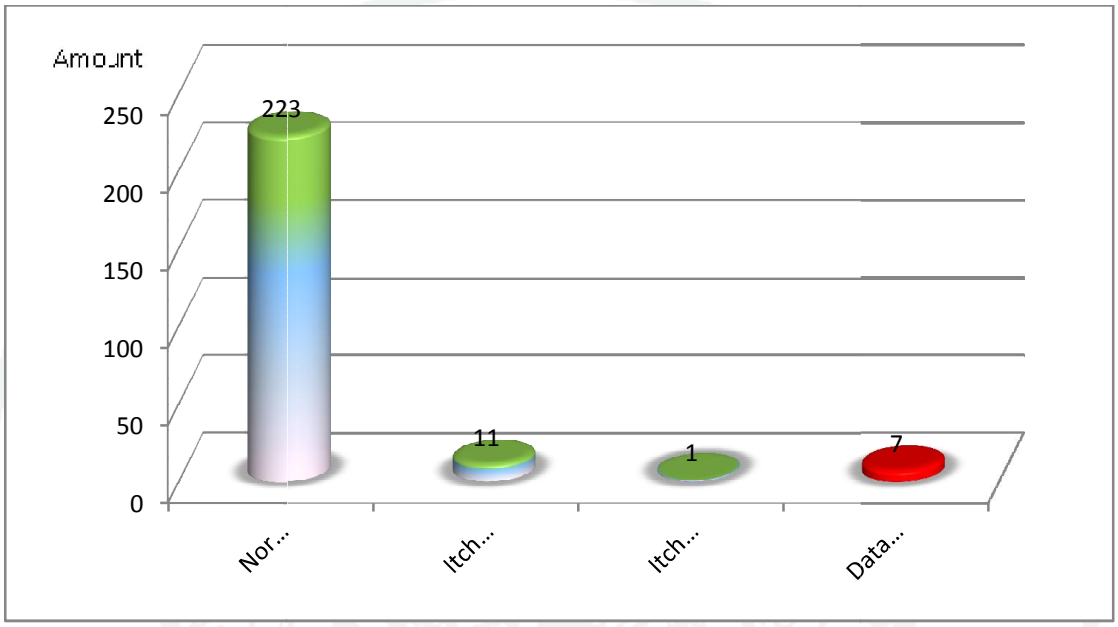


Figure 55 Observation by interviewers Symptom of participants reported by observation.

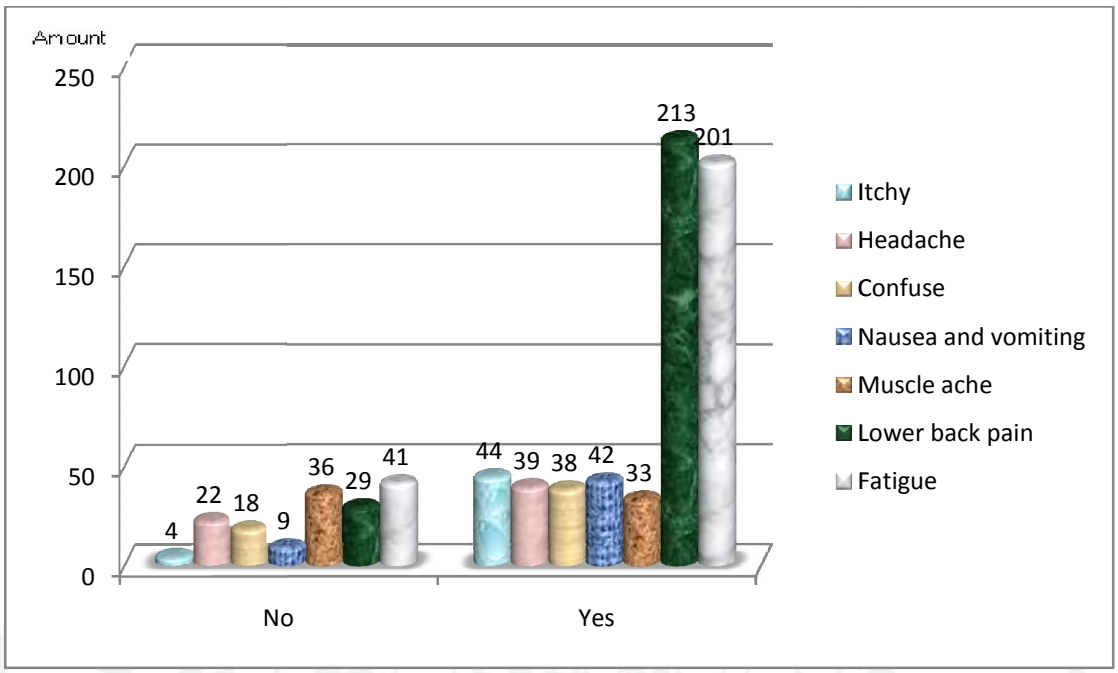


Figure 56 Observation by interviewers : Health history.

5. Environmental samples

TCE concentration from the environment samples were derived from GC-FID analysis. This study used triplicate sampling to ensure the accuracy concentration. A total of 10 measurements were analyzed. Environment sampling in 8 hour of working time, both personal and area sampling showed an equally wide range of TCE concentrations with none of the samples exceeding the maximum contaminant levels (guideline values. The concentration was generally less than 100 ppm, in all ten samples. The distribution of environmental samples by location is presented in Table 3-4.

Table 3 Results of air quality monitoring in the working environment (2010).

No	Area	Sampling Media	Flow rate (mL./min)	Time (min)	Trichloroethylene (TCE) / ppm.		Agency Standard (TWA) ; Ceilling
					TWA	Ceilling	
1.	Stamping 710 (P)	Charcoal Tube	102	17	-	19.637	200 (Ceilling)
2.	Stamping 710 (A)	Charcoal Tube	103	16	-	3.881	200 (Ceilling)
3.	CIB.750 (P) polishing	Charcoal Tube	101	18	-	40.696	200 (Ceilling)
4.	CIB.750 (A) polishing	Charcoal Tube	103	17	-	19.969	200 (Ceilling)
5.	CIB.750 (P) polishing	Charcoal Tube	101	167	42.571	-	100 (TWA)
6.	CIB.750 (A) polishing	Charcoal Tube	101	170	10.282	-	100 (TWA)
7.	Cleaning room751 (P)	Charcoal Tube	103	164	20.435	-	100 (TWA)

Table 3 (Continued)

No	Area	Sampling Media	Flow rate (mL./min)	Time (min)	Trichloroethylene (TCE) / ppm.		Agency Standard (TWA) ; Ceilling
					TWA	Ceilling	
8.	Cleaning room 751 (A)	Charcoal Tube	100	162	20.994	-	100 (TWA)
9.	Stamping 710 (P)	Charcoal Tube	101	152	41.376	-	100 (TWA)
10.	Stamping 710 (A)	Charcoal Tube	100	153	22.789	-	100 (TWA)

The standard in the workplace: ACGIH TLV (2006): TWA = 10 ppm, STEL = 25 ppm, Carcinogenicity = A2 NIOSH REL: Notation = Carcinogen, IDLH = Carcinogen, IARC Classification (1995) = Group 2A OSHA PEL: TWA = 100 ppm, Ceiling 200 ppm, Ministry of Interior Health and Safety in the work on the Environment (Chemical),Thailand BE 2520: TWA = 100 ppm, Ceiling = 200 ppm.

Table 4 Results of air quality monitoring in the working environment (2011).

No	Area	Sampling Media	Flow rate (mL/min)	Time (min)	Trichloroethylene(ppm) TWA	Agency Standard (TWA)
1.	750 Line #2 between machine 198 - 186 (A)	Charcoal Tube	200	71	2.145	100
2.	750 Line #4 between machine 197 - 139 (A)	Charcoal Tube	0.2	71	0.054	100
3.	Stamping #710 (A)	Charcoal Tube	0.2	75	0.545	100
4.	CNC 720 machine 74 (A)	Charcoal Tube	0.2	65	0.580	100
5.	CNC 720 between machine 73 – 39 (A)	Charcoal Tube	0.2	55	0.738	100
6.	Polishing #751 Line 1 between machine 5 – 125 (A)	Charcoal Tube	0.2	72	7.717	100
7.	Polishing #751 Line 1 between machine 32 – 128 (A)	Charcoal Tube	0.2	71	9.315	100
8.	Polishing #751 Line 3 machine 28 - 80 (A)	Charcoal Tube	0.2	70	9.131	100

Table 4 (Continued)

No	Area	Sampling Media	Flow rate (mL/min)	Time (min)	Trichloroethylene(ppm) TWA	Agency Standard (TWA)
9.	Polishing #751 Line 5 between machine 6 – 7 (A)	Charcoal Tube	0.2	69	7.524	100
10.	Polishing #751 Line 5 (center) the last 4machine(A)	Charcoal Tube	0.2	69	8.684	100

The standard in the workplace

ACGIH TLV (2006) : TWA = 10 ppm, STEL = 25 ppm, Carcinogenicity = A2 NIOSH REL: Notation = Carcinogen, IDLH = Carcinogen IARC Classification (1995) = Group 2A OSHA PEL: TWA = 100 ppm,, Ceiling 200 ppm, Ministry of Interior Health and Safety in the work on the Environment (Chemical), Thailand BE 2520 : TWA = 100 ppm, Ceiling 200 ppm

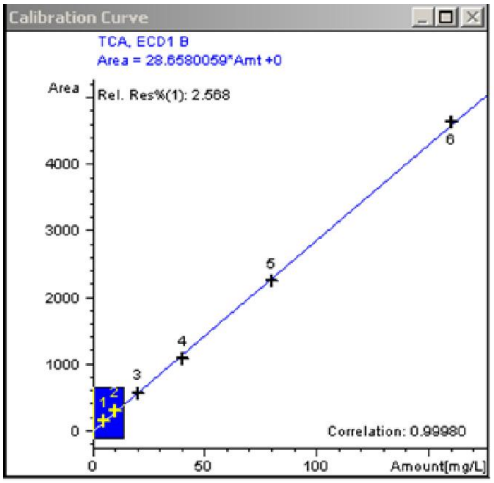
6. Method validation and Determination of TCA in Urine Sample

6.1 Method validation of TCA

6.1.1 Linearity and Range

TCA were determined using the linear regression equation of the calibration curves consisted of seven different concentrations which were 1, 5, 10, 20, 40, 80 and 160 mg/L. Calibration curve was constructed using peak area ratios of standard/internal standard (y axis) and concentration of TCA in a unit of mg/L (x axis) by correlation and linear regression equation program. The correlation of calibration curve was equal 0.9998 (R^2) and the linearity was range from 1-320 mg/L (Figure 57).

#	RT	Signal	Compound	Lvl	Amt[mg/L]	Area	Rsp.Factor	Ref	ISTD	#
1	9.121	ECD1 E	TCA	1	5.000	146.970	3.4021e-2	No	No	
				2	10.000	308.100	3.2457e-2			
				3	20.000	555.730	3.5989e-2			
				4	40.000	1070.800	3.7355e-2			
				5	80.000	2240.200	3.5711e-2			
				6	160.000	4631.100	3.4549e-2			



Trichloroacetic acid at exp. RT: 5.124
ECD1B,
Correlation: 0.99980
Residual Std. Dev.: 47.60915
Formula: y = mx
m: 28.65801
x: Amount[mg/L]
y: Area

Figure 57 Linearity and Range of TCA calibration curve.

6.1.2 Accuracy and Precision

The accuracy and precision of GC-ECD-HS were determined by adding sample blank with TCA standard solution of concentration 1, 80 and 160 mg/L and injected to GC-ECD-HS under the optimum conditions and repeated for 10 times. The signals of TCAs from GC-ECD-HS were converted to concentrations by comparison of the external standard curves and calculated to %RSD as shown in Table 5. From the resulting 10 determinations of each concentration, the mean value was calculated and is shown in Table 5. From the resulting 10 determinations of each concentration, the mean value was calculated and is shown in Table 5. The calculation shows an acceptable precision for values of 1, 80 and 160 mg/L with % recovery of 100.83 %, 100.18 % and 100.01 %, respectively. The % recoveries were in the typical range (90 – 110%) which assessed by USP. The calculated precision using Horwitz's equation, called the measured RSD, was 6.06, 1.70 and 1.20 for TCA concentration of 1, 80 and 160 mg/L. The predicted RSD precision was determined using Horrat ratio. (Figure 58) The predicted RSD value of 1, 80 and 160 mg/L was 0.625, 0.321 and 0.265, respectively. As shown in the results above, the accuracy and precision of the method developed were within the range of acceptance criteria recommended by international industry guidelines for method validation.

Predicted RSD = $0.66x2^{(1-5\log c)}$

C = 1

Predicted RSD = $0.66x2^{(1-5\log(1x10-6))}$

= $0.66x2^{(4)}$

= 10.56

Measured RSD = 0.051

Horrat Ratio = $\frac{\text{Measured RSD}}{\text{Predicted RSD}}$

= $\frac{6.60}{10.56}$

= 0.625

*Measured RSD = $\frac{SD \times 100}{Mean}$

Figure 58 Calculation of Precision using Horrat Ratio.

Table 5 Accuracy and Precision of TCA analysis by GC-ECD-HS.

	Trichloroacetic acid (mg/L)		
	1	**80**	**160**
1	0.985	78.574	159.679
2	0.954	80.998	158.766
3	1.067	79.655	161.554
4	0.968	82.671	158.091
5	0.952	79.021	162.477
6	1.092	81.454	159.304
7	0.933	79.635	157.211
8	1.093	81.335	162.012
9	0.958	79.002	158.773
10	1.081	79.152	162.435
Mean	**1.008**	**80.150**	**160.030**
SD	**0.066**	**1.365**	**1.931**

Table 5 (Continued)

	Trichloroacetic acid (mg/L)		
	1	**80**	**160**
%RSD	**6.60**	**1.70**	**1.20**
%Recovery	**100.83**	**100.18**	**100.01**
Horrat Ratio	**0.625**	**0.321**	**0.261**

Table 6 Detection Limit of TCA Analysis.

Number	**Trichloroacetic acid (mg/L)**
1	0.132
2	0.136
3	0.128
4	0.131
5	0.125
6	0.129
7	0.126
8	0.126
9	0.127
10	0.124
Mean	**0.128**
SD	**0.004**
LOD	**0.139**
LOQ	**0.165**

6.1.3 Selectivity and Sensitivity

The ability of the method with the specificity to analyze the substances as shown in Figure 59 and ability to analyze the substance in very small quantities which can be detected with an acceptable statistical significance. (Table 7)

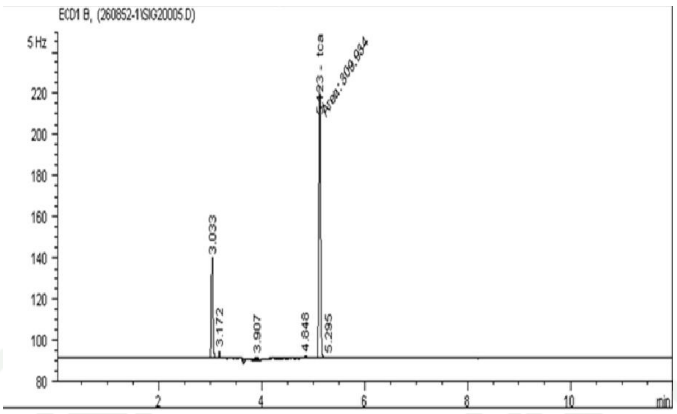


Figure 59 Selectivity of method for TCA analysis.

Table 7 Reproducibility and Sensitivity.

No. of injection	Trichloroacetic acid (mg/L)
1	0.985
2	0.954
3	1.067
4	0.968
5	0.952
6	1.092
7	0.933
8	1.093
9	0.958
10	1.081
Mean	1.008
SD	0.066
RSD	0.066
%Recovery	100.83

6.2 Determination of TCA in Urine Sample

The urine sample were analyzed using Gas chromatography - Electron Capture Detector Headspace (GC-ECD-HS) for determination TCA, TCE metabolites. The results of TCA concentration from urine were shown in Table 8. The participant in study group were 51 men and 191 women, between the median age range of 26 and 58 years. The mean age of study group was 41.9 ± 9.1 years old. All study participants were TCE exposed workers. For the 242 urine samples from exposed group, 113 (46.7%) had detectable levels of TCA which less than the standard level (<15 mg/L). Fifty three percent of participants demonstrated the level of TCA concentration higher than standard level (>15 mg/L) Most of sample detected the presence of TCA in urine range frome 0-50 mg/L as showin Table 9. The average concentration of TCA was 38.56 mg/L which was exceed the standard level (Figure 60 and Table 9).

Table 8 Result of TCA concentration.

TCA concentration	Number	Percent
Within standard level) ≤ 15mg/L)	113	46.7
Higher than standard level(> 15 mg/L)	129	53.3

Table 9 Duration of exposure to TCA.

Data	Average	Minimum	Maximum
TCA concentration (mg/L)	38.56 mg/L	0.46 mg/L	265.39 mg/L
Duration of work	10.41 years	1 week	40 years
Duration per day	10.85 hours	8 hours	15hours
Duration per week	6.59 days	6 days	7days

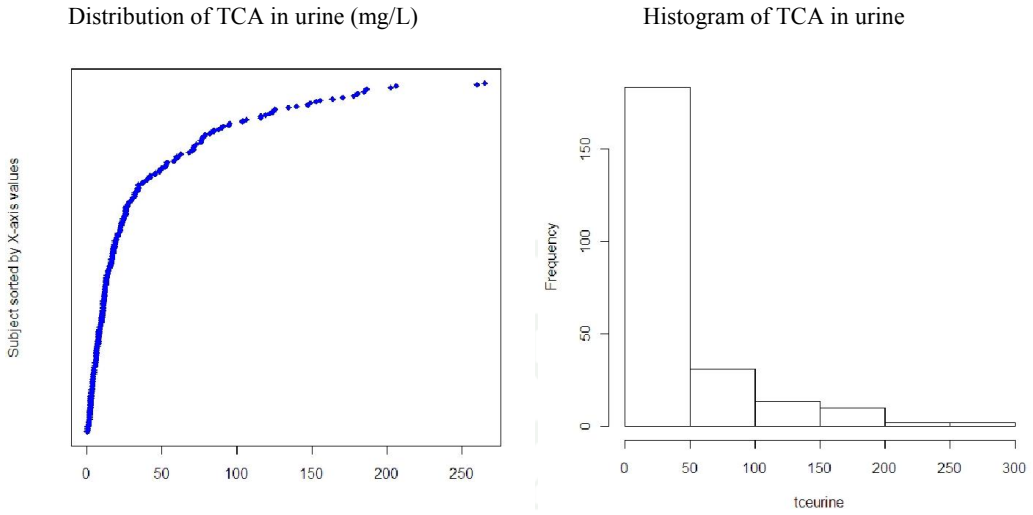


Figure 60 Distribution of TCA in urine.

6.3 Comparison of TCA level between the first and second determination

For the 242 participants who had TCA in urine, the average concentration of first TCA in urine detection was 38.56 mg/L which was exceed the standard level (Table 9).

Comparative analysis between TCA in urine is only possible for those 230 participants who underwent testing for TCA in urine at the time of first test and then underwent second urine test in six months later. The result shown that some participants had the level of TCA at the second test was lower than that at the first (Figure 61).

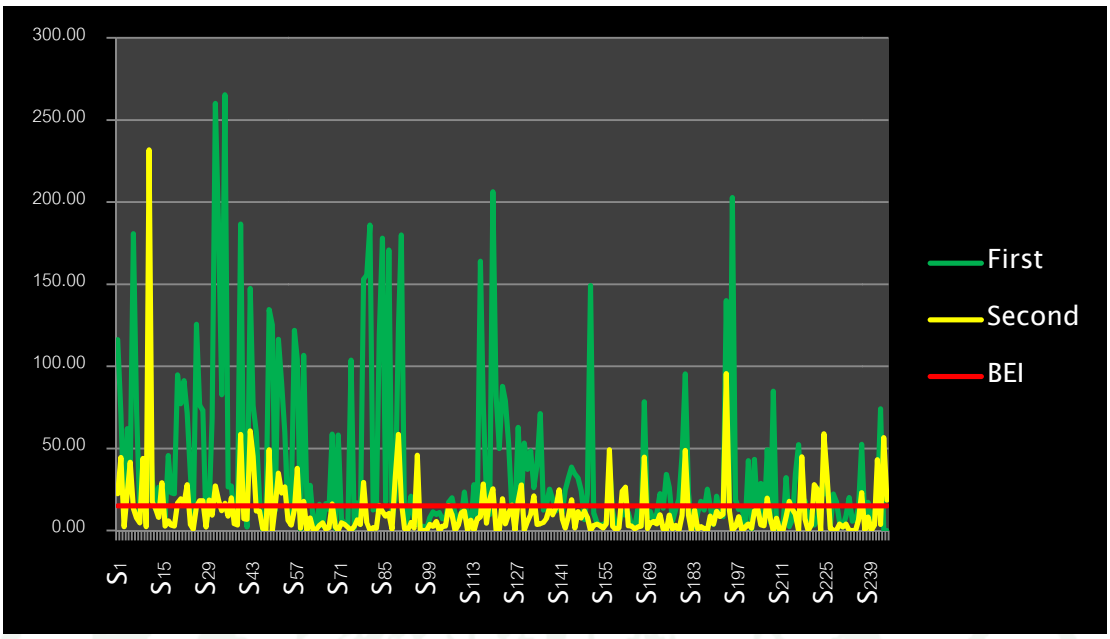


Figure 61 Comparison of TCA level between first and second determination.

7. Micronucleus assay

For the 134 participants who had exposed to TCE and 66 participants, control group. Comparative MN frequency between exposed group and control group: the result shown that the mean of DNA damage was higher in exposed group than in control group (Table10). MN picture from the study shown in Figure 62.

Table 10 Comparison of MN frequency between exposed group and control group.

Group	**Sample size**	**MN** frequency **(Statistical average)**
Expose/ Study	134	5.778
Control	64	1.339

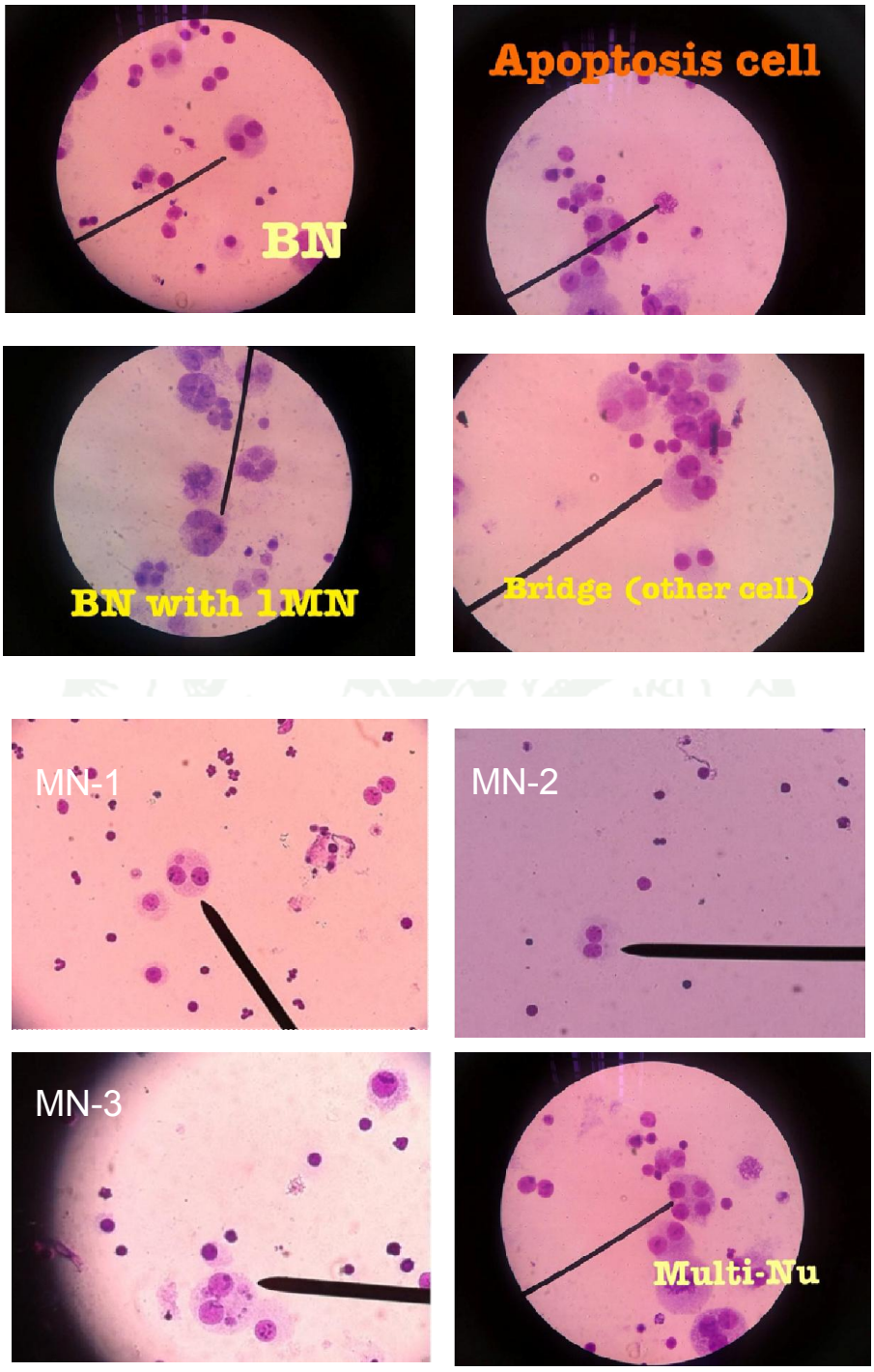


Figure 62 Characteristic of binucleate cell, Apoptosis cell, Binucleate with 1 MN, Binucleate with bridge, Binucleate with 2 MN , Binucleate with 3 MN and Multinucleate.

8. Inferential Statistics

Comparisons were made between level of TCA in urine and the level of DNA damage in blood of control and exposed groups. Statistically significant correlations were determined using paired sample t-test to identify the differences between control and exposed groups. The correlation between the TCA and DNA damage was determined using the Pearson correlation. Statistical significance is taken at $p \leq 0.05$ or less. The results are shown in Table 11-16.

Table 11 Comparison of TCA in urine between exposed group and control group using paired sample t-test.

Concentration of TCA in urine	Mean	Mean Differences	t	Sig
		8.854	5.166	.000*
Exposed group	11.1305			
Control group	2.276			

**P<0.01*

The descriptive statistics of exposed and control groups are given in Table 12 including average concentration of TCA in urine. The average TCA level of exposed group was 11.1305 and 2.276 of control group. A statistically significant difference ($p < 0.01$) was found between exposed and control groups.

Table 12 Comparison of DNA damage in exposed group and control group using paired sample t-test.

Level of DNA damage	Mean	Mean Differences	t	Sig
		4.438	9.105	.000*
Exposed group	5.778			
Control group	1.339			

**P<0.01*

The paired sample t-test was used to compare the DNA damage of the harmful effects of TCA between control and exposed groups and the mean DNA damage of both groups are shown in Table 12. The Mean DNA damage was higher in exposed group (5.778) than in control group (1.339). A significant difference was found between control and exposed groups with mean difference of 4.438 ($p < 0.01$) and t value is 9.105.

Table 13 Comparison of DNA damage and TCA in urine of exposed group using the paired sample t-test.

Exposed group	Mean	Mean Differences	t	Sig
		5.352	3.390	.001*
DNA damage	5.778			
TCA concentration	11.130			

**P<0.01*

The paired sample t-test was used to compare the DNA damage and TCA in urine of exposed groups and the mean DNA damage and TCA concentration are shown in Table 13. A significant difference was found with mean difference of 5.352 ($p < 0.01$) and t value is 3.390.

Table 14 Comparison of DNA damage and TCA in urine of control group using the paired sample t-test.

Control group	Mean	Mean Differences	t	Sig
		0.937	3.707	.000*
DNA damage	1.339			
TCA concentration	2.276			

**P<0.01*

The paired sample t-test was used to compare the DNA damage and TCA in urine of control groups and the mean DNA damage and TCA concentration are shown in Table 14. A significant difference was found with mean difference of 0.937 ($p < 0.01$) and t value is 3.707.

Table 15 presents the correlation coefficients (r) between TCA in urine level and independent variable among study subjects using Pearson Correlation. A positive correlation was found betpween sex and urine TCA levels (r=0.127). Negative correlation coefficients were found between the work hour per day and urine TCA levels (r=-0.169). The associations between urine TCA levels and work hour per day were found to be significant at $P< 0.01$.

Table 15 The correlation coefficients (r) between urine TCA level and independent variable among study subjects using Pearson Correlation.

Factors	Age	Sex	Marital status	Education	Respiratory symptom	Skin symptom	Nervous symptom	Life style	Duration of work	Work hour per day	TCA
Age	1	- 0.109	0.268**	-0.478**	0.011	0.043	0.066	0.056	0.832**	-0.122	-.035
Sex		1	-0.168**	0.078	-0.018	-0.169**	-0.145*	-0.132*	-0.095	-0.056	.127*
Marital status			1	-0.122	-0.035	0.028	0.027	0.046	0.216**	0.087	-.096
Education				1	-0.062	-0.073	0-.045	-0.083	-0.416**	0.092	-.143*
Respiratory symptom					1	0.394**	0.541**	0.347**	0.055	-0.025	-.033
Skin symptom						1	0.328**	0.241**	0.016	-0.023	-.055
Nervous symptom							1	0.595**	0.074	0.107	-.049
Life style								1	0.068	0.125	-.017
Duration of work									1	-0.157*	.042
Work hour per day										1	-.169**
TCA											1

**P<0.01*

Table 16 Multiple regression analyses were conducted to examine the relationship between variables to TCA urine and DNA damage.

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	40020.340	3	13340.113	5.631	.001**[c]
Residual	540138.288	228	2369.028		
Total	580158.628	231			

** p < 0.01

Correlation and multiple regression analyses were conducted to examine the relationship between variables such as age (X1), sex (X2), marital status (X3), education level (X4), respiratory tract symptom (X5), skin symptom (X6), nervous system symptom (X7), life style (X8), duration of work (X9), work hour per day (X10) and dependent variable which were urine TCA level and DNA damage (Y). The Analysis of variance demonstrated that urine TCA level and DNA damage had significant linear relationship (p<0.01) as shown in Table17.

Table 17 Multiple correlation coefficient (R) of variables and TCA in urine or DNA Damage.

Predictors	R	R-Square	F
X10	0.170	0.029	6.807**
X10, X4	0.227	0.051	6.210**
X10, X4, X1	0.263	0.069	5.631**

** p < 0.01

Table 17 summarizes the descriptive statistics and analysis results of multiple regression analysis. As can be seen each of the predictor is positively and significantly correlated with p value < 0.01. The first selected variable was duration of work per

day (X10) tend to be the best predictor, however, adding the second predictor, level of education (X4), the result showed that the multiple correlation coefficient significant increase ($p<0.01$). Using three variables together, the multiple regression value was statistically significant increase however, adding other variables, the result showed no significant difference.

Table 18 Multiple regression weight of predictors by Stepwise method.

Predictors	Beta	b	SE_b
Duration of work per day (X10)	- 0.170	- 4.493	1.704
Level of education(X4)	- 0.224	- 13.127	4.284
Age (X1)	- 0.152	- 0.762	0.368
a = - 0.170 R = 0.263 R2 = 0.069 SE = 0.368 F = 5.631**			

** $p < 0.01$

Table 18 indicated that the multiple regression model with all three predictors produced $R^2 = 0.069$, $F = 5.631$, $p <0.001$. As can be seen the predictor; duration of work per day, education level and age is positively correlated with the urine TCA level and had multiple regression weight equal - 0.170, - 0.224 and - 0.152 respectively. The result of multiple correlation (multiple R: b) of duration of work per day, education level and age were -4.493,-13.127,-0.762respectively. These three predictors can predict 6.9% of the level of TCA in urine and level of DNA damage in blood and had the error about 36.8%. The predicted equation of TCA level and DNA damage as shown below:

$$Y' = 18.382 + - 4.493\ (X_{10}) + - 13.127\ (X_4) + - 0.762\ (X_1)$$

Discussion

Trichloroethylene is used in many factories as a general solvent for cleaning and degreasing. The compound is used extensively in dry cleaning and it also produced by waste incinerators and the atmospheric half-life is 70-100 days. The exposure route are inhalation, ingestion, eye contact, and to a small extent, by absorption through the skin, and there are a lot of studies about TCE toxicity. In recent year, TCE is classified as group 2A substances, which the International Agency for Research on Cancer (IARC) has determined that TCE is "probably carcinogenic to humans." This classification is based on limited evidence in humans and sufficient evidence in animals. Ingestion or breathing high levels of trichloroethylene may cause nervous system effects, liver and lung damage, abnormal heartbeat, coma, and possibly death. The most typical clinical feature of TCE-induced disorder is skin symptom and respiratory symptom. In this study, the results from the questionnaire demonstrated that the most prevalent symptoms reported by workers were itchy, headache, fatigue and poor vision. These findings are agreement with a previous study, which revealed that low concentrations of TCE caused drowsiness, dizziness, fatigue and headaches (P. Kjellstrand, 1983).Many studies showed that TCE exposure mostly associates with headache. In our study we found the other prevalence of symptoms such as cough, phlegm, tightness, dyspnea and eye burning related to duration of employment in workers upper than 1 years.

Detection of TCE in exhaled air or blood is recommended as a confirmatory test for monitoring of TCE exposure. Some reviews demonstrated that determination of TCE metabolites in urine is the best indicator of integrated exposure for all the workweek. In this study, the development method for appropriate analysis of the TCA was evaluated. The Gas Chromatography (GC) analytical method proposed for TCE in environment and TCA in urine. The method is highly sensitive and specific and can be performed with equipment available in most laboratories. The reliability characteristic such as LOQ, precision and linearity obtained are reliable as recommendation of ISO/IEC 17025. This method is usefulness in routine bio monitoring analysis and having lower levels of detection for solvent exposure. The

spike of various TCA standards concentration of 1, 80 and160 mg/L showed the reliability of percent recovery of 100.8%, 100.1%and 100.0%, respectively. The percent recoveries of the procedure are in the range of acceptable, percent recovery of validation method which ranges from 90-110. The calibration graphs produced were linear within the range from 1 to 160 mg/mL and with the correlation coefficient 0.9998. The working range of the method is from 1 to 160 mg/L of TCA concentration. The working range is wide enough to determine the TCA level in the urine samples. Limit of detection was determined on repeatability of sample blank, the result of % recovery was 100.8 and precision was 0.066. Repeatability and reproducibility were detected to be sufficient and acceptable. In conclusion, it may be stated that the validated method is appropriate for quantification of TCA and TCE in urine and environment.

The personal air sample monitoring was carried out for the entire shift as the level of time-weighted average (TWA) and ceiling values. The levels of TCE detected by personal sampling reflected the exposure to this chemical of the employees working directly with the TCE (cleaners or instrument packers or washer). Personal sampling detected TCE in all 10 samples which mean level of TWA was 26.41 ± 5.24 ppm and ceiling values was 21.05 ± 7.55 ppm but the TCE concentration was still in the standard concentration of Safety standards in the workplace which declared by Ministry of the Interior. The highest concentration of TCE detected by personal sampling of the workers was 42.57 ppm. The levels of TCE by static sampling at washing room and baking room were performed. The highest level of TCE detected by static sampling was 40.69 ppm and personal sampling was 42.57 ppm, however, the result demonstrated over the occupational exposure limits for 8h TWA which recommended by the Scientific Committee on Occupational Exposure Limits for Trichloroethylene (10 ppm). Based on these findings, the cleaners (the person working directly with the TCE) was identified as the job category most exposed to TCE in this type of industry.

Biological markers of exposure to TCE include concentrations of TCE and its metabolites in body tissues and fluids, such as urine is well established for a long

time. These compounds have short biological half-life and levels may vary depending on levels of metabolizing enzymes (Boyes *et al.*, 2000). TCE metabolites are mainly excreted via the urine, namely 29-50% of TCE as Trichloroethanol (TCOH) and 10-24% of absorbed TCE as Trichloroacetic acid (TCA) (Monster *et al.*, 1976, 1979; Soucek and Vlachova, 1960). Other studies reported that up to 44% of TCE could be excreted as TCA (Nomiyama and Nomiyama, 1971). TCA in urine has been used as a biological marker in many occupational studies; however it only reflects relatively recent exposure (Anttila *et al*., 1995). The biological half-life of TCOH is longer in children and infants than adults, 8 days in adults, 10 days in children, 28 days in full-term infants, and 40 days in preterm infants (Renwick 1998).In this study. TCE metabolites in urine of exposed workers and control participants were determined. A measured mean concentration of TCA in urine of exposed workers for the first determination is 38.56 mg/L. The results demonstrated that 53.3 % of exposed group had urine TCA level higher than standard limit values, 15 mg/L. The second determination of TCA urine in the same group is 11.13 mg/L, the results demonstrated that TCA concentration decrease in all participants when compared to the first determination after the participants were introduced to use the protection equipment and told about knowledge of safety precaution. The results demonstrated that only 39.99 % of exposed group had urine TCA level higher than standard limit values in the second urine determination. In addition, urine TCA levels reported in this study are similar to those reported in other literatures (MM. Moore and K. Harrington-Brock, 2000). TCA levels in TCE exposed workers were significantly higher than those in controls (38.56 and 11.13 versus 2.277 mg/L, $P < 0.01$).

As mentioned previously, TCE is classified as group 2A substances, which is "probably carcinogenic to humans"; exposure to TCE could be associated with chromosome damage. Micronucleus in the smear of lymphocytes was one of the biological indicators of chromosomal damage in term of cytokinesis-block micronucleus (CBMN) assay. The detection of MN in binucleated cells by this assay both in vivo and in vitro (Fenech *et al.*, 2005) has been successfully employed as a reliable biomarker of exposure to chemical agents (Danitsja *et al.*, 2008; Bonassi *et al.*, 2007). The present study was designed to use this CBMN assay to determine

chromosome damage among workers who are occupationally exposed to TCE. Frequencies of micronucleus in TCE exposed workers and control were 5.778 and 1.339/1000 MN, respectively. The result demonstrated that TCE exposure was associated with the increase of micronucleus. TCE exposure has been reported associated with chromosome abbreviation and damage (P. Kumar, 2002; JL.Wang JL.Wang, 2001) by the other methods such as SCE. There is some evidence that exposure to TCE in humans is associated with alterations in levels of certain cytokines. There is also some evidence that TCE exposure is associated with several autoimmune diseases in humans such as systemic sclerosis, systemic lupus erythematosis, rheumatoid arthritis and dermatitis.

The results of micronucleus assay in the present study indicate a genotoxic activity in workers exposed to TCE. The correlation of micronucleus and TCE level was demonstrated four associations. The exposed group which had high level over the standard TCA limit (> 15mg/L) in first urine determination, and the second determination showed the decrease of TCA concentration. Frequency of MN still higher than the frequency of MN found in control group (11.77 compared to 1.33 mg/L of control) (Figure 63).

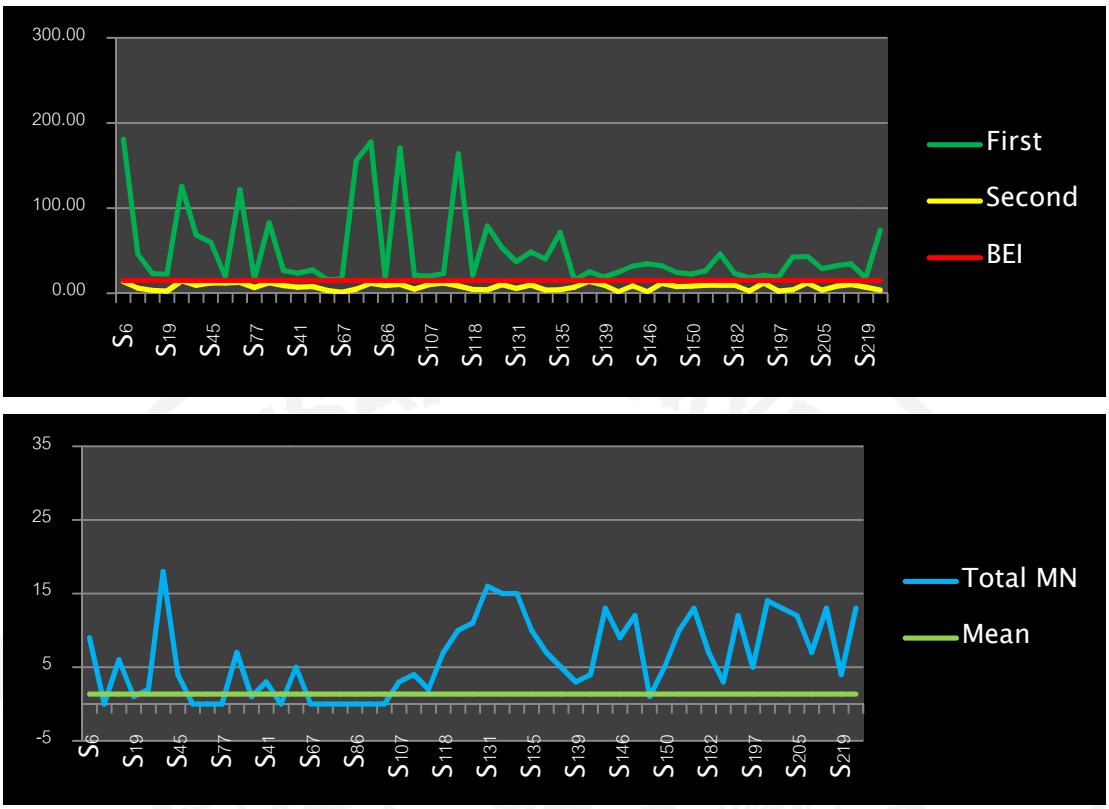


Figure 63 Comparison of TCA level between the first and second determination combinewith MN Frequency MN (Group 1).

In exposed group which second TCA determination higher than standard limit, frequency of MN still higher than the frequency of MN found in control group (14.00 compared to 1.33 mg/L of control) (Figure 64). The results indicate MN has a potential to be good biomarker for chronic TCE exposure. Previous studies also offered strong evidence in support of a dose–response relationship between TCE exposure and the genetic biomarker (P. Kumar, 2002).

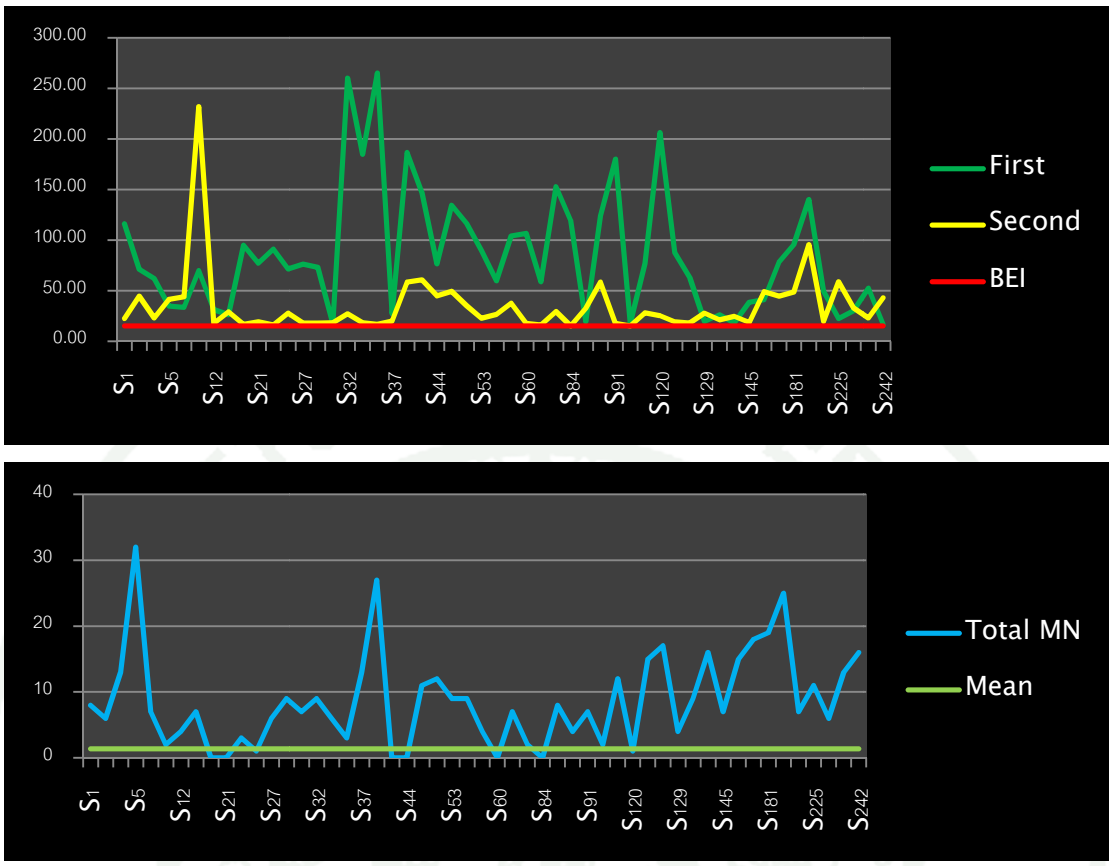


Figure 64 Comparison of TCA level between the first and second determination combine with MN Frequency MN (Group 2).

The exposed group which had the level TCA not exceeding the standard limit (< 15mg/L) in the first test of urine determination but the level TCE of the second test showed higher than the standard limit. Frequency of MN also higher than the frequency of MN found in control group (9.20 compared to 1.33 of control). In these cases, the results indicate MN has a potential to be a good biomarker for acute TCE exposure (Figure 65).

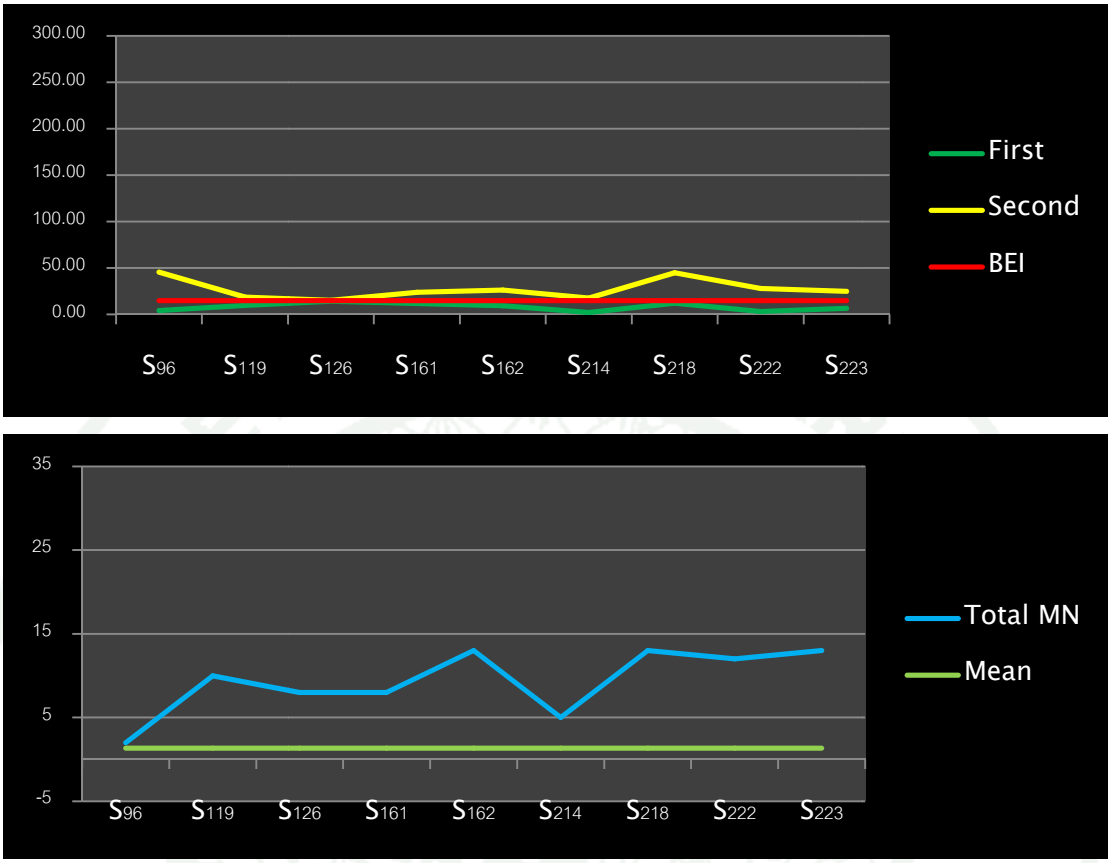


Figure 65 Comparison of TCA level between the first and second determination combine with MN Frequency MN (Group 3).

The exposed group which had the level TCA not exceeding the standard limit (< 15mg/L) in both of urine determination, the frequency of MN also higher than the frequency of MN found in control group (8.937 compared to 1.33 mg/L of control). In these cases, the results indicated that the workers had more exposure with the other harmful materials, the likely occurrence of their MN increase. Evaluating a possible association between MN and TCA as biomarkers of TCE exposure, assuming that they reflect better long-term cumulative TCE exposures to humans (Figure 66).

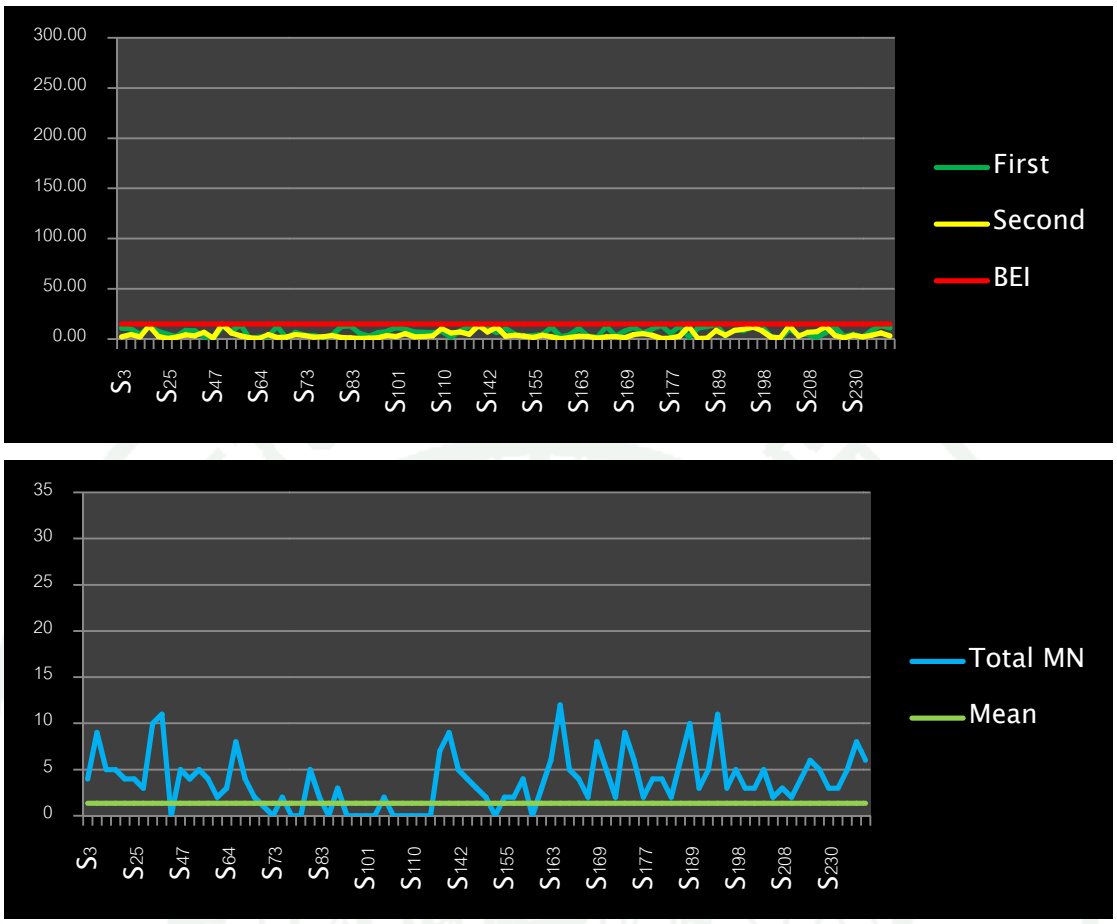


Figure 66 Comparison of TCA level between the first and second determination combine with MN Frequency MN (Group 4).

Multiple regression analysis in the study revealed that the duration of employment and TCA concentration in urine positively influenced the frequencies of MN.As can be seen the predictor; duration of work per day, education level and age is positively correlated with the urine TCA level. We also observed that urine TCA level and DNA damage had significant linear relationship ($p<0.01$).

In conclusion, low exposure to TCE induces genotoxic effects in workers as assessed by well-validated methodologies of GC-ECD-HS and CBMN. Individuals with a higher biologically effective dose (evaluated by TCA in urine) showed a higher frequency of MN. This study also confirms the importance of evaluating the duration of exposure in assessing the results of genotoxicity biomonitoring studies.

Biomonitoring studies on a greater number of individuals will be necessary to confirm these results.

A major limitation of the study was the retain response rate of the survey. Thirty four employees (20%) disappeared in the second determination. Although the retain rate was not great, it was good enough. A higher response rate would have given the study more power (the bigger number of participants is the more power given to the study) for statistical purposes and generalizability. More female participants in the study than males because the factory community is more populated with women.

CONCLUSION AND RECOMMENDATION

Conclusion

In this study, constant exposed to the low concentration of trichloroethylene caused damaged to the DNA. The damage level determined by the exposed level of Trichloroacetic acid (TCA) which were metabolized of trichloroethylene in urine from GC-ECD-HS and counting the number of micronuclei by CBMN, micronucleus assay technique. It was proved that the worker whose were exposed to the trichloroethylene can cause the damage to DNA level. The results were shown that the level of Trichloroacetic acid concentration were directly proportional by the amount of micronucleus. The result from the selected population sample were shown that the concentration of Trichloroacetic acid were higher than standard concentration (15 mg/L) for the first monitoring. After the studied population were exposed to trichloroethylene, the results of trichloacetic acid concentration were 11.130 mg/dl (control = 2.276 mg/dl). The average number of micronucleus were 5.778 (control = 1.339). The result of the first and second monitoring were statistically significantly different with the analysis by t- test between the result of TCA in urine and micronucleus assay technique. For the control population, the TCA in urine analysis shown the t-score at = 5.166 and the number of DNA damage at = 9.105 which were significantly difference ($p = 0.01$). They were also shown that the level of TCA in urine and level of DNA damaged in blood were directly proportional to the parameter of working time per day, education, and age.

Trichloroethylene has been used in various production processes of some industries. These chemicals can cause damage to the chromosome in both direct and indirect ways. Human body is normally can repair itself but if it were exposed to high concentration the cell will be permanently damages and may lead to cancer or genetic disorders. The industries standard of Thailand were chosen to analysis by the chemical approach to tested chemical or derivatives of chemical in urine and compare to the international standard level. This kind of technique cannot extrapolate the damage at the cell level and the level of the accumulation for the individual person.

However, cytogenetic is another alternatives biomarker technique to show genetic effects from chemical exposure prior since the beginning. It is also useful to evaluate the safety of the worker basis of treatment guidelines.

In this studied can be used as an indicator for the chemical exposed population. By combining the both data from the analysis technique and questionnaire can concluded to the risk assessment to effect of DNA damage from chemical exposure on the human life. The results had shown that the concentration of Trichloroacetic acid in urine sample were in acceptable range and after the second monitoring, the results were slightly decreased. Contrast to the data from the micronucleus analysis which had shown a vast damage in the cell level from a high number of multinucleated cells due to the concentration of these chemicals and from the other exposure such as working environment or wrong daily habits.

The early determination of cell damage from the chemical exposure can forecast the risk assessment for the cancer diseases. Combine the determination of the purpose technique with the questionnaires can reduce the risk and to increase more information further for the colleagues.

Recommendation

It is noteworthy that potentially harmful exposures in factories may concern relatively represent a considerable health risk such as toxic chemical substances. The effects of TCE on human health depend on how much individual is exposed to, and the length and frequency of exposure. Several studies have reported an increase of chromosomal damage in TCE exposed workers and the present study strongly supports the earlier findings. Our findings indicated the higher chromosome damage among the workers which may cause by continuous long-term exposure to TCE. The increased chromosome damage (MN frequency) may be caused by an accumulation of TCE in workplaces, due to inappropriate conditions such as ineffective or inadequate air circulation and ventilation. Health education on hazard exposures and appropriate prevention measures could reduce the risk. Although, we may not reduce

the accumulate of TCE in environment, but we may reduce exposure by encouraging the workers to give high priority on their health by using appropriate personal protective equipment (PPE) such as mask, gloves and safety goggle while working at factories. In addition, the biological monitoring of occupational exposure to TCE is very important. Urine and blood tests can indicate recently exposed to a large amount of TCE and also the toxic effect to cells. The medical surveillance program is to prescribe baseline and periodic health evaluations of workers exposed to TCE and the workers should be advised to have the medical examinations annually.

LITERATURE CITED

Aardema, M.J. and. M.K. Volders. 2001. The *in vitro* micronucleus assay. **Genetic Toxicology and Cancer Risk Assessment**. 163-186.

Aitio, A. 1999. Biomarkers and their use in occupational medicine. **Scandinavian Journal of Work, Environment and Health**. 25: 521-528.

Albertini, R.J., D. Anderson, G.R. Douglas, L. Hagmar, K. Hemminki, F. Merlo, A.T. Natarajan, H. Norppa, D.E. Shuker, R. Tice, M.D. Waters and A. Aitio. 2000. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International Programme on Chemical Safety. **Mutation Research**. 463: 111-172.

Alexander, D.D., P.J. Mink and H.O. Adami. 2006. A meta-analysis of occupational trichloroethylene exposure and multiple myeloma or leukemia. **Journal of Occupational Medicine**. 56: 485-493.

Alexander, D.D., M.A. Kelsh, P.J. Mirk, J.H. Mandel, R. Basu and M. Weingart. 2007. A meta-analysis of occupational trichloroethylene exposure and liver cancer. **International Archives of Occupational and Environmental Health**. 81: 127-143.

Andrianopoulos, C., G. Stephanou and N.A. Demopoulos. 2006. Genotoxicity of hydrochlorothiazide in cultured human lymphocytes. I. Evaluation of chromosome delay and chromosome breakage. **Environmental and Molecular Mutagenesis**. 47: 169-178.

Aranyi, C., W.J. O'Shea and J.A. Graham. 1986. The effects of inhalation of organic chemical air contaminants on murine lung host defenses. **Fundamental and Applied Toxicology**. 6: 713-720.

ATSDR (Agency for Toxic Substances and Disease Registry). 1997. **Toxicological Profile for Trichloroethylene**. Atlanta.

Bakke, B., P.A. Stewart and MA. Waters. 2007. Uses of and exposure to trichloroethylene in U.S. industry: a systematic literature review. **Journal of Occupational and Environmental Hygiene**. 4: 375-390.

Barton, H.A. and H.J. III. Clewell. 2000. Evaluating noncancer effects of trichloroethylene: dosimetry, mode-of-action, and risk assessment. **Environ Health Perspect**. 108 (suppl 2): 323-334.

Becker, P., H. Scherthan and H. Zankl. 1990. Use of a centromere-specific DNA probe (p82H) in nonisotopic in situ hybridization for classification of micronuclei. **Genes Chromosomes Cancer**. 2: 59-62.

Berman, E., M. Schlicht and V.C. Moser. 1995. A multidisciplinary approach to toxicological screening: I. Systemic toxicity. **Journal of Toxicology and Environmental Health**. 45: 127-143.

Boei, J. and A. Natarajan. 1995. Detection of chromosome malsegregation to the daughter nuclei in cytokinesis-blocked transgenic mouse splenocytes. **Chromosome Research**. 3: 45-53.

Bonassi, S., M. Neri, C. Lando, M. Ceppi, Y.P. Lin, W.P. Chang, N. Holland, M. Kirsch-Volders, E. Zeiger and M. Fenech. 2003. Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human MicroNucleus project. **Mutation Research**. 543: 155-166.

Bonassi, S., D. Ugolini, M. Kirsch-Volders, U. Stromberg, R. Vermeulen and J. Tucker. 2005. Human population studies with cytogenetic biomarkers: review of the literature and future prospective. **Environmental and Molecular Mutagenesis**. 45: 258-270.

Bonassi, S., A. Znaor, M. Ceppi, C. Lando, W.P. Chang, N. Holland, M. Kirsch-Volders, E. Zeiger, S. Ban, R. Barale, M.P. Bigatti, C. Bolognesi, A. Cebulska-Wasilewska, E. Fabianova, A. Fucic, L. Hagmar, G. Joksic, A. Martelli, L. Migliore, E. Mirkova, M.R. Scarfi, A. Zijno, H. Norppa and M. Fenech. 2007. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. **Carcinogenesis**. 28: 625-631.

Bovenzi, M., F. Barbone and A. Betta. 1995. Scleroderma and occupational exposure. **Scandinavian Journal of Work, Environment & Health**. 21: 289–292.

Brüning, T., H. Mann and H. Melzer. 1999a. Pathological excretion patterns of urinary proteins in renal cell cancer patients exposed to trichloroethylene. **Occupational Medicine**. (Lond) 49: 299–305.

Brüning, T., A.G. Sundberg and G. Birner. 1999b. Glutathione transferase alpha as a marker for tubular damage after trichloroethylene exposure. **Archives of Toxicology**. 73: 246–254.

Bull, R.J. 2000. Mode-of-action of liver tumor induction by trichloroethylene and its metabolites, trichloroacetate and dichloroacetate. **Environ Health Perspect**. 108 (suppl 2): 241–259.

Chen, H., D. Rupa, R. Tomar and D. Eastmond. 1994.Chromosomal loss and breakage in mouse bone marrow and spleen cells exposed to benzene *in vivo*. **Cancer Research**. 54: 3533-3539.

Cheng, T.J., D.C. Christiani, X. Xu, J.C. Wain, J.K. Wiencke and K.T. Kelsey. 1996. Increased micronucleus frequency in lymphocytes from smokers with lung cancer. **Mutation Research**. 349: 43-50.

Chia, S.E., V.H.H. Goh and C.N. Ong. 1997. Endocrine profiles of male workers with exposure to trichloroethylene. **American Journal of Industrial Medicine**. 32: 217–222.

Collins, A.R. 2004. The Comet assay for DNA damage and repair principles, applications, and limitations. **Molecular Biotechnology**. 26: 249–260.

Collins, A.R., A.A. Oscoz, G. Brunborg, I. Gaivão, L. Giovannelli, M. Kruszewski, C.C. Smith and R. Štětina. 2008. The comet assay: topical issues. **Mutagenesis.** 23: 143-151.

da Cruz, A.D., A.G. McArthur, C.C. Silva, M.P. Curado and B.W. Glickman. 1994 Human micronucleus counts are correlated with age, smoking, and cesium-137 dose in the Goiânia (Brazil) radiological accident. **Mutation Research**. 313: 57-68.

Dhawan, A., M. Bajpayee and D. Parmar. 2009. Comet assay: A reliable tool for the assessment of DNA damage in different models. **Cell Biology and Toxicology**. 25: 5-32.

Driscoll, T.R., H.H. Hamdan and G. Wang. 1992. Concentrations of individual serum or plasma bile acids in workers exposed to chlorinated aliphatic hydrocarbons. **British Journal of Industrial Medicine**. 49: 700-705.

Droz, P.O. and J.G. Fernandez. 1978. Trichloroethylene exposure: biological monitoring by breath and urine analysis, **British Journal of Industrial Medicine**. 35: 35-42.

Dusinska, M. and A. Collins. 2008 The comet assay in human biomonitoring: gene-environment interactions. **Mutagenesis.** 23: 191-205.

DuTeaux, S.B., M.J. Hengel, D.E. DeGroot, K.A. Jelks and M.G. Miller. 2003. Evidence for trichloroethylene bioactivation and adduct formation in the rat epididymis and efferent ducts. **Biology of Reproduction**. 69: 771–779.

Eastmond, D.A., M. Schuler and D.S. Rupa. 1995. Advantages and limitations of using fluorescence in situ hybridization for the detection of aneuploidy in interphase human cells. **Mutation. Research**. 348: 153-162.

Efthimiou, M., C. Andrianopoulos, G. Stephanou, N.A. Demopoulos and S.S. Nikolaropoulos. 2007. Aneugenic potential of the nitrogen mustard analogues melphalan, chlorambucil and p-N,N-bis(2-chloroethyl) aminophenylacetic acid in cell cultures *in vitro*. **Mutation Research**. 617: 125-137.

Ergene, S., A. Celik, T. Cavaş and F. Kaya. 2007. Genotoxic biomonitoring study of population residing in pesticide contaminated regions in Göksu Delta: micronucleus, chromosomal aberrations and sister chromatid exchanges. **Environment International**. 33: 877-885.

Fahrig, R. S. Madle and H. Baumann. 1995. Genetictoxicology of trichloroethylene, **Mutation Research**. 340: 1–36.

Fairbairn, D.W., P.L. Olive and K.L. O'Neill. 1995. The comet assay: a comprehensive review. **Mutation Research**. 339: 37-59.

Fisher, J.W. and B.C. Allen. 1993. Evaluating the risk of liver cancer in humans exposed to trichlorethylene using physiological models**. Risk Analysis**. 13: 87–95.

Fenech, M., N. Holland, W.P. Chang, E. Zeiger and S. Bonassi. 1999. The Human Micro Nucleus Project-An international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. **Mutation Research**. 428: 271-283.

Fenech, M. and J. Crott. 2002. Micronuclei, nucleoplasmic bridges and nuclear buds induced in folic acid deficient human lymphocytes evidence for breakage-fusion- bridge cycles in the cytokinesis-block micronucleus assay. **Mutation Research**. 504: 131-136.

Fenech, M., M. Kirsch-Volders, A.T. Natarajan, J. Surralles, J.W. Crott, J. Parry, H. Norppa, D.A. Eastmond, J.D. Tucker and P. Thomas. 2011. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. **Mutagenesis**. 26 (1): 125–132.

Ford, E.S., S. Rhodes and M. McDiarmid. 1995. Deaths from acute exposure to trichloroethylene. **Journal of Occupational and Environmental Medicine**. 37: 749-754.

Forkert, P.G., B. Millen, L.H. Lash, D.A. Putt and B.I. Ghanayem. 2006. Pulmonary bronchiolar cytotoxicity and formation of dichloroacetyl lysine protein adducts in mice treated with trichloroethylene. **Journal of Pharmacology and Experimental Therapeutics**. 316: 520–529.

Frenzilli, G., M. Nigro and B.P. Lyons. 2009. The Comet assay for the evaluation of genotoxic impact in aquatic environments. **Mutation Research**. 681: 80-92.

Gauthier, J., H. Dubeau, E. Rassart, W. Jarman and R. Wells. 1999. Biomarkers of DNA damage in marine mammals. **Mutation Research**. 444: 427-439.

Goh, V.H-H., S-E. Chia and C-N. Ong. 1998. Effects of chronic exposure to low doses of trichloroethylene on steroid hormone and insulin levels in normal men. **Environ Health Perspect**. 106: 41–44.

Goldman, J. 1996. Connective tissue disease in people exposed to organic chemical solvents: systemic sclerosis (scleroderma) in dry cleaning plant and aircraft industry workers. **Journal of Clinical Rheumatology**. 2: 185–190.

Gosden, C.M., C. Davidson and M. Robertson. 1992. Lymphocyte culture. In: Rooney D.E., Czepulkowski B.H. Human cytogenetics, a practical approach. 2nd edition, Vol I. Oxford: **Oxford University Press**. 31-54.

Hagmar, L., A. Brogger, I.L. Hansteen, S. Heim, B. Hogstedt, L. Knudsen, B. Lambert, K. Linnainmaa, F. Mitelman and I. Nordenson. 1994. Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: nordic study group on the health risk of chromosome damage. **Cancer Research**. 54: 2919-2922.

Hamdan, H. and N.H. Stacey. 1993. Mechanism of trichloroethylene-induced elevation of individual serum bile acids. I. Correlation of trichloroethylene concentrations to bile acids in rat serum. **Toxicology and Applied Pharmacology**. 121: 291-295.

Henschler, D., W. Romer and H.M. Elasser. 1980. Carcinogenicity study of trichloroethylene by long-term inhalation in three animal species. **Archives of Toxicology**. 43: 237-248.

Hessel, H., K. Radon, A. Pethran, B. Maisch, S. Gröbmair, I. Sautter and G. Fruhmann. 2001. The genotoxic risk of hospital, pharmacy and medical personnel occupationally exposed to cytostatic drugs-evaluation by the micronucleus assay. **Mutation Research.** 497: 101-109.

Holmén, A., A. Karlsson, I. Bratt and B. Högstedt. 1995. Increased frequencies of micronuclei in T8 lymphocytes of smokers. **Mutation Research**. 334: 205-208.

IARC (International Agency for Research in Cancer. Dry Cleaning). 1995. **Some Chlorinated Solvents and Other Industrial Chemicals**. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. World Health Organization: Lyon, France.

Ikeda, M. 1977. Metabolism of trichloroethylene and tetrachloroethylene in human subjects, Environ. **Health Perspect**. 21: 239-245.

Infante, P.F., E. Schwartz and R. Cahill. 1990. Benzene in petrol: a continuing hazard. **The Lancet**. 336: 814-815.

Jenssen, C. and C. Ramel. 1980. The micronucleus test as a part of a short-term mutagenicity test program for the prediction of carcinogenicity evaluated by 143 agents test. **Mutation Research**. 75: 191-202.

Joos, S., T.M. Fink, A. Rätsch and P. Lichter. 1994. Mapping and chromosome analysis: the potential of fluorescence in situ hybridization. **Journal of Biotechnology**. 35: 135-153.

Kaina, B. 2004. Mechanisms and consequences of methylating agent induced SCEs and chromosomal aberrations: a long road traveled and still a far way to go. Cytogenet. **Genome Research**. 104: 77-86.

Kaneko, T., P.Y. Wang and A. Sato. 1997. Assessment of the health effects of trichloroethylene. **Industrial Health**. 35: 301–324.

Kim, D. and B.I. Ghanayem. 2006. Comparative metabolism and disposition of trichloroethylene in Cyp2e1-/- and wild-type mice Drug Metabolism and Disposition. **Journal of Occupational and Environmental Medicine**. 34: 2020-2027.

Khan, F.M., B.S. Kaphalia and B.S. Prabhakar. 1995. Trichloroethene-induced autoimmune response in female MRL +/+ mice. **Toxicology and Applied Pharmacology**. 134: 155-160.

Kirsch-Volders, M., T. Sofuni, M. Aardema, S. Albertini, D. Eastmond, M. Fenech, M. Ishidate, E. Jr. Lorge, H. Norppa, J. Surrallés, W. von der Hude and A. Wakata. 2000. Report from the *In vitro* Micronucleus Assay Working Group. **Environmental and Molecular Mutagenesis**. 35: 167-172.

Kjellstrand, P., B. Holmquist and P. Alm. 1983a. Trichloroethylene: Further studies of the effects on body and organ weights and plasma butyrylcholinesterase activity in mice. **Acta Pharmacol Toxicol (Copenh)**. 53:375-384.

Kligerman, A.D., MF. Bryant, C.L. Doerr, G.L. Erexson, P.A. Evansky, P. Kwanyuen and J.K. McGee. 1994. Inhalation studies of the genotoxicity of trichloroethylene to rodents. **Mutation Research**. 322: 87–96.

Klobučar, G.I., M. Pavlica, R. Erben and D. Papeš. 2003. Application of the micronucleus and comet assays to mussel Dreissena polymorpha haemocytes for genotoxicity monitoring of freshwater environments. **Aquatic Toxicology**. 64: 15-23.

Krishnadasan, A., N. Kennedy, Y. Zhao, H. Morgenstern and B. Ritz. 2007. Nested case-control study of occupational chemical exposures and prostate cancer in aerospace and radiation workers. **American Journal of Industrial Medicine**. 50: 383-390.

Kumar, M., S. Tewari, P. Sharma, V.K. Verma, L.K.S. Chauchan, S.K. Agarwal, U.N. Dwivedi and S.K. Goel. 2009. Study of genetic polymorphism in solvent exposed population and its correlation to *in vitro* effect of trichloroethylene on lymphocytes**. Journal of Environmental Biology**. 30: 685-691.

Lash, L.H., J.W. Fisher, J.C. Lipscomb and J.C. Parker. 2000. Metabolism of trichloroethylene. **Environ Health Perspect**. 108: 177–200.

Lash, L.H., J.C. Parker and C.S. Scott. 2000b. Modes of action of trichloroethylene for kidney tumorigenesis. **Environ Health Perspect**. 108 (suppl 2): 225–240.

Lavin, A.L., C.F. Jacobson and J.M. DeSesso. 2000. An assessment of the carcinogenic potential of trichloroethylene in humans. **Human and Ecological Risk Assessment**. 6: 575–641.

Lindberg, H., X. Wang, H. Jarventaus, C. Ghita, M. Falck, M. Norppa and M. Fenech. 2007. Origin of nuclear buds and micronuclei in normal and folate-deprived human lymphocytes. **Mutation Research**. 617: 33-45.

Maltoni, C., G. Lefemine and G. Cotti. 1988. Long-term carcinogenicity bioassays on trichloroethylene administered by inhalation to Sprague-Dawley rats and Swiss and B6C3F, mice. **Annals of the New York Academy of Sciences**. 534: 316-342.

Mateuca, R., N. Lombaert, P.V. Aka, I. Decordier and M. Kirsch-Volders. 2006. Chromosomal changes: induction, detection methods and applicability in human biomonitoring. **Biochimie**. 88: 1515-1531.

Maull, E.A., V.J. Cogliano, C.S. Scott, H.A. Barton, J.W. Fisher, M. Greenberg, L. Rhomberg and S.P. Sorgen. 1997. Trichloroethylene health risk assessment: A new and improved process. **Drug and Chemical Toxicology**. 20: 427–42.

McCarthy, T.B. and R.D. Jones. 1983. Industrial gassing poisonings due to trichloroethylene, perchloroethylene, and l,l,l-trichloroethane, 1961-80. **British Journal of Industrial Medicine.** 40:450-455.

Merdink, J.L., A. Gonzalez-Leon, R.J. Bull and I.R. Schultz. 1998. The extent of dichloroacetate formation from trichloroethylene, chloral hydrate, trichloroacetate, and trichloroethanol in B6C3F1 mice. **The Journal of Toxicological Sciences**. 45: 33–41.

Moore, M.M. and K. Harrington-Brock. 2004. Mutagenicity of Trichloroethylene and Its Metabolites: Implications for the Risk Assessment of Trichloroethylene. **Environmental Health Perspectives**. 108: 215-223.

Motovali-Bashi, M., Zohreh. Hojati and R.M. Walmsley. 2004. Unequal sister chromatid exchange in the rDNA array of Saccharomyces cerevisiae. **Mutation Research**. 564: 129-137.

Müller, W.U. 2007. Comet Assay. In: Chromosomal Alterations: Methods, Results and Importance in Human Health. **Springer-Verlag Berlin Heidelberg**. 161-176.

Mussali-Galante, P., M. Ávila-Costa, G. Piñón-Zarate, G. Martínez-Levy, V. Rodríguez-Lara and M. Rojas-Le-mus. 2005. DNA damage as an early biomarker of effect in human health. **Toxicology and Industrial Health**. 21: 55-66.

Mutti, A. 1999. Biological monitoring in occupational and environmental toxicology. **Toxicology Letters.** 108: 77-89.

Nagaya, T., N. Ishikawa and H. Hata. 1989. Sister-chromatid exchanges in lymphocytes of workers exposed to trichloroethylene. **Mutation Research**. 222: 279-282.

Nagaya, T., N. Ishikawa and H. Hata. 1993. Subclinical and reversible hepatic effects of occupational exposure to trichloroethylene. **International Archives of Occupational and Environmental Health.** 64: 561-563.

National Research Council (NRC). 1989. **Biologic Markers in Pulmonary Toxicology**. National Academy Press, Washington, DC.

Neghab, M., S. Qu and C.L. Bai. 1997. Raised concentration of serum bile acids following occupational exposure to halogenated solvents, 1,1,2-trichloro-1,2,2-trifluoroethane and trichloroethylene. **International Archives of Occupational and Environmental Health**. 70: 187–194.

Nietert, P.J., S.E. Sutherland and R.M. Silver. 1998. Is occupational organic solvent exposure a risk factor for scleroderma. **Arthritis & Rheumatology**. 41: 1111–1118.

NTP. 1990. **Carcinogenesis Studies of Trichloroethylene (Without Epichlorohydrin) (CAS No. 79-01-6) in F344/N Rats and B6C3F1 Mice (Gavage Study).** NTP TR 243. Research Triangle Park, NC: U.S. Department of Health and Human Services.

Obe, G., P. Pfeiffer, J. Savage, C. Johannes, W. Goedecke, P. Jeppesen, A. Natarajan, A. Martinez-López, G. Folle and M. Drets. 2002. Chromosomal aberrations: formation, identification and distribution. **Mutation Research**. 504: 17-36.

Ojajarvi, A., T. Partanen and A. Ahlbom. 2001. Risk of pancreatic cancer in workers exposed to chlorinated hydrocarbon solvents and related compounds: a meta-analysis. **American Journal of Epidemiology**. 153: 841-850.

Ostling, O. and K.J. Johanson. 1984. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. **Biochemical and Biophysical Research Communications**. 123: 291-298.

Parks, P.A., M.I. Gilmour and M.K. Selgrade. 1993. Pulmonary defenses to streptococcal infection following acute exposure to trichloroethylene (TCE) or chloroform (CHCl3). **Toxicologist**. 13: 107.

Pastor, S., S. Gutiérrez, A. Creus, A. Cebulska-Wasilewska and R. Marcos. 2001. Micronuclei in peripheral blood lymphocytes and buccal epithelial cells of Polish farmers exposed to pesticides. **Mutation Research**. 495: 147-156.

Pfeiffer, P., W. Goedecke and G. Obe. 2000. Mechanisms of DNA double-strand break repair and their potential to induce chromosomal aberrations. **Mutagenesis**. 15: 289-302.

Rao, X., Y. Zhang, Q. Yi, H. Hou, B. Xu, L. Chu, Y. Huang, W. Zhang, M. Fenech and Q. Shi. 2008. Multiple origins of spontaneously arising micronuclei in HeLa cells: direct evidence from long-term live cell imaging. **Mutation Research**. 646: 41-49.

Rasmussen, K., S. Sabroe and M. Wohlert. 1988. A genotoxic study of metal workers exposed to trichloroethylene: Sperm parameters and chromosome aberrations in lymphocytes. **International Archives of Occupational and Environmental Health**. 60: 419-423.

Reinhardt, C.F., L.S. Mullen and M.E. Maxfield. 1973. Epinephrine-induced cardiac arrhythmia potential of some common industrial solvents. **Jounal of Occupational Medicine**. 15: 953-955.

Revazova, J., V. Yurchenko, L. Katosova, V. Platonova, L. Sycheva, L. Khripach, F. Ingel, T. Tsutsman and V. Zhurkov. 2001. Cytogenetic investigation of women exposed to different levels of dioxins in Chapaevsk town. **Chemosphere**. 43: 999-1004.

Rojas, E. 2009. Special issue on the 20th anniversary of the comet assay. **Mutation. Research**. 681: 1-2.

Rowland, R.E., L.A. Edwards and J.V. Podd. 2007. Elevated sister chromatid exchange frequencies in New Zealand Vietnam War veterans. **Cytogenetic and Genome Research**. 116: 248–251.

Sanders, V.M., A.N. Tucker and K.L. White. 1982. Humoral and cell-mediated immune status in mice exposed to trichloroethylene in drinking water. **Toxicology and Applied Pharmacology**. 62: 358–368.

Schulte, P.A. 1991. Contribution of biological markers to occupational health. **American Journal of Industrial Medicine**. 20: 435-446.

Schulte, P.A. 2005. The use of biomarkers in surveillance, medical screening, and intervention. **Mutation Research**. 592: 155-163.

Scott, C.S. and W.A. Chiu. 2006. Trichloroethylene cancer epidemiology: a consideration of select issues. **Environ Health Perspect**. 114: 1471-1478.

Seiji, K., C. Jin and T. Watanabe. 1990. Sister chromatid exchanges in peripheral lymphocytes of workers exposed to benzene, trichloroethylene, or tetrachloroethylene, with references to smoking habits. **International Archives of Occupational and Environmental Health**. 62: 171-176.

Singh, N.P., M.T. McCoy, R.R. Tice and E.L. Schneider. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. **Experimental Cell Research**. 175: 184-191.

Sjogren, B., N. Plato and R. Alexandersson. 1991. Pulmonary reactions caused by welding-induced decomposed trichloroethylene. **Chest**. 99: 237-238.

Slacik-Erben, R., R. Roll and G. Franke. 1980. Trichloroethylene vapors do not produce dominant lethal mutations in male mice. **Archives of Toxicology**. 45: 37.

Spencer, H.B., W.R. Hussein and P.B. Tchounwo. 2006. Growth inhibition in Japanese medaka (Oryzias Latipes) fish exposed to tetrachloroethyne. **Journal of Environmental Biology**. 27: 1-5.

Sudha, S., S. Prathyman, S. Joseph and K.S. Keyan. 2001. Micronucleus Test in Exfoliated Buccal Cells from Chromium Exposed Tannery Workers. **International Journal of Bioscience, Biochemistry and Bioinformatics**. 1: 58-62.

Sung, T.I., P.C. Chen and Lee L. Jyuhn-Hsiarn. 2007. Increased standardized incidence ratio of breast cancer in female electronics workers. **BMC Public Health**. 7: 102.

Tao, L., R. Ge and M. Xie. 1999. Effect of trichloroethylene on DNA methylation and expression of early intermediate protooncogenes in the liver of B6C3F1 mice. **Journal of Biochemical and Molecular Toxicology**. 13: 231–237.

Tice, R.R., E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.C. Ryu and Y.F. Sasaki, 2000. Single cell gel/comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. **Environmental and Molecular Mutagenesis.** 35: 206-221.

Timbrell, J.A. 1998. Biomarkers in toxicology. **Toxicology** 129: 1-12.

Troutman, W.G. 1988. Additional deaths associated with the intentional inhalation of typewriter correction fluid. **Veterinary and human toxicology**. 30: 130-132.

Tucker, J.D. and R.J. Preston. 1996. Chromosome aberrations, micronuclei, aneuploidy, sister chromatid exchanges, and cancer risk assessment. **Mutation Research**. 365: 147-159.

U.S. EPA (U.S. Environmental Protection Agency). 2009a. **Toxicological Review of Trichloroethylene (CAS No. 79-01-6) In Support of Summary Information on the Integrated**. USA.

Victor, R., A. R.P., P. Vasudev, D. Sebastian , V.A. Rao and S. Rajangam. 2002. Use of Porcine Serum in Lymphocyte Culture. **International Journal of Human Genetics**. 2: 273-274.

Wang, G. and N.H. Stacey. 1990. Elevation of individual serum bile acids on exposure to trichloroethylene or a-naphthylisothiocyanate. **Toxicology and Applied Pharmacology**. 105: 209-215.

Wartenberg, D., D. Reyner and C.S. Scott. 2000. Trichloroethylene and cancer: epidemiologic evidence. **Environ Health Perspect**. 108 (suppl 2): 161–176.

Waterfield, C.J. and J.A. Timbrell. 2000. **Biomarkers- An Overview**. MacMillan Reference, London.

Westerick, J.J., Mello, J.W. and Thomas, R.F. 1984. The groundwater supply survey. **Journal of the American Water Works Association**. 76: 52–62.

White, J.F. and G.P. Carlson. 1982. Epinephrine-induced cardiac arrhythmias in rabbits exposed to trichloroethylene: potentiation by caffeine. **Fundamental and Applied Toxicology**. 2: 125-129.

Wilson, D. and L. Thompson. 2007. Molecular mechanisms of sister-chromatid exchange. **Mutation Research**. 616: 11-23.

Windemuller, F.J.B. and J.H. Ettema. 1978. Effect of combined exposure to trichloroethylene and alcohol on mental capacity. **International Archives of Occupational and Environmental Health**. 41: 77-85.

Wolman, S.R. 1994. Fluorescence in situ hybridization: a new tool for the pathologist. **Human Pathology**. 25: 586-590.

APPENDICES

Appndix A

Questionnaire data

Appendix Table A1 Demographic statistics of study group.

Characteristics	Number	Percentage (%)
Age		
20-18year	9	3.7
21-30 year	94	38.8
40-31year	71	28.3
50-41year	47	19.4
≥ 51year	21	8.7
Gender		
Men	52	21.5
Women	190	78.5
Marital status		
Single	70	28.9
Couple	152	62.8
Widowed	7	2.9
Divorced	8	3.3
Separated	5	2.1
Education level		
Primary school	88	36.4
Junior high school	83	34.3
High school	66	27.3
Diploma	5	2.1
Length of stay) year(		
> 1year	30	12.4
5-2year	80	33.4
10-6year	49	20.2
11-15 year	26	10.7
16-20 year	9	3.7
> 21year	41	16.9

Appendix Table A1 (Continued)

Characteristics	Number	Percentage (%)
Domicile		
North region	36	14.9
North-east	118	48.8
Middle	63	26.0
East	15	6.2
West	2	0.8
South	5	2.1

Appendix Table A2 Percentage of study group differentiated by occupational history.

Characteristics	Number	Percentage (%)
1. Type of factory		
Chemical exposed group	92	38.0
Non chemical exposed group	150	62.0
Work duty (description)		
Non chemical exposed	170	70.2
chemical exposed	72	29.8
Duration of employ		
< 1year	48	19.8
2-5 year	81	33.5
10-6year	27	11.20
> 11 year	31	12.8
Health risk factor		
Chemical substances	72	29.8
Other risk	26	10.7
Chemical substances and other risk	144	59.7

Appendix Table A2 (Continued)

Characteristics	Number	Percentage (%)
Personal protective equipments		
Gloves	9	3.7
Mask	75	31.0
Gloves and mask	2	0.8
Other	3	1.2
Not use	3	1.2
2. Type of factory		
Chemical exposed group	82	33.9
Non chemical exposed group	2	0.8
Work duty		
Non chemical exposed	81	33.5
chemical exposed	9	3.7
Duration of employ		
(Number of working years in factory)		
< 1year	11	4.5
2-5year	39	16.1
10-6year	14	5.8
> 11 year	24	9.9
Health risk factors		
Exposure to trichloroethylene	44	18.2
Exposure to polishing substances	16	6.6
Other	15	6.2
3. TCE	9	3.7
Job duty		
Polishing section	6	2.5
Line puncture section	3	1.2

Appendix Table A2 (Continued)

Characteristics	Number	Percentage (%)
Duration of employ		
< 1year	1	0.4
2-5year	3	1.2
10-6year	3	1.2
Health risk factors		
Exposure to trichloroethylene	2	0.8
Exposure to polishing substances	3	1.2
Other	4	1.7

Appendix Table A3 Percentage of study group differentiated by healthy history.

Health history	Number	Percentage (%)
Cancer		
No	207	85.5
Yes	35	14.5
Respiratory diseases		
No	198	81.8
Yes	10	4.1
Skin diseases		
No	199	82.2
Yes	9	3.7
Nervous system		
No	201	83.1
Yes	9	3.7

Appendix Table A3 (Continued)

Health history	Number	Percentage (%)
Chronologically ill		
Allergy	35	14.5
Anemia	4	1.7
High blood pressure	10	4.1
Other (Please indicate)	19	7.9
Drinking		
Yes	56	23.1
No	154	63.6
Quit	18	7.4
Regular medicine		
No	177	73.1
Yes	44	18.2
-Respiratory diseases	1	0.4
- Nervous system diseases	12	5.0
- Other	18	7.4

Appendix Table A4 Percentage of study group differentiated by family healthy history.

Family healthy history	Number	Percentage (%)
Cancer		
No	28	11.6
Yes	35	14.5
High blood pressure		
No	29	12.0
Yes	51	21.10

Appendix Table A4 (Continued)

Family healthy history	Number	Percentage (%)
Asthma		
No	30	12.4
Yes	19	7.9
Allergy		
No	32	13.2
Yes	10	4.10
Anemia		
No	232	95.9
Yes	10	4.10
Tuberculosis		
No	36	14.9
Yes	5	2.1
Family healthy history	Number	Percentage (%)
Other diseases		
No	25	10.3
Yes (indicate)	54	22.3
- Gastrointestinal tract diseases	5	2.1
- Cardiovascular diseases	9	3.7
- Hormone disorder	37	15.3
- Other	2	0.8

Appendix Table A5 Current health status and disease

Health status and disease	Number	Percentage(%)
1. Respiratory symptom		
Cough		
No	124	51.2
Low prevalence	98	40.5
High prevalence	20	8.30
Nasal discharge and sputum		
No	128	52.9
Low prevalence	91	37.6
High prevalence	22	9.10
2. Respiratory symptom		
Impairment of smell		
No	211	87.2
Low prevalence	30	12.4
High prevalence	1	0.4
Tightness in the chest		
No	168	69.4
Low prevalence	64	26.4
High prevalence	10	4.10
Wheezing in the chest		
No	192	79.3
Low prevalence	41	16.9
High prevalence	9	3.7
Shortness of breath		
No	185	76.4
Low prevalence	44	18.20
High prevalence	13	5.4

Appendix Table A5 (Continued)

Health status and disease	Number	Percentage(%)
Bronchitis		
No	201	83.1
Low prevalence	35	14.5
High prevalence	6	2.5
3. Skin symptom		
Hands or fingers with fissures		
No	193	79.8
Low prevalence	44	18.2
High prevalence	5	2.1
Skin itchy		
No	166	68.6
Low prevalence	61	25.2
High prevalence	15	6.2
White scar		
No	232	95.9
Low prevalence	10	4.10
4. Nervous system symptom		
Diplopia		
No	155	64.1
Low prevalence	69	28.5
High prevalence	18	7.4
Poor vision		
No	155	64.0
Low prevalence	69	28.5
High prevalence	18	7.4

Appendix Table A5 (Continued)

Health status and disease	Number	Percentage(%)
Irritation to eyes and tear		
No	170	70.2
Low prevalence	60	24.8
High prevalence	12	5.0
Limb numbness		
No	150	62.0
Low prevalence	75	31
High prevalence	17	7.0
Generalized muscle weakness		
No	185	76.4
Low prevalence	50	20.7
High prevalence	7	2.9
Loss of balance		
No	202	83.5
Low prevalence	38	15.7
High prevalence	2	0.4
Face numbness		
No	235	97.1
Low prevalence	6	2.5
High prevalence	1	0.4
Dysarthria		
No	231	95.5
Low prevalence	9	3.7
High prevalence	2	0.8
Cardiac arrhythmia		
No	206	85.1
Low prevalence	32	13.2
High prevalenc	1	1.2

Appendix Table A5 (Continued)

Health status and disease	Number	Percentage(%)
Palpitation		
No	162	66.9
Low prevalence	76	31.4
High prevalence	4	1.7
Headache		
No	78	32.2
Low prevalence	129	53.3
High prevalence	35	14.5
Dizziness		
No	105	43.4
Low prevalence	108	44.6
High prevalence	29	12.0
Nausea and vomiting		
No	182	75.2
Low prevalence	51	21.1
High prevalence	8	3.3
Fatigue		
No	134	55.4
Low prevalence	93	38.4
High prevalence	15	6.2
Sleepy		
No	160	66.1
Low prevalence	74	30.6
High prevalence	8	3.3
Inability to sleep		
No	153	63.2
Low prevalence	70	28.9
High prevalence	19	7.9

Appendix Table A5 (Continued)

Health status and disease	Number	Percentage(%)
Restless		
No	186	76.9
Low prevalence	48	19.8
High prevalence	8	3.3
Confusion		
No	185	76.4
Low prevalence	53	21.9
High prevalence	4	1.7
Temperamental		
No	147	60.7
Low prevalence	86	35.5
High prevalence	9	3.7
Confuse		
No	176	72.7
Low prevalence	56	23.1
High prevalence	10	4.1
Memory loss		
No	138	57.0
Low prevalence	86	35.5
High prevalence	18	7.4
Sluggish		
No	140	57.9
Low prevalence	96	39.7
High prevalence	6	2.5
Depress		
No	184	76.0
Low prevalence	54	22.3
High prevalence	4	1.7

Appendix Table A5 (Continued)

Health status and disease	Number	Percentage(%)
Lost of appetite		
No	175	72.3
Low prevalence	64	26.4
High prevalence	3	1.2
Bad decision		
No	194	80.2
Low prevalence	46	19.0
High prevalence	2	0.8
Inability to concentrate		
No	180	74.4
Low prevalence	57	23.6
High prevalence	5	2.1

Appendix Table A6 Percentage of participants differentiated by lifestyle

Behavior	Number	Percentage (%)
Problems with memory		
No	156	64.5
Yes	86	35.5
Forgetfulness		
No	162	66.9
Yes	80	33.1
Using tools to remember		
No	188	77.7
Yes	54	22.3

Appendix Table A6 (Continued)

Behavior	Number	Percentage (%)
Forgetful symptoms : forgot your house key, forgot to turn off the gas stove.		
No	158	65.3
Yes	84	34.7
Not understand when reading.		
No	172	71.1
Yes	70	28.9
Irritable		
No	196	81.0
Yes	46	19.0
Inability to concentrate		
No	203	83.9
Yes	39	16.1
Depress without any reason		
No	216	89.3
Yes	26	10.7
Unusualfatigue		
No	176	72.7
Yes	66	27.3
Less interested in sex than usual		
No	199	82.2
Yes	43	17.8
Attack of cardiac arrhythmia without exercise	198	81.8
No	44	18.2
Yes		

Appendix Table A6 (Continued)

Behavior	Number	Percentage (%)
Get regular trouble with the breathing, but it always gets completely better		
No	188	77.7
Yes	54	22.3
Sweating without any reason		
No	199	82.2
Yes	43	17.8
Get headache at least 1 time per week		
No	142	58.7
Yes	100	41.3
Muscle pain		
No	177	73.1
Yes	65	26.9
Get trouble with buttoned or unbuttoned		
No	237	97.9
Yes	5	2.1

Appendix Table A7 Health history of TCA exposed participants

Health history	Number	Percentage(%)
Duration of work		
< 1 year	35	14.5
2-5 years	82	33.9
6-10 years	29	12.0
> 11 years	87	36.0

Appendix Table A7 (Continued)

Health history	Number	Percentage(%)
Department		
Finishing	25	10.3
Turning/Cutting	12	5.0
Crude-polishing/Fine-polishing	174	71.9
Quality Control	11	4.5
Job description		
Turning/Cutting	16	6.6
Crude-polishing/Fine-polishing	175	72.3
Turning-polishing	24	9.9
Chance to be exposure to TCA		
Always	111	45.9
Sometime	117	48.3
Exposure to TCA		
Few	18	7.4
Medium	31	12.8
Heavy	36	14.9
Duration of TCE exposure		
8hours	4	1.7
Once per week	2	0.8
Six days per week	1	0.4
Used to work with TCE		
No	204	84.3
Yes	38	15.7
Used to expose to TCE		
Few	2	0.8
Medium	8	3.3
Heavy	18	7.4

Appendix Table A7 (Continued)

Health history	Number	Percentage(%)
Work in shift		
No	25	10.3
Yes	206	85.1
Have a holiday		
No	139	57.4
Yes	100	41.3
A holidays per week		
No	84	34.7
Yes	155	64.0
Two holidays per week		
No	238	98.3
Yes	1	0.4
Other holidays		
No	202	83.5
Yes	37	15.3
Using cloth mask		
No	4	1.7
Yes	238	98.3
Frequency of using cloth mask		
Always	209	86.4
Sometime	22	9.1
Using of eye glasses		
No	2	0.8
Yes	26	10.7
Frequency of using eye glasses		
Always	9	3.7
Sometime	14	5.8

Appendix Table A7 (Continued)

Health history	Number	Percentage(%)
Using of gloves		
No	11	4.5
Yes	231	95.5
Frequency of wearing gloves		
Always	194	80.2
Sometime	28	11.6
Frequency of using chemical protective cloth		
Always	65	26.9
Sometime	3	1.2
Cleaning hands or skin with TCE		
No	127	52.5
Yes	113	46.7
Frequency of using TCE to clean hands or skin		
Every day	18	7.4
Sometime	90	37.2
Smelling of TCE during work period		
No smell	32	13.2
Strong smell	153	63.2
A little smell	49	20.2
Other	5	2.1
Medical history in the last six months		
No	55	22.7
Yes	26	10.7
Headache		
No	22	9.1
Yes	115	47.5
Dizziness		
No	28	11.6
Yes	75	31.0

Appendix Table A7 (Continued)

Health history	Number	Percentage(%)
Nausea		
No	38	15.7
Yes	32	13.2
Vomiting		
No	44	18.2
Yes	23	9.5
Weary		
No	26	10.7
Yes	95	39.3
Fatigue		
No	28	11.6
Yes	82	33.9
Sleepless		
No	191	78.9
Yes	51	21.1
Forgetfulness		
No	198	81.8
Yes	44	18.2
Eye irritate		
No	175	72.3
Yes	67	27.7
Itchy Skin		
No	37	15.3
Yes	44	18.2
Itchy eyes		
No	173	71.5
Yes	69	28.5

Appendix Table A7 (Continued)

Health history	Number	Percentage (%)
Poor vision		
No	217	89.7
Yes	25	10.3
Lower back pain		
No	147	60.7
Yes	95	39.3
Numbness in hands and feet		
No	193	79.8
Yes	49	20.2
Easy to catch a cold		
No	208	86.0
Yes	34	14.0
Allergic rhinitis		
No	34	14.0
Yes	39	16.1
Anosmia		
No	239	98.8
Yes	3	1.2
Other (indicate)		
No	48	19.8
Yes	5	2.1

Appendix Table A8 Observation by interviewers

List	Number	Percentage (%)
Symptom of participants reported by observation		
Normal	223	92.1
Itchy maculopapularrash on skin	11	4.5
Itchy maculopapularrash on body	1	0.4
Symptom at the first day of working		
Itchy		
Yes	4	1.7
No	44	18.2
Headache		
Yes	22	9.1
No	39	16.1
Confuse		
Yes	18	7.4
No	38	15.7
Nausea and vomiting		
Yes	9	3.7
No	42	17.4
Muscle ache		
Yes	36	14.9
No	33	13.6
Lower back pain		
Yes	29	12.0
No	213	88.0
Fatigue		
Yes	41	16.9
No	201	83.1

Appndix B

Questionnaire

ใบยินยอมด้วยความสมัครใจ (กลุ่มศึกษา)

¶ **การวิจัยเรื่อง การประเมินความเป็นพิษต่อเซลล์และสารพันธุกรรมของไตรคลอโรเอทิลีนในคนงานไทย**

วันที่ให้คำยินยอม วันที่..... เดือน ..... พ.ศ. .....

ก่อนที่จะลงนามในใบยินยอมให้ทำการศึกษาวิจัยนี้ ข้าพเจ้าได้รับคำอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย ความเสี่ยง อันตรายหรืออาการที่อาจเกิดขึ้นจากการวิจัย รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยได้ตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้าเข้าร่วมโครงการวิจัยนี้โดยสมัครใจ และสามารถบอกเลิก ยุติ หรือถอนตัวจากการศึกษานี้เมื่อใดก็ได้ และไม่ว่าข้าพเจ้าจะเข้าร่วมในการศึกษาหรือไม่ก็ตาม หรือถอนตัวจากการศึกษานี้ในภายหลังจะไม่มีผลต่อการเข้ารับบริการป้องกันและรักษาโรคที่ข้าพเจ้าพึงจะได้รับตามสิทธิต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับ จะเปิดเผยข้อมูลได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย หรือในการเปิดเผยข้อมูลต่อผู้มีหน้าที่เกี่ยวข้องกับการสนับสนุนและ/หรือกำกับดูแลการวิจัยเท่านั้น

ข้าพเจ้าได้อ่านคำอธิบายโครงการวิจัยรวมทั้งใบหนังสือยินยอมด้วยความสมัครใจ และได้รับคำตอบทุกข้อสงสัยทั้งหมดแล้ว ข้าพเจ้ามีความเข้าใจดีทุกประการและได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม.....ผู้ยินยอม

ลงนาม.....ผู้วิจัย

ลงนาม.....ผู้ที่ได้รับมอบหมาย

ใบยินยอมด้วยความสมัครใจ (กลุ่มควบคุม)

การวิจัยเรื่อง การประเมินความเป็นพิษต่อเซลล์และสารพันธุกรรมของไตรคลอโรเอทิลีนในคนงานไทย

วันที่ให้คำยินยอม วันที่..... เดือน ..... พ.ศ. .....

ก่อนที่จะลงนามในใบยินยอมให้ทำการศึกษาวิจัยนี้ ข้าพเจ้าได้รับคำอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย ความเสี่ยง อันตรายหรืออาการที่อาจเกิดขึ้นจากการวิจัย รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยได้ตอบคำถามต่าง ๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้าเข้าร่วมโครงการวิจัยนี้โดยสมัครใจ และสามารถบอกเลิก ยุติ หรือถอนตัวจากการศึกษานี้เมื่อใดก็ได้ และไม่ว่าข้าพเจ้าจะเข้าร่วมในการศึกษาหรือไม่ก็ตาม หรือถอนตัวจากการศึกษานี้ในภายหลังจะไม่มีผลต่อการเข้ารับบริการป้องกันและรักษาโรคที่ข้าพเจ้าพึงจะได้รับตามสิทธิต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับ จะเปิดเผยข้อมูลได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย หรือในการเปิดเผยข้อมูลต่อผู้มีหน้าที่เกี่ยวข้องกับการสนับสนุนและ/หรือกำกับดูแลการวิจัยเท่านั้น

ข้าพเจ้าได้อ่านคำอธิบายโครงการวิจัยรวมทั้งใบหนังสือยินยอมด้วยความสมัครใจ และได้รับคำตอบทุกข้อสงสัยทั้งหมดแล้ว ข้าพเจ้ามีความเข้าใจดีทุกประการและได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม.....ผู้ยินยอม

ลงนาม.....ผู้วิจัย

ลงนาม.....ผู้ที่ได้รับมอบหมาย

ใบยินยอมด้วยความสมัครใจ
สำหรับผู้บริหารโรงงาน

การวิจัยเรื่อง การประเมินความเป็นพิษต่อเซลล์และสารพันธุกรรมของไตรคลอโรเอทิลีนในคนงานไทย

วันที่ให้คำยินยอม วันที่..... เดือน ..... พ.ศ. .....

ชื่อโรงงาน.....

ก่อนที่จะลงนามในใบยินยอมให้ทำการศึกษาวิจัยนี้ ข้าพเจ้าได้รับคำอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย ความเสี่ยง อันตรายหรือผลที่อาจเกิดขึ้นจากการวิจัย รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยได้ตอบคำถามต่าง ๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้าในฐานะผู้บริหารของโรงงาน ได้เข้าร่วมโครงการวิจัยนี้โดยสมัครใจ และสามารถบอกเลิก ยุติ หรือถอนตัวจากการศึกษานี้เมื่อใดก็ได้ และไม่ว่าข้าพเจ้าจะเข้าร่วมในการศึกษาหรือไม่ก็ตาม หรือถอนตัวจากการศึกษานี้ในภายหลังจะไม่มีผลต่อการดำเนินงานของหน่วยงานของข้าพเจ้า

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับหน่วยงานของข้าพเจ้าเป็นความลับ จะเปิดเผยข้อมูลได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย หรือในการเปิดเผยข้อมูลต่อผู้มีหน้าที่เกี่ยวข้องกับการสนับสนุนและ/หรือกำกับดูแลการวิจัยเท่านั้น

ข้าพเจ้าได้อ่านคำอธิบายโครงการวิจัยรวมทั้งใบหนังสือยินยอมด้วยความสมัครใจ และได้รับคำตอบทุกข้อสงสัยทั้งหมดแล้ว ข้าพเจ้ามีความเข้าใจดีทุกประการและได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม.....ผู้ยินยอม หรือผู้ที่ได้รับมอบหมาย

(.....)

ตำแหน่ง.....

ลงนาม.....ผู้วิจัย

แบบสอบถามอาการทั่วไปและประวัติสุขภาพของผู้ที่สัมผัสกับไตรคลอโรเอธิลีน (กลุ่มศึกษา)

<u>ประวัติทั่วไป</u>

ชื่อโรงงาน.....โทรศัพท์.....

อายุ ..... ปี รหัส (สำหรับเจ้าหน้าที่) .....

เพศ ☐ ชาย ☐ หญิง สถานภาพ ☐ โสด ☐ คู่ ☐ หม้าย ☐ หย่า ☐ แยกกันอยู่

ระดับการศึกษาสูงสุด ☐ ประถมศึกษา ☐ มัธยมศึกษาตอนต้น

☐ มัธยมศึกษาตอนปลาย / ปวช ☐ อนุปริญญา / ปวท / ปวส

☐ ตั้งแต่ปริญญาตรีขึ้นไป ☐ อื่นๆ ระบุ.....

ที่อยู่ปัจจุบัน.....อาศัยในที่อยู่ปัจจุบันมานาน (ปี).....

ภูมิลำเนาเดิม จังหวัด.....

<u>ประวัติการทำงานจากอดีตจนถึงปัจจุบัน</u>

ชื่อโรงงาน	ลักษณะงานที่ทำ	ระยะเวลา (ปี)	สารหรือปัจจัยเสี่ยงต่อสุขภาพ	มีเครื่องป้องกันตน	อาชีพเสริม

ระยะเวลาปฏิบัติงานเฉลี่ยต่อวัน .....ช.ม./วัน ระยะเวลาปฏิบัติงานเฉลี่ยต่อสัปดาห์ .....วัน/สัปดาห์

<u>ประวัติสุขภาพส่วนตัว</u> ท่านมีประวัติเป็นโรคดังต่อไปนี้หรือไม่

1. โรคมะเร็ง ☐ ไม่เป็น ☐ เป็น มะเร็งที่.....
2. โรคระบบทางเดินหายใจ ☐ ไม่เป็น ☐ เป็น ระบุโรค.....
3. โรคผิวหนัง ☐ ไม่เป็น ☐ เป็น ระบุโรค.....
4. โรคทางระบบประสาท ☐ ไม่เป็น ☐ เป็น ระบุโรค.....
5. โรคประจำตัวอื่นๆ ☐ ภูมิแพ้ ☐ โรคเลือด ☐ ความดันโลหิตสูง

(ตอบได้มากกว่า 1 ข้อ) ☐ อื่นๆ ระบุ.....

6. สูบบุหรี่ ☐ สูบ ☐ ไม่เคยสูบ ☐ เคยสูบขณะนี้เลิกสูบ
7. ดื่มสุรา ☐ ดื่ม ☐ ไม่ดื่ม ☐ เคยดื่มขณะนี้เลิกดื่ม
8. การกินยาประจำ ☐ ไม่มี ☐ มี ระบุโรค.....

<u>ประวัติสุขภาพครอบครัว</u> คนในครอบครัวของท่านมีประวัติเป็นโรคดังต่อไปนี้หรือไม่
(ตอบได้มากกว่า 1 ข้อและระบุคนที่เป็นด้วย)

☐ โรคมะเร็ง มีความสัมพันธ์เป็น.....
☐ ความดันโลหิตสูง มีความสัมพันธ์เป็น.....
☐ หอบหืด มีความสัมพันธ์เป็น.....
☐ ภูมิแพ้ มีความสัมพันธ์เป็น.....
☐ โรคเลือด มีความสัมพันธ์เป็น.....
☐ วัณโรค มีความสัมพันธ์เป็น.....
☐ อื่นๆ ระบุ..... มีความสัมพันธ์เป็น.....

<u>อาการทั่วไป</u>

โปรดทำเครื่องหมาย ✓ ในช่องตรงกับระดับอาการเจ็บป่วยในปัจจุบันของท่าน

0 = ไม่เป็น 1 = เป็นบางครั้ง 2 = เป็นบ่อย

อาการทางระบบทางเดินหายใจ

อาการ	0	1	2
1. ไอ			
2. มีน้ำมูกและเสมหะมาก			
3. จมูกไม่ได้กลิ่น			
4. แน่นหน้าอกหายใจไม่ออก			
5. หายใจมีเสียง			
6. หายใจถี่			
7. หลอดลมตอนบนอักเสบ			

อาการทางผิวหนัง

อาการ	0	1	2
1. เป็นผื่นแดงแสบร้อนที่ผิวหนัง			
2. เป็นผื่นคันที่ผิวหนัง			
3. เป็นปื้นขาวที่ผิวหนัง			

อาการทางระบบประสาท

อาการ	0	1	2
1. มีอาการตามัว			
2. มองไม่ชัด			
3. น้ำตาไหล			
4. มีอาการชาตามปลายมือ,เท้า			
5. มีอาการแขนขาอ่อนแรง			
6. เดินยืนไม่ตรง เดินเซ			
7. ชาบริเวณใบหน้า			
8. พูดไม่ชัด			
9. หัวใจเต้นผิดปกติ			
10. ใจสั่น			
11. ปวดศีรษะ			
12. มีอาการเวียนศีรษะ			
13 คลื่นไส้อาเจียน			
14. เหนื่อย,อ่อนเพลียง่าย			
15. ง่วงซึม			
16. นอนไม่หลับ			
17. กระสับกระส่าย			
18. สับสน			
19. อารมณ์แปรปรวน			
20. มึนงง			
21. หลงลืมง่าย			
22. เหนื่อยหน่าย			
23. มีอารมณ์เศร้า			
24. เบื่ออาหาร			
25. การตัดสินใจไม่ดี			
26. ขาดสมาธิ			

กรุณาตอบคำถามต่อไปนี้ตามความเป็นจริง โดยขีดเครื่องหมาย ✓ ในช่องตรงกับความจริงของท่าน

ลำดับที่	คำถาม	ใช่	ไม่ใช่
1	คุณเป็นคนขี้ลืมง่าย		
2	ญาติหรือเพื่อนสนิทของคุณบอกว่าคุณเป็นคนขี้ลืม		
3	คุณจำเป็นต้องจดบันทึกเรื่องราวที่คุณต้องจำเสมอๆ		
4	เมื่อออกนอกบ้านคุณต้องย้อนกลับไปตรวจเช็คเกี่ยวกับสิ่งที่คุณได้ทำแล้วซ้ำอีกเสมอๆ เช่น ตรวจดูเตา ตรวจดูว่า ล๊อค ประตูหรือยัง ฯลฯ		
5	คุณรู้สึกเป็นการยากที่จะเข้าใจความหมายต่างๆจากการอ่านหนังสือพิมพ์หรือหนังสืออื่นๆ		
6	คุณเป็นคนไม่มีสมาธิ		
7	คุณรู้สึกหงุดหงิดเสมอๆ โดยไม่มีเหตุผล		
8	คุณรู้สึกซึมเศร้าเสมอๆ โดยไม่มีเหตุผล		
9	คุณรู้สึกเหนื่อยง่ายผิดปกติ		
10	คุณมีความสนใจเรื่องทางเพศน้อยกว่าปกติ		
11	คุณรู้สึกใจสั่นทั้งๆที่คุณอยู่เฉยๆไม่ได้ออกกำลังอะไรเลย		
12	คุณรู้สึกแน่นหน้าอกหรือเหมือนมีอะไรมากดทับหน้าอกเป็นบางครั้ง		
13	คุณมีอาการเหงื่อแตกโดยไม่มีเหตุผล		
14	คุณมีอาการปวดหัวอย่างน้อยสัปดาห์ละ 1 ครั้ง		
15	คุณมีอาการเจ็บปวดจี๊ดๆตามเนื้อตามตัว		
16	คุณมีปัญหาเรื่องกลัดกระดุมหรือปลดกระดุม		

ผลการตรวจทางห้องปฏิบัติการ

รายการตรวจ	ผลการตรวจ
1. ไตรคลอโรเอธิลีนในอากาศ (ค่า TWA 10 ppm)	……..... ……..
2. ไตรคลอโรอะซิติกแอซิดในปัสสาวะ (ค่าวันสุดท้ายของการทำงานในสัปดาห์ 15 mg/L)	……... ……..
3. DNA damage • MN Assay • Comet assay	……. ...……..

สรุป

……
……
……
……
……
……
……
……
……
……
……
……
……
……

แบบสอบถามอาการทั่วไปและประวัติสุขภาพของผู้ที่ไม่ได้สัมผัสกับไตรคลอโรเอธิลีน (กลุ่มควบคุม)

<u>ประวัติทั่วไป</u>

ชื่อโรงงาน.....โทรศัพท์.....

อายุ ..... ปี รหัส (สำหรับเจ้าหน้าที่) .....

เพศ ☐ ชาย ☐ หญิง สถานภาพ ☐ โสด ☐ คู่ ☐ หม้าย ☐ หย่า ☐ แยกกันอยู่

ระดับการศึกษาสูงสุด ☐ ประถมศึกษา ☐ มัธยมศึกษาตอนต้น

☐ มัธยมศึกษาตอนปลาย / ปวช ☐ อนุปริญญา / ปวท / ปวส

☐ ตั้งแต่ปริญญาตรีขึ้นไป ☐ อื่นๆ ระบุ.....

ที่อยู่ปัจจุบัน.....อาศัยในที่อยู่ปัจจุบันมานาน (ปี).....

ภูมิลำเนาเดิม จังหวัด.....

<u>ประวัติการทำงานจากอดีตจนถึงปัจจุบัน</u>

ชื่อโรงงาน	ลักษณะงานที่ทำ	ระยะเวลา (ปี)	สารหรือปัจจัยเสี่ยงต่อสุขภาพ	มีเครื่องป้องกันตน	อาชีพเสริม

ระยะเวลาปฏิบัติงานเฉลี่ยต่อวัน .....ช.ม./วัน ระยะเวลาปฏิบัติงานเฉลี่ยต่อสัปดาห์ .....วัน/สัปดาห์

<u>ประวัติสุขภาพส่วนตัว</u> ท่านมีประวัติเป็นโรคดังต่อไปนี้หรือไม่

1. โรคมะเร็ง ☐ ไม่เป็น ☐ เป็น มะเร็งที่.....
2. โรคระบบทางเดินหายใจ ☐ ไม่เป็น ☐ เป็น ระบุโรค.....
3. โรคผิวหนัง ☐ ไม่เป็น ☐ เป็น ระบุโรค.....
4. โรคทางระบบประสาท ☐ ไม่เป็น ☐ เป็น ระบุโรค.....
5. โรคประจำตัวอื่นๆ ☐ ภูมิแพ้ ☐ โรคเลือด ☐ ความดันโลหิตสูง

(ตอบได้มากกว่า 1 ข้อ) ☐ อื่นๆ ระบุ.....

6. สูบบุหรี่ ☐ สูบ ☐ ไม่เคยสูบ ☐ เคยสูบขณะนี้เลิกสูบ
7. ดื่มสุรา ☐ ดื่ม ☐ ไม่ดื่ม ☐ เคยดื่มขณะนี้เลิกดื่ม
8. การกินยาประจำ ☐ ไม่มี ☐ มี ระบุโรค.....

<u>ประวัติสุขภาพครอบครัว</u> คนในครอบครัวของท่านมีประวัติเป็นโรคดังต่อไปนี้หรือไม่
(ตอบได้มากกว่า 1 ข้อและระบุคนที่เป็นด้วย)

☐ โรคมะเร็ง มีความสัมพันธ์เป็น.....
☐ ความดันโลหิตสูง มีความสัมพันธ์เป็น.....
☐ หอบหืด มีความสัมพันธ์เป็น.....
☐ ภูมิแพ้ มีความสัมพันธ์เป็น.....
☐ โรคเลือด มีความสัมพันธ์เป็น.....
☐ วัณโรค มีความสัมพันธ์เป็น.....
☐ อื่นๆ ระบุ..... มีความสัมพันธ์เป็น.....

<u>อาการทั่วไป</u>

โปรดทำเครื่องหมาย ✓ ในช่องตรงกับระดับอาการเจ็บป่วยในปัจจุบันของท่าน

0 = ไม่เป็น 1 = เป็นบางครั้ง 2 = เป็นบ่อย

อาการทางระบบทางเดินหายใจ

อาการ	0	1	2
1. ไอ			
2. มีน้ำมูกและเสมหะมาก			
3. จมูกไม่ได้กลิ่น			
4. แน่นหน้าอกหายใจไม่ออก			
5. หายใจมีเสียง			
6. หายใจถี่			
7. หลอดลมตอนบนอักเสบ			

อาการทางผิวหนัง

อาการ	0	1	2
1. เป็นผื่นแดงแสบร้อนที่ผิวหนัง			
2. เป็นผื่นคันที่ผิวหนัง			
3. เป็นปื้นขาวที่ผิวหนัง			

อาการทางระบบประสาท

อาการ	0	1	2
1. มีอาการตามัว			
2. มองไม่ชัด			
3. น้ำตาไหล			
4. มีอาการชาตามปลายมือ,เท้า			
5. มีอาการแขนขาอ่อนแรง			
6. เดินยืนไม่ตรง เดินเซ			
7. ชาบริเวณใบหน้า			
8. พูดไม่ชัด			
9. หัวใจเต้นผิดปกติ			
10. ใจสั่น			
11. ปวดศีรษะ			
12. มีอาการเวียนศีรษะ			
13 คลื่นไส้อาเจียน			
14. เหนื่อย,อ่อนเพลียง่าย			
15. ง่วงซึม			
16. นอนไม่หลับ			
17. กระสับกระส่าย			
18. สับสน			
19. อารมณ์แปรปรวน			
20. มึนงง			
21. หลงลืมง่าย			
22. เหนื่อยหน่าย			
23. มีอารมณ์เศร้า			
24. เบื่ออาหาร			
25. การตัดสินใจไม่ดี			
26. ขาดสมาธิ			

กรุณาตอบคำถามต่อไปนี้ตามความเป็นจริง โดยขีดเครื่องหมาย ✓ ในช่องตรงกับความจริงของท่าน

ลำดับที่	คำถาม	ใช่	ไม่ใช่
1	คุณเป็นคนขี้ลืมง่าย		
2	ญาติหรือเพื่อนสนิทของคุณบอกว่าคุณเป็นคนขี้ลืม		
3	คุณจำเป็นต้องจดบันทึกเรื่องราวที่คุณต้องจำเสมอๆ		
4	เมื่อออกนอกบ้านคุณต้องย้อนกลับไปตรวจเช็คเกี่ยวกับสิ่งที่คุณได้ทำแล้วซ้ำอีกเสมอๆ เช่น ตรวจดูเตา ตรวจดูว่า ล๊อค ประตูหรือยัง ฯลฯ		
5	คุณรู้สึกเป็นการยากที่จะเข้าใจความหมายต่างๆจากการอ่านหนังสือพิมพ์หรือหนังสืออื่นๆ		
6	คุณเป็นคนไม่มีสมาธิ		
7	คุณรู้สึกหงุดหงิดเสมอๆ โดยไม่มีเหตุผล		
8	คุณรู้สึกซึมเศร้าเสมอๆ โดยไม่มีเหตุผล		
9	คุณรู้สึกเหนื่อยง่ายผิดปกติ		
10	คุณมีความสนใจเรื่องทางเพศน้อยกว่าปกติ		
11	คุณรู้สึกใจสั่นทั้งๆที่คุณอยู่เฉยๆไม่ได้ออกกำลังอะไรเลย		
12	คุณรู้สึกแน่นหน้าอกหรือเหมือนมีอะไรมากดทับหน้าอกเป็นบางครั้ง		
13	คุณมีอาการเหงื่อแตกโดยไม่มีเหตุผล		
14	คุณมีอาการปวดหัวอย่างน้อยสัปดาห์ละ 1 ครั้ง		
15	คุณมีอาการเจ็บปวดจี๊ดๆตามเนื้อตามตัว		
16	คุณมีปัญหาเรื่องกลัดกระดุมหรือปลดกระดุม		

ผลการตรวจทางห้องปฏิบัติการ

รายการตรวจ	ผลการตรวจ
1. ไตรคลอโรเอธิลีนในอากาศ (ค่า TWA 10 ppm)	……..... ……..
2. ไตรคลอโรอะซิติกแอซิดในปัสสาวะ (ค่าวันสุดท้ายของการทำงานในสัปดาห์ 15 mg/L)	……... ……..
3. DNA damage • MN Assay • Comet assay	…… ...……..

สรุป

……
……
……
……
……
……
……
……
……
……
……
……

หมายเหตุ : รหัสโครงการวิจัย 3/52-292

Version 6/2553 วันที่ 19 เมษายน 2553

Appndix C

NIOSH 1022

TRICHLOROETHYLENE **1022**

CCl2=CHCl MW: 131.39 CAS: 79-01-6 RTECS: KX4550000

METHOD: **1022**, Issue 2 EVALUATION: PARTIAL Issue 1: 15 August 1987
Issue 2: 15 August 1994

OSHA: 100 ppm; C 200 ppm;
P 300 ppm
NIOSH: 25 ppm; C 2 ppm/1 h
(waste anesthetic);
suspect carcinogen;
Group 1 Pesticide
ACGIH: 50 ppm; STEL 200 ppm;
suspect carcinogen
(1 ppm = 5.37 mg/m 3 @ NTP)

PROPERTIES: liquid; d 1.46 g/mL @ 20 °C; BP 87 °C; MP •86 °C; VP 9.9 kPa (74 mm Hg; 9.8% v/v) @ 25 °C; explosive range 11 to 41% v/v in air

SYNONYMS: trichloroethene; ethylene trichloride; triclene

SAMPLING

SAMPLER:	SOLID SORBENT TUBE (coconut shell charcoal, 100 mg/50 mg)
FLOW RATE:	0.01 to 0.2 L/min
VOL-MIN:	1 L @ 100 ppm
MAX:	30 L
SHIPMENT:	routine
SAMPLE STABILITY:	not determined
BLANKS:	2 to 10 field blanks per set

MEASUREMENT

TECHNIQUE:	GAS CHROMATOGRAPHY, FID
ANALYTE:	trichloroethylene
DESORPTION:	1 mL CS 2; stand 30 min
INJECTION VOLUME:	5 µL
TEMPERATURE	
-INJECTION:	225 °C
-DETECTOR:	250 °C
-COLUMN:	70 °C
CARRIER GAS:	N_2, 30 mL/min
COLUMN:	3 m x 3-mm OD stainless steel, packed with 10% OV-101 on 100/200 mesh Chromosorb WHP
CALIBRATION:	standard solutions of trichloroethylene in CS_2
RANGE:	0.5 to 10 mg per sample
ESTIMATED LOD:	0.01 mg per sample [2]
PRECISION (S_r):	0.038 @ 1.6 to 6.4 mg per sample [1]

APPLICABILITY: The working range is 27 to 875 ppm (150 to 4700 mg/m 3) for a 3.4-L air sample. The method is applicable to STEL determinations. The method was used for samples containing 0.5 to 5 mg trichloroethylene from a tool-degreasing operation [2].

INTERFERENCES: None studied. Alternate columns which have been used are stainless steel, 6 m x 3 mm OD, packed with 10% SP-1000 on 80/100 mesh Supelcoport [2] and fused silica capillary, 60 m x 0.32 mm, coated with 0.25 µm OV-351 [3].

REAGENTS:

1. Carbon disulfide (CS_2), chromatographic quality.*
2. Trichloroethylene (TCE), reagent grade.*
3. Nitrogen, purified.
4. Hydrogen, prepurified.
5. Air, filtered, compressed.

*See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Sampler: glass tube, 7 cm long, 6-mm OD, 4- mm ID, flame-sealed ends with plastic caps, containing two sections of 20/40 mesh activated (600 °C) coconut shell charcoal (front = 100 mg; back = 50 mg) separated by a 2-mm urethane foam plug. A silylated glass wool plug precedes the front section and a 3-mm urethane foam plug follows the back section. Pressure drop across the tube at 1 L/min airflow must be less than 3.4 kPa. Tubes are commercially available.
2. Personal sampling pump, 0.01 to 0.2 L/min, with flexible connecting tubing.
3. Gas chromatograph, flame ionization detector, integrator, and column (see page 1022-1).
4. Vials, 2-mL, PTFE-lined septum caps.
5. Syringes, 10-µL, readable to 0.1 µL.
6. Volumetric flasks, 10-mL.
7. Pipet, TD, 1-mL.

SPECIAL PRECAUTIONS: Carbon disulfide is toxic and a serious fire and explosion hazard (flash point= •30 °C). Trichloroethylene is a suspect carcinogen and a narcotic [6,7,8]. Work with these substances only in a hood.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Break the ends of the sampler immediately before sampling. Attach sampler to personal sampling pump with flexible tubing.
3. Sample at an accurately known flow rate between 0.01 and 0.2 L/min for a total sample size of 1 to 30 L.
4. Cap the samplers. Pack securely for shipment.

SAMPLE PREPARATION:

5. Place the front and back sorbent sections of the sampler tube in separate vials. Discard the glass wool and foam plugs.
6. Add 1.0 mL CS 2 to each vial. Cap each vial.
   NOTE: A suitable internal standard, such as ethylbenzene [1], undecane [2], or octane [3] at 0.1% (v/v) may be added at this step.
7. Allow to stand 30 min with occasional agitation.

CALIBRATION AND QUALITY CONTROL:

8. Calibrate daily with at least six working standards.
   a. Add known amounts of TCE to CS 2 in 10-mL volumetric flasks and dilute to the mark. Use serial dilutions as needed to obtain TCE concentrations in the range 0.01 to 10 mg/mL.
   b. Analyze with samples and blanks (steps 11 and 12).
   c. Prepare calibration graph (peak area vs. mg TCE)
9. Determine desorption efficiency (DE) at least once for each lot of sorbent used for sampling in the range of interest. Prepare three tubes at each of five concentrations plus three media blanks.
   a. Remove and discard back sorbent section of a media blank sampler.
   b. Inject a known amount (2 to 20 μL) of TCE, or a standard solution thereof in

CS_2, directly onto front sorbent section with a microliter syringe.

c. Cap the tube. Allow to stand overnight.

d. Desorb (steps 5 through 7) and analyze with working standards (steps 11 and 12). e. Prepare a graph of DE vs. mg TCE recovered.

10. Analyze three quality control blind spikes and three analyst spikes to ensure that the calibration graph and DE graph are in control.

MEASUREMENT:

11. Set gas chromatograph according to manufacturer's recommendations and to conditions given on page 1022-1. Inject sample aliquot manually using solvent flush technique or with autosampler.

NOTE: If peak area is above the linear range of the working standards, dilute an aliquot of the desorbed liquid with CS 2, reanalyze, and apply the appropriate dilution factor in calculations.

12. Measure peak area.

CALCULATIONS:

13. Determine the mass, mg (corrected for DE) of TCE found in the sample front (W_f) and back (W_b) sorbent sections and in the average media blank front (B_f) and back (B_b) sorbent sections.

NOTE: If $W_b > W_f/10$, report breakthrough and possible sample loss.

14. Calculate concentration, C, of TCE in the air volume sampled, V (L):

$$\boldsymbol{C = \frac{\left(W_f + W_b - B_f + B_b\right) \cdot 10^3}{v}, mg/m^3}$$

EVALUATION OF METHOD:

Method S336 was issued on June 6, 1975 [4], and validated with generated atmospheres using a calibrated syringe drive [1]. Average recoveries were 92 to 94% (16 samples) in the range 477 to 2025 mg/m^3 for 3.4-L samples. Breakthrough volume of 18.5 L (effluent = 5% of test concentration) occurred after sampling for 99 min at 0.187 L/min from an atmosphere containing 2266 mg/m^3 trichloroethylene in dry air. Desorption efficiency for SKC Lot 105 activated coconut charcoal in the range 1.6 to 6.4 mg per sample averaged 96.4% with S_r = 0.7% (18 samples). n-Octane was used as an internal standard in the chromatographic measurements. The semi-quartile ranges of desorption efficiencies in two rounds of the Proficiency Analytical Testing (PAT) program were 0.97 to 1.0 for charcoal tubes spiked with 0.6 to 1.1 mg trichloroethylene [9].

REFERENCES:

[1] Documentation of the NIOSH Validation Tests, S336, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 77-185 (1977), available at Stock No. PB 274-248 from NTIS, Springfield, VA 22161.

[2] UBTL Report for NIOSH Sequence #4266-R (NIOSH, unpublished, March 26, 1984).

[3] UBTL Report for NIOSH Sequence #4266-N (NIOSH, unpublished, March 14, 1984**)**

[4] NIOSH Manual of Analytical Methods, 2nd ed., Vol. 3, S336, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 77-157-C (1977).

[5] Ibid., Vol. 1., P&CAM 127, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 77-157-A (1977).

[6] Criteria for a Recommended Standard...Occupational Exposure to Trichloroethylene, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 73-11025 (1973).

[7] NIOSH Current Intelligence Bulletin 2, Trichloroethylene (TCE), NIOSH

(June 6, 1975), U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 78-127 (1978).

[8] Special Occupational Hazard Review with Control Recommendations -- Trichloroethylene, U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 78-130 (1978).

[9] Groff, J. Personal communication, NIOSH (July, 1985).

METHOD REVISED BY:

G. David Foley, NIOSH/DPSE.

CURRICULUM VITAE

NAME : Ms. Siriporn Singthong

BIRTH DATE : 14 September 1966

BIRTH PLACE : Phichit, Thailand

EDUCATION :

YEAR	INSTITUTE	DEGREE/DIPLMA
1989	Kasetsart Univ.	B.Sc. (Biology)
2003	Kasetsart Univ.	M.S. (Environmental Science)

WORK PLACE : Reference Laboratory and Toxicology, Bureau of Occupational and Environmental Diseases, Department of Disease Control, Ministry of Public Health.