行政院國家科學委員會專題研究計畫　成果報告

氯乙烯工人世代生物標記研究(2/2)

計畫類別：個別型計畫
計畫編號：NSC92-2320-B-002-140-
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日
執行單位：國立臺灣大學公共衛生學院職業醫學與工業衛生研究所

計畫主持人：鄭尊仁

計畫參與人員：蕭舒庭、蕭敦仁、謝蕙宜

報告類型：完整報告
報告附件：出席國際會議研究心得報告及發表論文
處理方式：本計畫可公開查詢

中　華　民　國 93 年 8 月 31 日

行政院國家科學委員會補助專題研究計畫成果報告

氯乙烯工人世代生物標記研究

計畫類別：☑個別型計畫□整合型計畫

計畫編號：NSC92-2320-B-002-140

執行期間：91 年 8 月 1 日至 93 年 7 月 31 日

計畫主持人：鄭尊仁 教授

計畫參與人員： 謝蕙宜，蕭敦仁，蕭舒庭

執行單位：台大職業醫學與工業衛生研究所

行政院國家科學委員會專題研究計畫成果報告

氯乙烯工人世代生物標記研究

計畫編號：NSC92-2320-B-002-140
執行期限：91 年 8 月 1 日至 93 年 7 月 31 日
主持人：鄭尊仁 教授
計畫參與人員：謝蕙宜，蕭敦仁，蕭舒庭

中文摘要

氯乙烯為人類已知的致癌物質，已被國際癌症研究院 (IARC) 歸類為第一類致癌物質,我們過去的研究也發現氯乙烯暴露與肝功能異常及慢性肝疾病有關，然而劑量-反應關係並不清楚；此外，台灣地區為 B 型肝炎病毒帶原率高的地區，B 型或 C 型肝炎病毒感染是肝功能異常及肝硬化的重要因子,若同時暴露化學物質如酒精及黃麴毒素對肝臟疾病可具有協同作用,但目前有關肝炎病毒感染合併氯乙烯暴露，是否會造成交互作用並不清楚。因此本研究第一年以血清麩草醋酸轉胺酶(AST)及麩丙醋酸轉胺酶(ALT)為肝功能指標,探討氯乙烯及二氯乙烯（與氯乙烯結構類似且具肝毒性的化合物）暴露與肝功能異常之相關，然後進一步探討 B 型或 C 型肝炎病毒感染，對於肝功能異常是否存有交互作用。本研究第二年主要是探討氯乙烯暴露與肝纖維化的劑量反應關係。我們利用杜氏所發展之數學模式計算個人累積暴露劑量,探討氯乙烯暴露與肝纖維化與肝硬化之劑量-反應關係，然後進一步探討氯乙烯暴露與 B 型或 C 型肝炎病毒感染對肝纖維化的發生是否也有交互作用。另外，氯乙烯暴露代謝後所產生的活化性中間產物亦可能對肝細胞功能產生影響,因此本研究也進一步分析易感性基因多形性包括代謝基因 *CYP2E1*、*ALDH2*、*GSTT1* 及 DNA 修補基因 *XRCC1* 於氯乙烯暴露造成慢性肝纖維化與硬化的影響。

研究對象包括台灣 5 家聚氯乙烯工廠、4 家氯乙烯製造工廠的男性員工,我們採集其血液及收集健檢資料及詳細問卷資料,包括生活習慣 (抽煙及喝酒情形等)及詳細工作史，根據工作史建立累積暴露劑量,並利用超音波診斷慢性肝纖維與肝硬化，。

研究結果顯示（附錄一），肝炎病毒感染與身體質量指數偏高是血清轉胺酶異常最重要的影響因子。當工人未有肝炎病毒感染時,不同暴露組間的血清轉胺酶異常比例並未有明顯差異,但在感染肝炎病毒的工人中,高暴露組較低暴露組,血清轉胺酶異常比例均顯著增加（OR=6.5、6.2, p<0.01），中暴露組也有增加的趨勢，雖然未達顯著意義。進一步分析發現 B 型肝炎病毒 e 抗原陽性者其肝功能異常比率較高,並且在高暴露組特別明顯。在肝纖維化（包括纖維化與肝硬化）的研究中（附錄二），發現曾經從事高暴露工作比未曾從事高暴露工作對肝纖維化的危險性有顯著增加 (OR=5.5, 95%CI=1.7-25.4)，而 B 型或 C 型肝炎病毒感染和身體質量指數(≧25)都是肝纖維化的重要危險因子。另外，氯乙烯暴露與 B 型或 C 型肝炎病毒感染對肝纖維化的發生有交互作用,但是因為

有肝纖維化的研究對象數目較少，統計上並未達到顯著。我們進一步分析（附錄三），發現氯乙烯暴露工人之慢性肝疾病隨累積暴露劑量增加有上升的趨勢，以氯乙烯累積暴露劑量小於 40 ppm-years 工人為對照組，40-1000 ppm-years，1000-10000 ppm-years，大於 10000 ppm-years 工人的慢性肝疾病危險性分別為 OR=3.5, 95％CI=0.4-32.1, OR=4.1, 95％CI=0.4-43.9, OR=5.6 , 95％CI=0.5-68.4；我們也發現 B 型或 C 型肝炎病毒感染是慢性肝疾病重要的危險因子 (OR=6.2, 95%CI=2.3-16.9)。在易感性基因分析方面，以氯乙烯累積暴露劑量小於 1000 ppm-years 具 *XRCC1 Arg-Arg/Arg-Gln* 基因型者為對照，氯乙烯累積暴露劑量小於 1000 ppm-years 具 *XRCC1 Gln-Gln* 基因型者對慢性肝疾病的危險性為 2.7 (95％CI=0.3-29.3)，在氯乙烯累積暴露劑量大於 1000 ppm-years 中，具 *XRCC1 Arg-Arg/Arg-Gln* 基因型者對慢性肝疾病的危險性為 1.3 (95％CI=0.4-4.2)，而氯乙烯累積暴露劑量大於 1000 ppm-years 且具有 *XRCC1 Gln-Gln* 基因型者對慢性肝疾病則有較高的危險性 (OR=10.1, 95％CI=1.2-85.1)。在 *CYP2E1* 的分析中，以氯乙烯累積暴露劑量小於 1000 ppm-years 具 *CYP2E1 c1c1/c1c2* 基因型者為對照，氯乙烯累積暴露劑量小於 1000 ppm-years 具 *CYP2E1 c2c2* 基因型對慢性肝疾病的危險性為 3.3 (95％CI=0.3-33.7)，在氯乙烯累積暴露劑量大於 1000 ppm-years 中，具有 *CYP2E1 c1c1/c1c2* 基因型者對慢性肝疾病的危險性為 1.2 (95%CI=0.4-4.0)，而氯乙烯累積暴露劑量大於 1000 ppm-years 且具有 *CYP2E1 c2c2* 基因型者對慢性肝疾病有較高的危險性 (OR=7.8, 95％CI=1.3-46.1)。進一步分析，以 B 型或 C 型肝炎病毒感染呈現陰性具 *XRCC1 Arg-Arg/Arg-Gln* 基因型者為對照組，B 型或 C 型肝炎病毒感染呈現陰性且具 *XRCC1 Gln-Gln* 基因型對於慢性肝疾病的危險性為 3.9 (95％CI=0.4-36.9)，在呈現有 B 型或 C 型肝炎病毒感染陽性中，具 *XRCC1 Arg-Arg/Arg-Gln* 基因型者對慢性肝疾病的危險性為 6.2 (95％CI=2.1-18.3)，而有 B 型或 C 型肝炎病毒感染且具 *XRCC1 Gln-Gln* 基因型者有最高的危險性 (OR=28.0, 95%3.4-231.8)

根據本研究結果，B 型及 C 型肝炎病毒感染與氯乙烯或二氯乙烯暴露對血清轉胺酶活性存有交互作用，而在具 e 抗原之 B 型肝炎感染工人此作用更為明顯。在慢性肝病研究中，氯乙烯慢性暴露會增加肝纖維化的危險性，而且具劑量反應關係。我們的研究也發現具有易感性 *CYP2E1* 及 *XRCC1* 基因型的氯乙烯工人，較易發生慢性肝疾病。另外，具有 *XRCC1* 易感性基因型並有 B 型或 C 型肝炎病毒感染的工人比較容易發生慢性肝疾病。因此，具有易感性基因或有 B 型或 C 型肝炎病毒感染者，特別是 e 抗原陽性者，應盡量避免在氯乙烯暴露。

關鍵字：氯乙烯、二氯乙烯、血清轉胺酶、B 型肝炎表面抗原、B 型肝炎 e 抗原、C 型肝炎抗體、肝纖維化、肝硬化、脾腫大，XRCC1、CYP2E1、ALDH2、GSTT1。

Abstract

The association between the angiosarcoma of liver and vinyl chloride monomer (VCM) exposure has been established. Thus, VCM is classified as a group I carcinogen by International Agency for Research on Cancer (IARC). Recently, the association between VCM exposure and hepatocellular cancer is also reported. However, the relationships of VCM exposure with liver cirrhosis and abnormal liver function are less clear. Furthermore, hepatitis B infection is common in Taiwan, which has been reported to have synergistic effect with chemicals including alcohol and aflatoxin. In this study, we investigated the dose response relationship between VCM exposure and liver fibrosis (liver fibrosis and cirrhosis). Then we further tested the synergistic effects between hepatitis virus infection and VCM exposure on liver enzymes and liver fibrosis. Furthermore, genetic polymorphysims are also found to modify the chemical induced liver diseases. Here, we also examined the effects of metabolic genotypes on VCM related liver diseases.

A total of 568 male workers who were employed in 5 polyvinyl chloride (PVC) or 4 vinyl chloride monomer (VCM) manufacturing factories were included for analysis. Information relating to current job title, alcohol consumption and cigarette smoking was obtained by an interviewer-administered questionnaire. Exposure level of chemical mixtures was classified by hygienic effect (a summation of personal time weighted average / reference permissible exposure level of each chemical) into high, moderate and low exposure groups. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), and anti-hepatitis C antibody were assayed.

For studying the association between VCM exposure and liver fibrosis, a total of 347 workers with

occupational exposure to VCM were systemically examined using liver ultrasonography and routine liver function tests. Vinyl chloride monomer cumulative dose (ppm-month) was estimated by summing the products of air VCM concentration levels and months of employment. Liver fibrosis was defined in subjects with precirrhosis and cirrhosis of liver diagnosed using ultrasonography.

To assess the dose response relationship between VCM and chronic liver diseases, cumulative dose was calculated for each subject using a model developed by Du et al. The genetic polymorphisms for GSTT1, ALDH2, CYP2E1 and XRCC1 were determined using PCR-RFLP assay.

In the study of liver enzymes, hepatitis virus infection and increased body mass index were associated with abnormal serum aminotransferase activity. We also found that in workers with hepatitis virus infection, those with high exposure had a higher prevalence of abnormal AST and ALT as compared to low exposure group (odds ratio, 6.2, 6.5; $p<0.01$). While among those without hepatitis virus infection, the differences of prevalence of abnormal AST and ALT were not statistically significant between different chemical exposure groups. Such a synergistic effect was more prominent among HBeAg-positive workers.

In the subsequent study, significantly increased risks of developing liver fibrosis were found in workers who had history of high exposure jobs (O.R. 5.5, 95% C.I. 1.7-25.4) when compared with workers who did not have history of high exposure jobs.

There was dose-response relationship between VCM exposure and chronic liver diseases (liver fibrosis and splenomegaly). Analysis showed that susceptible CYP2E1 and XRCC1 modified the VCM related chronic liver diseases. Furthermore, we found that

XRCC1 modified the relationship of hepatitis virus infection related chronic liver diseases.

We conclude that VCM may cause abnormal liver function and chronic liver diseases including liver fibrosis, liver cirrhosis and splenomegaly. Further, there was a synergistic effect between hepatitis virus infection and VCM on liver enzyme abnormality and possibly for chronic liver diseases. Assessment of fitness for work should be considered in workers with hepatitis B and C infection, when they have potential exposure to hepatotoxin at workplace. Furthermore, in countries where hepatitis B and C virus infection is prevalent, more stringent occupational standard is needed to protect workers exposed to hepatotoxin.

Key terms: Liver enzyme, liver fibrosis, liver cirrhosis, vinyl chloride, genetic polymorphism

成果自評

本計畫研究成果與計畫目標符合。部分內容已發表在 Occup Environ Med. 或已被 J Occup Environ Med 接受。

附錄一

Synergistic effect by hepatitis virus infection and occupational exposures to vinyl chloride monomer and ethylene dichloride on serum aminotransferase activity

Hui-I Hsieh, Jung-Der Wang, Pau-Chung Chen, Tsun-Jen Cheng

Institute of Occupational Medicine and Industrial Hygiene, College of Public Health, National Taiwan University, Taipei, Taiwan (H-I Hsieh, J-D Wang, P-C Chen, T-J Cheng)

Center for the Research of Environmental and Occupational Diseases, College of Public Health, National Taiwan University, Taipei, Taiwan (J-D Wang, P-C Chen, T-J Cheng)

Department of Family Medicine, Cathay General Hospital, Taipei, Taiwan (H-I Hsieh)

Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan (J-D Wang)

Short running head: Chemical-viral interaction on serum aminotransferases

Key words: serum aminotransferase; hepatitis virus infection; occupational chemical exposure

Abbreviation: AST, aspartate aminotransferase; ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; anti-HCV, anti-hepatitis C antibody; VCM, vinyl chloride monomer; EDC, 1,2-ethylene dichloride; PEL, permissible exposure level; TWA, time weighted average; PVC, polyvinyl chloride; GSH, glutathione

Correspondence to: Dr. T-J Cheng, Institute of Occupational Medicine and Industrial Hygiene, College of Public Health, National Taiwan University, Taipei, Taiwan 10018; tcheng@ha.mc.ntu.edu.tw

Main Messages

- Hepatitis virus infection and increased body mass index are important non-occupational factors

that increase serum aminotransferase in asymptomatic chemical workers.

- Hepatitis virus infection has synergistic effect with exposure to vinyl chloride monomer and 1,2-ethylene dichloride on serum aminotransferase activity.
- Synergistic effect between hepatitis virus infection and chemical exposures on serum aminotransferase is more prominent in workers with positive HBeAg as compared to those with positive HBsAg but without HBeAg.

Policy implications

- Assessment of fitness for work should be considered in workers with hepatitis B and C infection, when they have potential exposure to hepatotoxin at workplace.
- In countries where hepatitis B and C virus infection is prevalent, more stringent occupational standard is needed to protect workers exposed to hepatotoxin.

Abstract

Objectives --To study the synergistic effect of occupational chemical exposure with hepatitis virus infection on serum aminotransferase activity.

Methods --A total of 568 male workers who were employed in 5 polyvinyl chloride (PVC) or 4 vinyl chloride monomer (VCM) manufacturing factories were included for analysis. Information relating to current job title, alcohol consumption and cigarette smoking was obtained by an interviewer-administered questionnaire. Exposure level of chemical mixtures was classified by hygienic effect (a summation of personal time weighted average / reference permissible exposure level of each chemical) into high, moderate and low exposure groups. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), and anti-hepatitis C antibody were assayed.

Results -- Hepatitis virus infection and increased body mass index were associated with abnormal serum aminotransferase activity. We also found that in workers with hepatitis virus infection, those with high exposure had a higher prevalence of abnormal AST and ALT as compared to low exposure group (odds ratio, 6.2, 6.5; $p<0.01$). While among those without hepatitis virus infection, the differences of prevalence of abnormal AST and ALT were not statistically significant between different chemical exposure groups. But there was a significant trend of increasing risks of elevated AST and ALT in moderate and high exposure groups with hepatitis virus infection. Such a synergistic effect was more prominent among HBeAg-positive workers.

Conclusions --We conclude that mixed exposures to EDC and VCM have positive synergistic effect with hepatitis virus infection on liver damage. Assessment of fitness for work should be considered in workers with hepatitis B and C infection, when they have potential exposure to hepatotoxin at workplace. Furthermore, in countries

where hepatitis B and C virus infection is prevalent, more stringent occupational standard is needed to protect workers exposed to hepatotoxin.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have been used extensively in the assessment of the liver damage. Abnormal serum aminotransferases have been associated with occupational and non-occupational factors.[1] Among nonoccupational hepatotoxins, hepatitis B and C virus infection have synergistic effects with alcohol consumption on serum aminotransferases.[2][3] However, it is not clear whether occupational chemical exposure and hepatitis B and C have synergistic effects on these hepatic enzymes or not. This question is needed to be resolved for the assessment of fitness of work, particularly in population with high prevalence of hepatitis B infection, including Taiwan.[4][5]

We have followed vinyl chloride monomer (VCM) and polyvinyl chloride (PVC) manufacturing workers for their liver disorders in the past decade. Either VCM (CAS No. 75-01-4) or 1,2-ethylene dichloride (EDC; CAS No. 107-06-2) exposure has been associated with serum aminotransferase abnormalities in our previous studies.[6][7] In the above studies, it seems that exposed workers with hepatitis B infection tend to have higher risk of abnormal serum aminotransferase as compared to those without hepatitis B infection. However, it was difficult to draw a conclusion because of the small number of subjects with abnormal serum aminotransferase. To increase the power of detection for the interaction between hepatitis virus infection and occupational chemical exposure, we included workers from both PVC and VCM manufacturing plants, who received medical examination between 1995 and 1997. Since workers with hepatitis B e antigen (HBeAg) also had higher prevalence of serum aminotransferase abnormality,[8] the interaction between HBeAg and occupational chemical exposure on serum aminotransferase was further assessed.

Methods

STUDY POPULATION

A total of 617 workers were eligible. Because 49 workers had incomplete data, 568 (92%) workers from 5 PVC (n = 292) and 4 VCM (n = 276) manufacturing factories were included for the analysis. Among them, 11 workers were office workers, who stayed indoor during most of their working hours, and 3 workers were guards who stayed away from the

manufacturing site. Most of these workers have been presented in previous studies to investigate the relationship between external chemical exposures and liver function or genotoxicity, or the effects of metabolic genotypes on liver function or genotoxicity.[6][7][9]

After informed consent was obtained, all study subjects were surveyed by an interviewer-administered questionnaire to obtain information on smoking, alcohol consumption, medicines, and medical and occupational histories. Alcohol consumption was calculated from drinking frequency and alcohol content of each beverage consumed. Those who drank at least once and had alcohol consumption with a minimum of 80 grams intake per week in past one month were defined as having drinking habit. Smoking behavior was defined as having smoked at least one cigarette per day within the preceding 6 months of data collection.

EXPOSURE ASSESSMENT

EDC is used in the production of VCM, and VCM is subsequently used for the polymerization to manufacture PVC. In VCM manufacturing plants, workers were exposed to both EDC and VCM, while workers in PVC plants were exposed to VCM only. Detailed occupational history included job title, daily activity and use of respirator in the current and previous jobs. Personal samplings were conducted to calculated EDC and VCM time weighted average (TWA) for each category of work.[9][10] If personal sampling data was not available, data of area sampling was used. Office workers and guards were presumably exposed to extreme low concentration of chemicals, thus 0 ppm of VCM and EDC were assumed as their TWA. To consider the combined effect of EDC and VCM, the hygienic effect was calculated by using the model of $(C_1/T_1) + (C_2/T_2)$, where C was the measured TWA and T was the permissible exposure levels or equivalents for each chemical. One ppm was used for both EDC and VCM in this study, which has been adopted by many institutions. Our previous study also found that EDC and VCM cause abnormal liver aminotransferase around 1 ppm. [7] Workers with hygienic effects below 1 were classified into the low exposure group. Workers with hygienic effects between 1 to 5 were classified into the moderate exposure group, and workers with exposures greater than 5 were classified into the high exposure group.

BIOCHEMICAL TESTS AND HEPATITIS VIRUS MARKERS

Markers of liver damage, including AST and ALT, were analyzed with a Hitachi 7050 autoanalyzer (Hitachi Co, Tokyo, Japan) at National Taiwan University Hospital (NTUH). Hepatitis B virus surface antigen (HBsAg) and anti-hepatitis C virus antibody (anti-HCV) were determined by enzyme-linked immunoassay (EIA, Abbott Laboratories, Chicago, IL, USA), respectively. HBeAg was also assayed with ELISA method in workers with positive HBsAg. Abnormal results for serum aminotransferases were defined as having values greater than reference provided by NTUH. In 1995, it was 31 for both ALT and AST. In 1996 and 1997, it was 37 for AST, and 41 for ALT. Subjects with positive hepatitis B infection were defined as having positive HBsAg, and subjects with positive hepatitis C infection were defined as having positive anti-HCV. Since the number of subjects exhibiting a positive titer for anti-HCV was small, HBsAg and anti-HCV were grouped together as hepatitis virus infection. BMI was calculated as weight in kilograms divided by the square of height in meters.

STATISTICAL ANALYSIS

PC/SAS statistical package (SAS Institute Inc., Cary, NC, USA) was used for the statistical analysis. χ^2 test was used to compare the differences of age, employment duration, hepatitis virus infections, body mass index and alcohol consumption between different exposure groups. Crude comparisons of abnormal AST and ALT by variables of interests were conducted in the univariate analysis. Subsequently, a multiple logistic regression model was used to determine the odds ratio (OR) of abnormal AST and ALT levels for different exposure groups (high, moderate and low chemical exposure), hepatitis virus infection (yes and no), body mass index ($\geq$ 25.0 and <25.0 kg/m^2) and habitual drinking (yes and no). OR of abnormal AST and ALT levels on different exposure groups stratified by hepatitis B and C virus infection was also calculated after controlling for potential confounders including age, BMI and alcohol drinking. OR of abnormal AST and ALT on the chemical exposure (low, moderate and high) was further calculated by HBeAg and HBsAg status (-/-, -/+, and +/+, respectively). All the *p*-values were quoted two-sided, and those values < 0.05 were regarded as statistically

significant.

Results

DESCRIPTIVE STATISTICS

The basic characteristics of study subjects stratified by different exposure groups are summarized in Table 1. The median TWA of VCM is 0.67 (range from 0.0 to 73.8) ppm and EDC is 0.35 (from 0.0 to 30.5) ppm. Most workers (83.6%) were less than 50 years of age, 29.8% of workers had BMI greater than 25, 11.1% of workers consumed more than 80 grams of alcohol per week, 17.3% of workers had HBsAg, and 3.5% of workers had HBeAg. High exposure group had more habitual drinkers than moderate and low exposure groups. Low exposure group was older than moderate and high exposure groups. All other characteristics of cigarette smoking, BMI, HBeAg, HBsAg and Anti-HCV were not statistically significant between these three exposure groups.

Overall, 112 workers (19.7%) showed elevations of AST or ALT. There were 22.4%, 20.4, and 18.6% of workers who having abnormal AST or ALT among high, moderate and low exposure groups. Workers of high exposure had more cases of abnormal AST or ALT when comparing with other workers, but this did not reach a statistical significance.

MULTIPLE LOGISTIC REGRESSION ANALYSIS

Multiple logistic regression analysis in Table 2 revealed that AST was associated with BMI and hepatitis virus infection. ALT also had similar findings but the association with hepatitis C infection and ALT did not reach a statistical significance. Increased chemical exposure was also associated with abnormal ALT or AST, but this association did not reach a statistical significance.

INTERACTION ANALYSIS OF FACTORS ON SERUM AMINOTRANSFERASE

The interactions between chemical exposure and each potential factor (hepatitis virus infection, BMI and alcohol drinking) on serum aminotransferase were calculated. Significant interactions were observed for chemical exposure and hepatitis virus infection (Table 3). When workers didn’t have hepatitis virus infection, all serum aminotransferases showed no difference among different exposure groups. The dose-dependent effects of chemicals on AST and ALT were found when workers had hepatitis virus infection. Workers

who had high chemical exposure and hepatitis virus infection had the highest risk on abnormal AST (OR, 10.6; 95% CI, 3.6-31.5) and ALT (OR, 6.4; 95% CI, 2.1-19.1) when comparing with workers who were low chemical exposure and lacked hepatitis virus infection. If we confined the analysis for those with hepatitis virus infection, high exposure group had the highest risk of abnormal AST (OR, 6.2; 95% CI, 1.8-21.4) and ALT (OR, 6.5; 95% CI, 1.8-23.6) when comparing with low exposure group.

Further analysis was also performed when workers with positive HBsAg were divided into 2 groups according to the presence of HBeAg status (Figure 1). After adjustment for age, drinking, and BMI, significant higher risks of abnormal AST (OR, 7.1; 95% CI, 0.6-81.2) and ALT (OR, 21.0; 95% CI, 1.4-320.7) were observed in high exposure group compared with low exposure group among workers with both HBsAg and HBeAg. Among workers who had positive HBsAg but did not have positive HBeAg, higher risk of abnormal AST (OR, 5.1; 95% CI, 1.1-24.3) or ALT (OR, 3.7; 95% CI, 0.7-20.0) was observed in high exposure group when comparing with low exposure group. Moreover, among workers without HBsAg, chemical exposure didn't increase the risk of abnormal liver function. Workers who had positive HBeAg and high chemical exposure had the highest risk of abnormal AST (OR, 29.5; 95% CI, 5.2-166.0) and ALT (OR, 25.6; 95% CI, 3.9-168.0) when comparing with low exposed workers without HBsAg. There is a statistically significant trend (Mantel extension test for trend, $p<0.01$) for increased frequency of abnormal AST and ALT along with severity of hepatitis virus infection among high exposure group.

Among workers whether consuming alcohol or not, prevalence of abnormal AST and ALT was not observed between different chemical exposure groups. It is interesting to observe that, after adjusted with hepatitis virus infection, age, and BMI, drinkers with hepatitis virus infection had higher rates of abnormal AST (OR, 9.1; 95% CI, 2.4-34.1) and ALT (OR, 2.9; 95% CI, 0.9-10.1) as compared to those without hepatitis virus infection and habitual drinking. The positive interaction effect of hepatitis virus infection and alcohol consumption on AST reached a statistical significance.

Discussion

The results reveal that occupational chemical exposure had

positive interaction with hepatitis B and/or C infection on serum AST and ALT. This synergistic effect was the most obvious in workers with positive HBeAg. However, a statistical association may not indicate a causal association. As the most commonly reported determinants for elevation of AST and/or ALT, including BMI, alcohol, and age, were all controlled; we suspected that such an association might be causal.

Hepatitis B and/or C infection have been associated with elevated serum aminotransferase activities.[11] Our results reveal similar findings, although the association between anti-HCV and ALT was not statistically significant. This is probably due to small numbers of workers with anti-HCV. Increased BMI is also associated with increased ALT and AST in our study (table 2). Increased BMI is a common etiology of abnormal liver function tests for healthy workers.[6][7][12] Our results corroborate such an association. Alcohol consumption has been reported to be associated with abnormal liver function,[11] but our results didn't show this relationship. This is most likely due to relative small amount of alcohol consumption by theses workers. Lack of association between alcohol consumption with abnormal ALT and AST was also observed in several studies conducted in Taiwanese workers.[10][15] A study conducted in Italy showed that those consumed 80 gm each day had greater risk of developing abnormal liver function among chronic symptomless HBV carriers.[2] Positive synergism was also observed between HCV infection and alcohol consumption.[3] Our study also revealed that HBV and HCV infection exacerbated the effect of alcohol on AST, although the effects of alcohol on ALT was less prominent. Again, this could result from the small amount of alcohol consumption in our study subjects. Additionally, AST elevation is usually more prominent than ALT in alcoholic hepatitis.[13][14] Thus, the relationship between abnormal serum aminotransferases and nonoccupational factors in our study is consistent with previous studies.

A recent study also showed that the relationship between occupational dimethylformamide exposure and abnormal liver function were enhanced in those with HBV infection.[15] Here, we demonstrated that VCM and EDC together could also have a more than additive interaction with HBV and HCV infection. Further analysis in our study indicated that workers with HBeAg were more likely to have abnormal ALT and

AST as compared to those with HBsAg alone, when they were exposed to occupational chemicals. As both VCM and EDC were reported to be hazardous to the liver, detection of such an effect is not surprised and the mechanism should be clarified.

Previous human and animal studies indicated that glutathione (GSH) depletion can be caused by hepatitis virus infection.[16][17][18] Glutathione S-transferases (GST) and glutathione play an important role in the metabolism of EDC and VCM, of which the electrophilic intermediate metabolites are conjugated with GSH to be detoxified.[19][20] Thus, GSH depletion caused by hepatitis virus infection may lead to an accumulation of active intermediate metabolites of EDC and VCM, then exacerbate EDC and VCM-induced hepatotoxicity. Previous study that conducted with 1,1-Dichloroethylene showed correlations between hepatocellular damage and magnitudes of both covalent binding and GSH depletion also supported this proposed hypothesis.[21] Our recent study also suggests that the GSTT1 genotype may play an important role on liver aminotransferase abnormality caused by vinyl chloride.[6] In patients with positive HBeAg, there is more active HBV replication and inflammation, which can reduce the level of GSH.[22] Therefore, they are at a high risk of showing elevation for AST and ALT, as showed in figure 1. We conclude that subjects with hepatitis B and/or C virus infections are more likely to be damaged by hepatotoxic agents, such a potential synergistic effect may be caused by GSH depletion after hepatitis virus infection.

Studies conducted in human and rats also found that ethanol significantly decreased the glutathione concentrations,[23][24][25] of which the synergistic hepatotoxic effect between alcohol and hepatitis virus infection could be also resulted from potential overloading of the oxidative damage through the generation of reactive intermediate and decreased radical scavenging.

It is our concern that VCM or EDC workers with HBV and/or HCV infections may not be well protected under current occupational standards. We suggest workers with HBeAg not to be involved in works exposed to hepatotoxins. For workers with anti-HCV and abnormal serum aminotransferases, we also advise them not be exposed to hepatotoxin. Workers with positive HBsAg but negative HBeAg need to be closely followed for their serum aminotransferases, if

exposed to higher levels of VCM or EDC. Most of all, more stringent occupational standard is needed to protect workers exposed to hepatotoxin in countries where hepatitis B and C virus infection is prevalent.

Acknowledgments

We thank Mr. Yen-Cheng Chen and Dr. Chung-Li Du for their technical assistance. We also thank Professor Robert Chen for his editorial assistance.

References

1 Chen JD, Wang JD, Tsai SY, et al. Effects of occupational and nonoccupational factors on liver function tests in workers exposed to solvent mixtures. *Arch Environ Health* 1997;**52**:270-4.

2 Villa E, Rubbiani L, Barchi T, et al. Susceptibility of chronic symptomless HBsAg Carriers to ethanol-induced hepatic damage. *Lancet* 1982;**2**:1243-4.

3 Nevins CL, Malaty H, Velez ME, et al. Interaction of alcohol and hepatitis C virus infection on severity of liver disease. *Dig Dis Sci* 1999;**44**:1236-42.

4 Chen DS. Natural history of chronic hepatitis B virus infection: new light on old story. *J Gastroenterol Hepato* 1993;**8**:470-5.

5 Huang KY, Lin SR. Nationwide vaccination: A success story in Taiwan. *Vaccine* 2000;**18**:S35-8.

6 Huang CY, Huang KL, Cheng TJ, et al. The GSTT1 and CYP2E1 genotypes are possible factors causing vinyl chloride induced abnormal liver function. *Arch Toxicol* 1997;**71**:482-8.

7 Cheng TJ, Huang ML, You NC, et al. Abnormal liver function in workers exposed to low levels of ethylene dichloride and vinyl chloride monomer. *J Occup Environ Med* 1999;**41**:1128-33.

8 Chu CM, Sheen IS, Lin SM, et al. Sex difference in chronic hepatitis B virus infection: studies of serum HBeAg and alanine aminotransferase levels in 10,431 asymptomatic Chinese HBsAg carriers. *Clin Infect Dis* 1993;**16**:709-13.

9 Cheng TJ, Chou PY, Huang ML, et al. Increased lymphocyte sister chromatid exchange frequency in workers with exposure to low level of ethylene dichloride. *Mutat Res* 2000;**470**:109-14.

10 Du CL, Kuo ML, Chang HL, et al. Changes in lymphocyte single strand breakage and liver function of workers exposed to vinyl chloride monomer. *Toxicol Lett* 1995;**77**:379-85.

11 Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N Engl J Med* 2000;**342**:1266-71.

12 Salvaggio A, Periti M, Miano L, et al. Body mass index and liver enzyme activity in serum. *Clin*

Chem 1991;**37**:720-3.

13 Clermont RJ, Chalmers TC. The transaminase tests in liver disease. *Medicine* 1967;**46**:197-207.

14 Sorbi D, Boynton J, Lindor KD. The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. *Am J Gastroenterol* 1999;**94**:1018-22.

15 Luo JC, Kuo HW, Cheng TJ, et al. Abnormal liver function associated with occupational exposure to dimethylformamide and hepatitis B virus. *J Occup Environ Med* 2001;**43**:474-82.

16 Swietek K, Juszczyk J. Reduced glutathione concentration in erythrocytes of patients with acute and chronic viral hepatitis. *J Viral Hepat* 1997;**4**:139-41.

17 Barbaro G, Di Lorenzo G, Soldini M, et al. Hepatic glutathione deficiency in chronic hepatitis C: quantitative evaluation in patients who are HIV positive and HIV negative and correlations with plasmatic and lymphocytic concentrations and with the activity of the liver disease. *Am J Gastroenterol* 1996;**91**:2569-73.

18 De Flora S, Hietanen E, Bartsch H, et al. Enhanced metabolic activation of chemical hepatocarcinogens in woodchucks infected with hepatitis B virus. *Carcinogenesis* 1989;**10**:1099-106.

19 ATSDR (Agency for Toxic Substances and Disease Registry). *Toxicological profile for vinyl chloride – Update.* Atlanta, Georgia: ATSDR, 1997.

20 World Health Organisation. *Environmental Health Criteria 176 1,2-Dichloroethane, Second Edition.* Geneva: WHO, 1995.

21 Dowsley TF, Forkert PG, Benesch LA, et al. Reaction of glutathione with the electrophilic metabolites of 1,1-dichloroethylene. *Chem Biol Interact* 1995;**95**:227-44.

22 Kaneko S, Miller RH, Di Bisceglie AM, et al. Detection of hepatitis B virus DNA in serum by polymerase chain reaction. Application for clinical diagnosis. *Gastroenterology* 1990;**99**: 799-804.

23 Michelet F, Gueguen R, Leroy P, et al. Blood and plasma glutathione measured in healthy subjects by HPLC: relation to sex, aging, biological variables, and life habits. *Clin Chem* 1995;**41**:1509-17.

24 Oh SI, Kim CI, Chun HJ, et al. Chronic ethanol consumption affects glutathione status in rat liver. *J Nutr* 1998;**128**:758-63.

25 Vendemiale G. Grattagliano I. Signorile A, et al. Ethanol-induced changes of intracellular thiol compartmentation and protein redox status in the rat liver: effect of tauroursodeoxycholate. *J Hepatol* 1998;**28**:46-53

TABLE 1

Frequency distribution of basic characteristics in percentage (%) among 568 male workers stratified by exposure categories

Characteristic	Chemical exposure			
	High (≥ 5) (n=76) (%)	Moderate (≥1 and < 5) (n=191) (%)	Low (<1) (n=301) (%)	Total (N=568) (%)
Manufacturing factory				
PVC	67.1	64.4	39.2	51.4**
VCM	32.9	25.6	60.8	48.6**
Age ≥ 40 years	46.1	46.1	59.1	53.0*
Duration of employment ≥ 15 years	43.4	45.0	56.5	50.9*
Body mass index ≥ 25.0 kg/m^2	29.0	33.0	27.9	29.8
Current cigarette smoking (yes)	48.7	43.5	36.9	40.7
Alcohol drinking (yes)	23.7	8.4	9.6	11.1**
Positive hepatitis B surface antigen (HBsAg)	19.7	15.7	17.6	17.3
Positive hepatitis B e antigen (HBeAg)	7.9	1.6	3.7	3.5
Positive anti-hepatitis C antibody (Anti-HCV)	1.3	4.7	3.7	3.7

* $p< 0.05$; ** $p< 0.01$ by χ^2 test.

TABLE 2

Odds ratio (OR) with 95% confidence intervals (C.I.) of multiple logistic regression modeling adjusted for major determinants: including body mass index (BMI), chemical exposure, hepatitis B surface antigen (HBsAg), anti-hepatitis C antibody (Anti-HCV), drinking, and smoking

		AST	ALT
Determinants	Definition	OR (95% C.I.)	OR (95% C.I.)
Age (years)	≥ 40.0 *v* < 40.0	0.8 (0.5-1.5)	0.6 (0.4-1.0)
BMI (Kg/M^2)	≥ 25.0 *v* < 25.0	2.2 (1.2-3.9)*	3.5 (2.2-5.5)*
Chemical exposure	High *v* Low	1.3 (0.6-2.9)	1.4 (0.7-2.6)
	Moderate *v* Low	0.8 (0.4-1.5)	1.0 (0.6-1.6)
HBsAg	Positive *v* Negative	3.5 (1.9-6.4)*	2.5 (1.5-4.2)*
Anti-HCV	Positive *v* Negative	5.9 (2.2-15.9)*	2.3 (0.8-6.3)
Drinking	Yes *v* No	1.3 (0.6-3.0)	0.9 (0.5-1.9)
Smoking	Yes *v* No	1.1 (0.6-2.1)	1.0 (0.6-1.6)

* $p < 0.01$ by χ^2 test.

TABLE 3

Frequencies and adjusted Odds ratio (OR) with 95% confidence intervals (C.I.) of abnormal serum aminotransferase activity stratified by exposure classification and hepatitis virus infection. All odds ratios were adjusted for age, body mass index and alcohol drinking.

	AST (n=58)			ALT (n=103)		
Category	%	OR (95%CI)†	OR (95% CI)‡	%	OR (95% CI)†	OR (95% CI)‡
HBsAg (+) or Anti-HCV (+)						
High Exposure	56.3	10.6 (3.6-31.5)*	6.2 (1.8-21.4)*	62.5	6.4 (2.1-19.1)*	6.5 (1.8-23.6)*
Moderate Exposure	23.5	3.0 (1.1-8.0)	1.6 (0.4-5.7)	35.3	2.1 (0.9-5.0)	2.3 (0.7-7.5)
Low Exposure	15.9	2.3 (0.9-5.4)	1.0 (Referent)	19.0	1.4 (0.7-3.0)	1.0 (Referent)
HBsAg (-) and Anti-HCV (-)						
High Exposure	1.7	0.5 (0.1-1.5)		11.7	0.5 (0.2-1.3)	
Moderate Exposure	5.7	0.6 (0.3-1.6)		14.6	0.7 (0.4-1.3)	
Low Exposure	8.8	1.0 (Referent)		16.4	1.0 (Referent)	

* $p < 0.01$ by Mantel-Haenszel χ^2 test for trend analysis

† The referent group (OR=1) refers to workers with low chemical exposure and negative hepatitis virus infection.

‡ The referent group (OR=1) refers to workers with low chemical exposure and positive hepatitis virus infection.

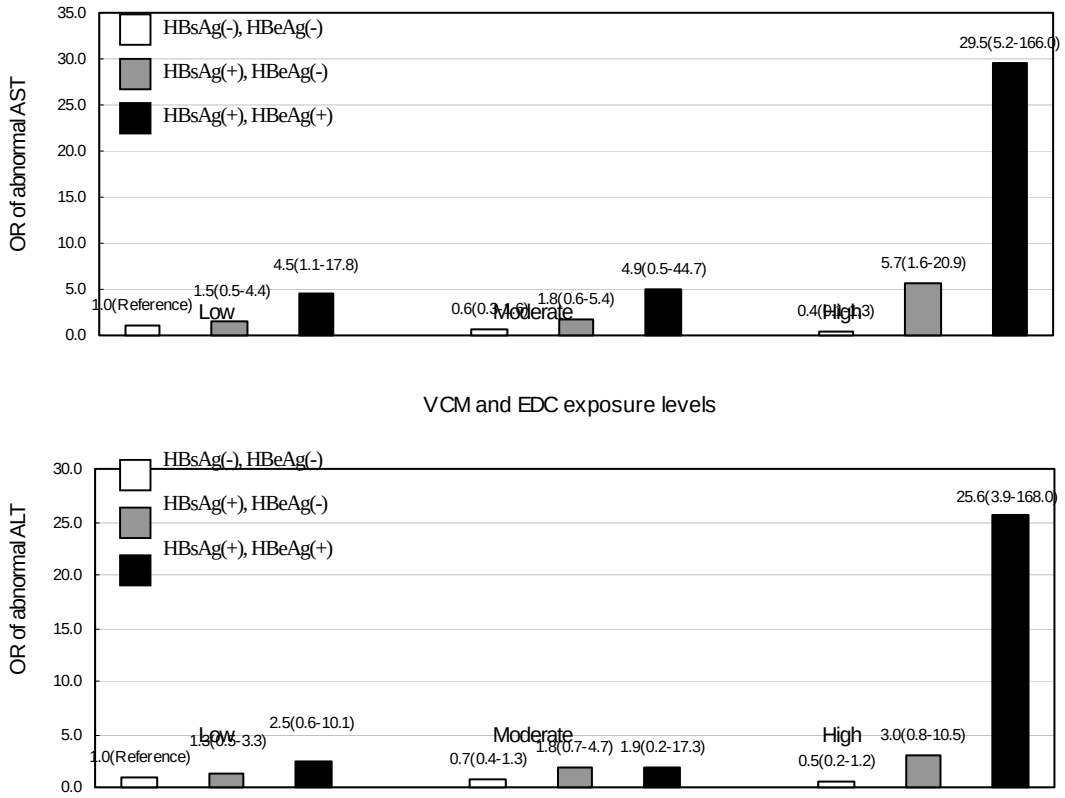

Figure 1

Odds ratio (OR) with 95% confidence intervals (CI) of abnormal AST (top) and ALT (bottom) among workers with different categories of exposure and hepatitis B infection; adjusted with age, drinking, Anti-HCV and BMI. The reference group (OR=1.0) refers to workers with low chemical exposure and negative hepatitis B infection.

附錄二

Liver Fibrosis in Asymptomatic Poly-vinyl Chloride Workers

Tun-Jen Hsiao, MD, MS[1,2] Jung-Der Wang, MD, ScD,[2,3] Pei-Ming Yang, MD, PhD,[3]

Pei-Jen Yang, MD,[1] Tsun-Jen Cheng, MD, ScD[2]

[1]Department of Internal Medicine, Tao-Yuan General Hospital

[2]Institute of Occupational Medicine and Industrial Hygiene, College of Public Health, National Taiwan University

[3]Department of Internal Medicine, National Taiwan University Hospital

Address correspondence to: Dr. Tsun-Jen Cheng,

Institute of Occupational Medicine and Industrial Hygiene,

College of Public Health, National Taiwan University,

No. 1 Jen-Ai Rd., Sec. 1, Taipei, 10018, Taiwan;

e-mail: tcheng@ha.mc.ntu.edu.tw

Running title: Liver fibrosis in PVC workers

Number of words: 3,624

ABSTRACT

This study was designed to determine whether vinyl chloride monomer (VCM) exposure is associated with liver fibrosis. A total of 347 workers with occupational exposure to VCM were systemically examined using liver ultrasonography and routine liver function tests. Vinyl chloride monomer cumulative dose (ppm-month) was estimated by summing the products of air VCM concentration levels and months of employment. Liver fibrosis was defined in subjects with precirrhosis and cirrhosis of liver diagnosed using ultrasonography. Significantly increased risks of developing liver fibrosis were found in workers who had history of high exposure jobs (O.R. 5.5, 95% C.I. 1.7-25.4) when compared with workers who did not have history of high exposure jobs. We concluded that there was an increased risk of developing liver fibrosis in PVC workers who had high exposure to VCM.

Key terms: Liver fibrosis, liver cirrhosis, obesity, vinyl chloride

INTRODUCTION

According to the results of epidemiological studies, angiosarcoma of the liver has been associated with vinyl chloride monomer (VCM) exposure (1). Thus, VCM has been classified as a group I carcinogen by International Agency for Research on Cancer (IARC)(2). However, the association between VCM exposure and liver cirrhosis is less clear. Cases of liver cirrhosis have been reported in PVC workers with high doses of VCM exposure (3,4). Recently, PVC workers were found to have an increased risk of liver cirrhosis as compared with control subjects (5). However, the effects of hepatitis viral infection on liver cirrhosis could not be separated from the VCM exposure in previous studies. Because the number of cirrhosis was small, we further included precirrhotic fibrosis of liver in this study to investigate whether VCM exposure leaded to liver fibrotic change, which consisted of precirrhosis and cirrhosis (6) as diagnosed by liver ultrasonography.

Liver function tests including AST (Aspartate aminotransferase), ALT (alanine aminotransferase), and GGT (gamma glutamyl transpeptidase) have been widely used in medical surveillance on those who are exposed to hepatic toxins. Liver function has been less reliable in detecting chronic liver diseases particularly for liver cirrhosis and liver cancer than other methods of diagnosis. Thus, we also compared the prevalence of abnormal results on liver function tests between individuals with and without liver fibrosis diagnosed using results of liver ultrasonography.

MATERIAL AND METHODS

After informed consent was obtained from each subjects, we performed liver ultrasonographic examinations on 382 workers from five polyvinyl chloride (PVC) manufacturing plants from 1994 through 1997. All these workers had no symptoms of liver disease and were currently working in the plants. Considering the induction period of chronic liver disease, we excluded the workers with working history of polyvinyl chloride production of less than 1 year. Female workers were also excluded because of small number. Thus, a total of 347 male workers were included for analysis.

Each worker completed an interviewer-administered questionnaire with questions which pertained to history of alcohol consumption, tobacco smoking, medicine, and work environment. We used air vinyl chloride concentrations as determined by Du et al. in 1995 to ascertain the association between VCM exposure and abnormal liver function (7). Briefly, personal samplings were performed to calculate median TWA concentrations of VCM for each category of work. If personal sampling was not available, data of area sampling was used. The VCM levels ranged between less than 1 ppm and 80 ppm. To further determine the association between cumulative VCM exposure and liver fibrosis, several exposure indices were used. Cumulative VCM exposure doses (ppm-month) for the study subjects were calculated by summing the product of air VCM concentration levels, as previously determined, and months of employment

(7). Since the above methodology may underestimate the exposure dosage of early years, history of high VCM exposure jobs including reaction tank cleaning, PVC unloading, and catalyst adding, were also used as exposure indices. The median of cumulative exposure dose among those workers with the history of high exposure jobs was about 2400 ppm-month. Thus, those with history of high exposure jobs were further divided into high and moderate exposure groups using 2400 ppm-month as the cutoff point, while the low cumulative VCM exposure group was defined as having never been involved in the high exposure jobs. The median of duration of employment for those with history of high exposure jobs was 40 months. Again, we divided those with the history of high exposure jobs into two groups using 40 months as the cutoff point.

Current alcohol drinking was defined as having consumed greater than or equal to one drink per week. Total amount of alcohol consumed for each worker was also calculated. Current tobacco smoking was defined as having at least one cigarette per day. Number of pack-years of cigarette smoking was also calculated for each worker. Overweight was defined as body mass index (BMI) greater than or equal than 25 (8). Liver enzymes of AST, ALT, GGT, and HBsAg, and Anti-HCV were determined at the National Taiwan University Hospital (NTUH). Abnormalities of AST, ALT,

and GGT were defined as having values greater than the reference ranges at NTUH, and positivity of HBsAg and anti-HCV was determined according to the manufacturers' recommendation. Ultrasonographic examinations of liver and spleen were also performed in each worker (Toshiba, model SAL-38B equipped with a 3.75 MHz convex-type transducer) by three hepatologists at NTUH, who were blind to the exposure status of these workers and applied the same criteria to make the diagnoses of liver fibrosis, fatty liver, and splenomegaly (6,9). Liver fibrosis includes precirrhosis and cirrhosis, both of which have heterogeneous echo patterns. Liver cirrhosis, a more advanced form of liver fibrosis, was diagnosed in the presence of coarse echo patterns with and without irregular surface outlines. Fatty liver was recognized by increased liver echogenicity.

Statistical analysis was performed using SAS (statistical analysis system) edition 6.12. ANOVA (analysis of variance) was used to compare the continuous variables, and chi-square test or Fisher's exact test was used to compare the interval variables among different exposure groups for abnormal liver functions and liver fibrosis. Subsequently, multiple logistic regression models were used to assess whether VCM exposure, hepatitis viral infection, body mass index, alcohol consumption, and tobacco smoking were associated with abnormal liver enzymes and liver fibrosis.

RESULTS

The basic characteristics of the 347 study subjects are shown in Table 1. The average age of the workers was 41±10 (mean±SD) years. The median cumulative dose of VCM exposure for the workers was around 2400 ppm-month. The average of body mass index was 24.0±2.6. Sixty-one (17.6%) workers consumed alcohol regularly. Positive results for HBsAg were found in 73 (21.0%) workers. The subjects were not tested for Anti-HCV before 1995. Among the 290 workers who had anti-HCV data, eight (2.8%) workers tested positive. The prevalence of abnormal AST, ALT and GGT were 6.3%, 10.7%, and 13.5%, respectively. Results for ultrasonogrpahic examinations among different exposure groups are compared in Table 2. Twenty subjects (5.8%) were diagnosed with liver fibrosis, and seven (2.0%) with cirrhosis of the liver. Among these 20 workers with liver fibrosis, only 10 had at least one abnormality in liver function tests. Proportion of liver fibrosis was significantly higher in the high exposure group as compared with the low exposure group. Splenomegaly was found in 32 (9.2%) workers. The association between splenomegaly and VCM exposure was not significant. Fatty liver was observed in 135 (38.9%) workers, which was the most common ultrasonographic finding among these workers. However, fatty liver was not associated with VCM exposure. Eight subjects had small hemangiomas. However, we did not observe any focal lesion which was consistent with either angiosarcoma of liver or hepatocellular cancer.

Multiple logistic regression models revealed that VCM exposure was not associated with fatty liver, and overweight was the only factor associated with fatty liver (O. R. 5.1, 95% C.I. 3.1- 8.6). Table 3 summarized the results of analysis, which observed that subjects with abnormal AST were associated with viral hepatitis (O.R. 7.3, 95% C.I. 2.8-20.4) and habitual drinking (O.R. 3.1, 95% C.I. 1.1-8.4). Subjects with abnormal ALT were associated with viral hepatitis (O.R. 3.0, 95% C.I. 1.4-6.4) and

overweight (O.R. 2.6, 95% C.I. 1.2-5.5). Furthermore, subjects with abnormal GGT were associated with habitual drinking (O.R. 3.9, 95% C.I. 1.9-8.0). Although those with VCM exposure greater than 10 ppm tended to have higher risk of abnormal ALT and AST as compared with those with VCM less than 10 ppm, the relationship was not statistically significant.

Logistic regression models were further used to analyze the association between VCM exposure and liver fibrosis (Table 4). Workers with history of high exposure jobs had an O.R. of 4.5 to develop pre-cirrhosis (95% C.I. 1.1-30.1) compared with workers without history of high exposure jobs. Similar results were also obtained for those with cirrhosis (O.R. 5.8; 95% C.I. 0.9-116.8). When we combined precirrhosis and cirrhosis together as liver fibrosis, workers with history of high exposure jobs had higher risk of developing liver fibrosis (O.R. 5.5; 95% C.I. 1.7-25.4). Viral hepatitis B and/or C infections and overweight were also found to be independent risk factors for liver fibrosis in these models. Three different exposure indices were further used to test the association between cumulative VCM exposure dose and liver fibrosis. Workers with history of high exposure jobs were divided into high and moderate cumulative VCM exposure groups. These groups had odds ratios for liver fibrosis of 5.9 (95% C.I.1.7-28.2) and 4.6 (95% C.I. 1.0-25.5) respectively as compared with the workers without history of high exposure jobs. Workers with history of high exposure jobs were also divided by the duration of work. Those with longer duration of high exposure jobs had O.R. of 3.7 (95% C.I. 1.0-18.3) and those with shorter duration had O.R. of 6.3 (95% C.I. 1.6-33.1) as compared with those without history of high exposure.

When low exposure workers with neither HBsAg nor anti-HCV were used as a reference group, risk of liver fibrosis was higher in low exposure workers with HBsAg and/or anti-HCV, and in high exposure workers with neither HBsAg nor anti-HCV

(O.R. 7.9 and 4.2, respectively). High exposure workers with HBsAg and/or anti-HCV experienced the highest risk (O.R. 40.8). However, the interaction term between hepatitis infection and VCM exposure on liver fibrosis was not significant.

DISCUSSION

Increased morbidity odds ratio of liver cirrhosis among Taiwanese vinyl chloride monomer-exposed workers has been previously reported (5). In this study, we further observed that high VCM exposure jobs were associated with precirrhosis, cirrhosis and liver fibrosis in asymptomatic workers. Data of anti-HCV were not available in 2 out of 20 cases with liver fibrosis. Assuming the worst scenario that these two subjects had positive anti-HCV, VCM exposure remained associated with liver fibrosis. As we have already controlled alcohol drinking, hepatitis B and/or C infections and BMI in our multiple logistic regression models, we suspect that the association may be a causal one and deserves further attention. Recently, increased periportal fibrosis of liver diagnosed by ultrasonography was also reported in Italian workers (10).

Although different exposure models consistently showed that workers with history of high exposure jobs had an increased risk of liver fibrosis, the dose response relationship was less prominent for cumulated exposure. Moreover, there was no such relationship in the analysis of work duration. Duration is not a sensitive indicator because it can't reflect the difference between current and remote exposure levels. The cumulative dose is also not sensitive enough, because the remote exposure is very likely under estimated. Thus, methodology of cumulated dose calculation needs to be improved.

Since our study was conducted in asymptomatic workers only, selection bias can't be completely ruled out. Those with advanced liver cirrhosis may leave their job earlier. Since they tend to have high VCM exposure, the real risk of liver cirrhosis from VCM exposure may be underestimated. When these workers leave their job because of HBV or HCV status, the real OR may remain, given the VCM exposure status is evenly distributed in these workers. In fact, the proportion of positive HBsAg and anti-HCV in the current study was

compatible with that in general population. Therefore, the true OR of liver fibrosis for VCM exposure may be higher.

In our study, hepatitis B and/or C viral infection carried an odds ratio of 10.7 for liver fibrosis, which is consistent with previous studies in Taiwan (11-13). Model fitting also revealed that there was a potential multiplicative effect between viral hepatitis and VCM exposure on liver fibrosis. Since the number of subjects with liver fibrosis was small in our study, further verification is needed.

The other independent risk factor for developing liver fibrosis was overweight, or BMI ≥ 25, as shown in table 4. In our present study, workers with liver fibrosis had higher BMI than those without fibrosis (25.9 ± 2.0 vs 23.9 ± 2.6, Student's *t*-test, $P < 0.001$). In a recent study by Ratziu et al. in 2000, 93 overweight patients without any known risk factors of liver damage had persistently elevated ALT levels. Among them, 28 patients (30%) had septal fibrosis including 10 subjects with cirrhosis (14). The possible mechanism was that the excess weight induced non-alcoholic steatohepatitis (NSAH) via lipid preoxidation (15, 16). Although the progression from NASH to liver fibrosis is slow, some study results showed that about 37% of patients with fatty liver had undergone this change (17-19). Thus, body weight reduction program may be considered in future health promotion for workers with increased BMI.

William et al. in 1976 were among the first to use ultrasonographic examinations for monitoring chronic liver disease in PVC manufacturing workers (20). They detected abnormal findings of ultrasonography in 5 out of 10 workers with normal liver function. However, they did not claim the finding to be a fibrosis probably because of the low resolution of the instrument, and they did not control other potential confounders for fibrosis. Since ultrasonographic machines with high resolution have become portable, liver ultrasonography should be considered in addition to traditional liver function tests in the medical surveillance for PVC workers,

especially those with hepatitis viral infection. The high resolution of our machine also allowed us to differentiate heterogeneous and coarse echotexture of liver fibrosis from increased echogenicity, which was a sign of fatty liver. However, these might still be some misclassification if the diagnosis of fibrosis is based solely on ultrasonography. A previous study showed that the sensitivity and specificity of detecting liver fibrosis was 57% and 88%, respectively (6). Because the hepatologists in our study performing the ultrasonographic examinations were blind to the VCM exposure status, the misclassification was assumed to be non-differential. Nonetheless, data analysis showed that the results were compatible with the prior knowledge that viral hepatitis infection, obesity and VCM exposure associated with liver fibrosis. We concluded that the accuracy of ultrasonography diagnosis is acceptable.

In our study, half of subjects with liver fibrosis diagnosed by ultrasonography could not be detected by traditional liver function tests. We suggest that ultrasonographic examination should be included in medical surveillance for PVC workers to detect chronic liver disease.

Acknowledgements

We are grateful to Ms. Yi-Ping Lin and Miss Chi-Ting Tzeng for their assistance with data management.

References

1. Creech JL, Johnson MN. Angiosarcoma of liver in the manufacture of polyvinyl chloride. *J Occup Med.* 1974;16:150-151.
2. IARC. Monographs on the elevation of the carcinogenic risk of chemicals in humans. Vinyl chloride, polyvinyl chloride and vinyl chloride-vinyl acetate copolymers. Lyon, France:WHO, IARC 19:377-401, 419-438.
3. Lelbach WK. A 25-year follow-up study of heavily exposed vinyl chloride workers in Germany. *Am J Ind Med.* 1996;29:446-458.
4. Creech JL, Makk L. Liver disease among polyvinyl chloride production workers. *Ann NY Acad Sci.* 1975;246:88-94.
5. Du CL, Wang JD. Increased morbidity odds ratio of primary liver cancer and cirrhosis of liver among vinyl chloride monomer workers. *Occup Environ Med.* 1998;55:528-532.
6. Saverymuttu SH, Joseph AEA, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. *Br Med J.* 1986;292:13-15.
7. Du CL, Kuo ML, Chang HL, Sheu TJ, WangJD. Changes in lymphocyte single strand breakage and liver function of workers exposed to vinyl chloride monomer. *Toxicol Lett.* 1995;77:379-385.
8. The Asia-pacific perspective: redefining obesity and its treatment. WPRO, WHO, IASO, and IOTF. 2000, Feb.
9. Yang PJ, Chen RC, Yang PM. Ultrasonography in predicting the existence of esophageal varices and the possibility of variceal bleeding. *J Ultrasound Med ROC.* 1991;18:383-390.
10. Maroni M, Mocci F, Visentin S, Preti G, Fanetti AC. Periportal fibrosis and other liver ultrasonography findings in vinyl chloride workers. *Occup Environ Med.* 2003;60:60-65.
11. Liaw YF, Tai DI, Chu CM, Chen TJ. The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. *Hepatology.* 1988;8:493-496.
12. Tsai JF, Chang WY, Jeng JE, et al. Hepatitis C virus infection as a risk factor for non-alcoholic liver cirrhosis in Taiwan. *J Med Virol.* 1993;41:296-300.
13. Yu MW, Hsu FC, Sheen IS, et al.

Prospective study of hepatocellular carcinoma and liver cirrhosis in asymptomatic chronic hepatitis B virus carriers. *Am J Epidemiol.* 1997;145:1029-1047.

14. Ratziu V, Giral P, Charlotte F, et al. Liver fibrosis in overweight patients. *Gastroenterology.* 2000;118:1117-1123.
15. Macdonald GA, Bridle KR, Ward PJ, et al. Lipid peroxidation in hepatic steatosis in humans is associated with hepatic fibrosis and occurs predominantly in acinar zone 3. *J Gastroenterol Hepatol.* 2001;16:599-606.
16. Letteron P, Fromenty B, Terris B, Degott C, Pessayre D. Acute and chronic hepatic steatosis lead to in vivo lipid peroxidation in mice. *J Hepatol.* 1996;24:200.
17. Bacon BR, Farahvash MJ, Janney CG, Neuschwander-tetri BA. Non-alcoholic steatohepatitis: an expanded clinical entity. *Gastroenterology.* 1994;107:1103.
18. Powell EE, Cooksly WGE, Hanson R et al: The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. *Hepatology*. 1990;11:74-80.
19. Lee RG. Nonalcoholic steatohepatitis:a study of 49 patients. *Huma pathol.* 1989;20: 594-598.
20. William DMJ, Smith PM, Taylor KJW, Crossley IR, Duck BW. Monitoring liver disorder in vinyl chloride monomer workers using grey-scale ultrasonography. *Br J Ind Med.* 1976;33:152-157.

Table 1

Basic Characteristics of Study Subjects According to Type of Exposure Groups

	Cumulative dose[†]			
	High	Moderate	Low	Total
Numbers	97	97	153	347
Age[**] (years)	43±9[‡]	38±9	42±10	41±10
Working duration[**] (years)	18.4±8.9	12.9±8.9	17.1±10.2	16.3±9.7
Body mass index (BMI)	24.4±2.6	23.6±2.7	23.9±2.6	24.0±2.6
Current alcohol drinking[*]	25 (25.8%)	15 (15.5 %)	21 (13.7 %)	61 (17.6%)
Current tobacco smoking	14 (14.4%)	22 (22.7 %)	34 (22.2 %)	70 (20.2%)
HBsAg (+)	28 (28.9 %)	16 (16.5 %)	29 (19.1%)	73 (21.0%)
Anti-HCV (+)	3 (3.7 %)	1 (1.3%)	4 (3.0%)	8 (2.8%)[¶]

[†] High, workers who had history of high exposure jobs and the cumulative dose of exposure equal to or greater than 2400 ppm-month (median 4316, range, 2402 - 20413); moderate, workers who had the history of high exposure jobs and the cumulative dose of high exposure jobs less than 2400 ppm-month (median 963; range, 88 - 2361); low, workers who never had high exposure jobs (median 147; range, 2 - 3498).

[‡] Mean±SD.

[**] $p < 0.01$, ANOVA test; [*] $p < 0.05$, chi-square test

[¶]Anti-HCV data available in 290 workers.

Table 2

Abnormal Ultrasonographic Findings of Different Cumulative VCM Exposure Groups

	Cumulative dose			
	High	Moderate	Low	Total
Number	97	97	153	347
Liver fibrosis**	12 (12.4%)	5 (5.2%)	3 (2.0%)	20 (5.8%)
Pre-cirrhosis*	8 (8.3%)	3 (3.1%)	2 (1.3%)	13 (3.8%)
Cirrhosis	4 (4.1%)	2 (2.1%)	1 (0.7%)	7 (2.0%)
Splenomegaly	11 (11.3%)	11 (11.3%)	10 (6.6%)	32 (9.2%)
Fatty liver	36 (37.1%)	38 (39.2%)	61 (39.9%)	135 (38.9%)

*$p < 0.05$, **$p < 0.01$, chi-square test

Table 3

Multiple Logistic Regression Models of Abnormal Liver Function Tests in PVC Workers

	AST		ALT		GGT	
	Abnormal (n)	Odds ratio[+] (95%C.I.)	Abnormal (n)	Odds ratio[+] (95%C.I.)	Abnormal (n)	Odds ratio[+] (95%C.I.)
Current VCM exposure						
≥ 10 ppm (n=61)	7	1.3 (0.4-3.9)[†]	12	2.0 (0.8-5.0)	9	1.1 (0.4-2.8)
1-10 ppm (n=151)	6	0.7 (0.2-2.1)	13	1.1 (0.4-2.5)	23	1.5 (0.7-3.2)
< 1 ppm (n=135)	9	1.0	12	1.0	15	1.0
Viral hepatitis[‡]						
Yes (n=79)	14	7.3[**](2.8-20.4)	16	3.0[**] (1.4-6.4)	16	1.9 (0.9-3.9)
No (n=268)	8	1.0	21	1.0	31	1.0
Body mass index						
≥ 25 (n=111)	10	1.9 (0.7-5.0)	18	2.6[*] (1.2-5.5)	18	1.1 (0.5-2.1)
< 25 (n=236)	12	1.0	19	1.0	29	1.0
Current alcohol drinking						
Yes (n=61)	9	3.1[*] (1.1-8.4)	10	1.5 (0.6-3.4)	19	3.9[*] [*](1.9-8.0)
No (n=286)	13	1.0	27	1.0	28	1.0

[+]Adjusted for tobacco smoking and age.

[‡]Viral hepatitis, HBsAg positive and/or Anti-HCV positive

*$p < 0.05$, ** $p < 0.01$

Table 4

Multiple Logistic Regression Models of pre-cirrhosis, cirrhosis and all fibrosis cases in PVC Workers

	Pre-cirrhosis[a]	Cirrhosis[a]	Fibrosis[b]
	Odds ratio[+] (95% C.I.)	Odds ratio[+] (95% C.I.)	Odds ratio[+] (95% C.I.)
Case number	13	7	20
History of high exposure jobs			
Yes (n=194)	4.5[*] (1.1-30.1)	5.8 (0.9-116.8)	5.5[*] (1.7-25.4)
No (n=153)	1.0	1.0	1.0
Viral hepatitis[‡]			
Yes (n=79)	8.8[**] (2.7-34.4)	9.2[**](1.8-69.9)	10.7[**](3.9-33.4)
No (n=268)	1.0	1.0	1.0
Body mass index			
≥ 25 (n=111)	2.9 (0.9-10.1)	2.5 (0.5-14.9)	3.1* (1.1-9.1)
< 25 (n=236)	1.0	1.0	1.0

[+]Adjusted for alcohol drinking, tobacco smoking and age.

*$p < 0.05$, ** $p < 0.01$

[‡]Viral hepatitis, HBsAg positive and/or Anti-HCV positive

[a] The case of pre-cirrhosis diagnosed by ultrasonography.

[b] The sum of cases of pre-cirrhosis and cirrhosis.

附錄三

聚氯乙烯工人慢性肝危害與基因多形性相關研究

一、緣由與目的

氯乙烯已經被國際癌症研究院(IARC) 歸類為第一類致癌物質，研究顯示氯乙烯暴露與肝血管肉瘤[i]及肝細胞癌症[ii]有關，除了癌症，亦有報告指出氯乙烯暴露與慢性肝疾病有關，包括肝功能異常[iii、iv]、肝纖維化 [3]、肝門脈高壓[v]、肝硬化[vi、vii]及脾腫大[viii]等。這方面的資料並不多，特別是慢性肝疾病劑量反應關係。此外，慢性肝疾病的發生與氯乙烯代謝後所產生的活化中間產物有關，因此，基因多形性包括代謝基因 *CYP2E1*、*ALDH2*、*GSTT1* 及 DNA 修補基因 *XRCC1* 可能影響氯乙烯暴露所造成的慢性肝疾病。本研究的目的主要是探討慢性肝疾病與氯乙烯累積暴露劑量之劑量-反應關係及基因多形性於慢性肝疾病中所扮演的角色。

二、材料與方法

研究對象為 1995-1999 年 327 名氯乙烯暴露的男性員工，我們採集其血液並收集健檢資料及詳細的問卷資料，包括生活習慣 (抽煙及喝酒情形等)及詳細工作史，並根據工作史建立累積暴露劑量，我們也利用超音波診斷慢性肝疾病包括肝纖維化、肝硬化及脾腫大，。

三、研究結果

研究結果發現，氯乙烯暴露工人之慢性肝疾病隨累積暴露劑量增加而有上升的趨勢，與氯乙烯累積暴露劑量小於 40 ppm-years 工人相比，40-1000 ppm-years，1000-10000 ppm-years，大於 10000 ppm-years 工人的慢性肝疾病危險性分別為 OR=3.5, 95％CI=0.4-32.1, OR=4.1, 95％CI=0.4-43.9, OR=5.6 , 95％CI=0.5-68.4。我們也發現 B 型或 C 型肝炎病毒感染是慢性肝疾病重要的危險因子 (OR=6.2, 95%CI=2.3-16.9)。在易感性基因分析方面，以氯乙烯累積暴露劑量小於 1000 ppm-years 具 *XRCC1 Arg-Arg/Arg-Gln* 基因型者為對照，氯乙烯累積暴露劑量小於 1000 ppm-years 具 *XRCC1 Gln-Gln* 基因型者對慢性肝疾病的危險性為 2.7 (95％CI=0.3-29.3)，在氯乙烯累積暴露劑量大於 1000 ppm-years 中，具 *XRCC1 Arg-Arg/Arg-Gln* 基因型者對慢性肝疾病的危險性為 1.3 (95％CI=0.4-4.2)，而在氯乙烯累積暴露劑量大於 1000 ppm-years 中且具有 *XRCC1 Gln-Gln* 基因型者對慢性肝疾病則有最高的危險性(OR=10.1, 95％CI=1.2-85.1)。相似的，以氯乙烯累積暴露劑量小於 1000 ppm-years 具 *CYP2E1 c1c1/c1c2* 基因型者為對照，氯乙烯累積暴露劑量小於 1000 ppm-years 具 *CYP2E1 c2c2* 基因型對慢性肝疾病的危險性為 3.3 (95％CI=0.3-33.7)，在氯乙烯累積暴露劑量大於 1000 ppm-years 中，具有 *CYP2E1 c1c1/c1c2* 基因型者對慢性肝疾病的危險性為 1.2 (95%CI=0.4-4.0)，而在氯乙烯累積暴露劑量大於 1000 ppm-years 中且具有 *CYP2E1 c2c2* 基因型者對慢性肝疾病有最高的危險性 (OR=7.8, 95％CI=1.3-46.1)。進一步分析，以 B 型或 C 型肝炎病毒感染呈現陰性具 *XRCC1 Arg-Arg/Arg-Gln* 基因型者為對照組，B 型或 C 型肝炎病毒感染呈現

陰性且具 *XRCC1 Gln-Gln* 基因型對於慢性肝疾病的危險性為 3.9 (95％CI=0.4-36.9)，具 B 型或 C 型肝炎病毒感染陽性者且有 *XRCC1 Arg-Arg/Arg-Gln* 基因型者對慢性肝疾病的危險性為 6.2 (95％CI=2.1-18.3)，而 B 型或 C 型肝炎病毒感染陽性且具 *XRCC1 Gln-Gln* 基因型者有最高的危險性 (OR=28.0, 95%3.4-231.8)。

四、討論

本研究發現具易感性 *XRCC1* 與 *CYP2E1* 基因型者比較容易發生慢性肝病。相較於其他 *CYP2E1* 基因型與氯乙烯累積暴露劑量排列組合，氯乙烯累積暴露劑量大於 1000 ppm-years 具 *CYP2E1 c2c2* 基因型對於慢性肝疾病的危險性最高，因為氯乙烯進入人體之後，經由 *CYP2E1* 代謝成 CEO，CEO 很快又變成 CAA，不管是 CEO 或 CAA 都是具有親電性的中間產物，除了與 DNA 形成鍵結，也會與大分子如 RNA、蛋白質及脂肪形成鍵結，引發細胞功能的障礙，因此具有 *CYP2E1 c2c2* 基因型的，相較於 *CYP2E1 c1c1/c1c2* 有較高的代謝能力[ix]，所以有較高的機會代謝成 CEO 或 CAA，因此發生慢性肝疾病的危險性較高。

有關 *XRCC1* 基因型與慢性肝病相關的作用機轉並不清楚。先前台灣的氯乙烯暴露工人發現 DNA 修補基因 *XRCC1* 與 *p53* 過度表現有關[x]，*p53* 正常的功能是調節細胞的生長週期及細胞凋亡，如果 *p53* 有突變，可能影響細胞的成長週期或細胞凋亡，進而影響不正常的細胞增生，例如肝硬化。

本研究也觀察到在 B 型或 C 型肝炎病毒感染者中具有 *XRCC1 Gln-Gln* 基因型的工人其發生慢性肝疾病的危險性較高。同樣的， *XRCC1* 影響 B 型或 C 型肝炎病毒造成慢性肝疾病的機轉仍然不清楚，可能的解釋是肝組織因 B 型或 C 型肝炎病毒感染而引起發炎反應，造成反應性氧化物種(active oxygen species, ROS) 增加[xi、xii、xiii]，在台灣氯乙烯暴露工人發現在 B 型或 C 型肝炎病毒感染者中尿液 8-OHdG 有較高的情形[xiv]；而 *XRCC1* 蛋白可以修復因 ROS 所造成的鹼基傷害，如果 *XRCC1* 的 DNA 修補效率較低，可能會造成基因的突變，如果這些突變發生於調控生長的重要基因如 *p53*，可造成慢性肝病變。

我們的研究顯示，氯乙烯暴露工人發生慢性肝疾病的危險性隨著累積暴露劑量增加而上升；具有易感性 *CYP2E1* 及 *XRCC1* 基因型的氯乙烯工人，較易發生慢性肝疾病；同時，具有 *XRCC1* 易感性基因型並有 B 型或 C 型肝炎病毒感染的工人更為容易發生慢性肝疾病。因此，具有易感性基因或有 B 型或 C 型肝炎病毒感染者，應避免暴露於氯乙烯。

五、參考文獻：

[i] Creech JL, Johnson MN. Angiosarcoma of liver in the manufacture of polyvinyl chloride. J Occup Med. 1974; 16(3):150-1

[ii] Evans DM, Williams WJ, Kung IT. Angiosarcoma and hepatocellular carcinoma in vinyl chloride workers. Histopathology. 1983; 7(3):377-88.

[iii] Lilis R, Anderson H, Nicholson WJ, Daum S, Fischbein AS, Selikoff IJ. Prevalence of disease among vinyl chloride and polyvinyl chloride workers. Ann N Y Acad Sci. 1975; 246:22-41.

[iv] Ho SF, Phoon WH, Gan SL, Chan YK. Persistent liver dysfunction among workers at a vinyl chloride monomer polymerization plant. J Soc Occup Med. 1991; 41(1):10-6.

[v] Smith PM, Crossley IR, Williams DM. Portal hypertension in vinyl chloride production workers. Lancet 1976; 2(7986):602-4.

[vi] Popper H, and Tomas LB. Alteration of liver and spleen among workers exposed to vinyl chloride. Ann. N. Y. Acad. Sci. 1975; 246:172-194.

[vii] Gedigk P, Muller R, Bechtelsheimer H. Morphology of liver damage among polyvinyl chloride production workers. A report of 51 cases. Ann N Y Acad Sci. 1975; 246:278-85.

[viii] Berk PD, Martin JF, Young RS, Creech J, Selikoff IJ, Falk H, Watanabe P, Popper H, Thomas L. Vinyl chloride-associated liver disease. Ann Intern Med. 1976; 84(6):717-31.

[ix] Hayashi S, Watanabe J, Kawajiri K. Genetic polymorphisms in the 5'-flanking region change transcriptional regulation of the human cytochrome P450 2E1 gene. J. Biochem. 1991; 110(4):559-565.

[x] Wong RH, Du CL, Wang JD, Chan CC, Luo JCJ, and Cheng TJ. XRCC1 and CYP2E1 polymorphisms as susceptibility factor of plasma mutant p53 protein and anti-p53 antibody expression in vinyl chloride monomer-exposed polyvinyl chloride workers. Cancer Epid. Biom. Prev. 2002; 11:475-482.

[xi] Hagen TM, Huang S, Curnutte J, Fowler P, Martinez V, Wwhr CM, Ames B N, Chisari FV. Extensive oxidative DNA damage in hepatocytes of transgenic mice with chronic active hepatitis destined to develop hepatocellular carcinoma. Proc. Natl. Acad. Sci. U. S. A. 1994; 91:12808-12812.

[xii] Shimoda R, Nagashima M, Sakamoto M, Yamaguchi N, Hirohashi S, Yokota J, Kasai H. Increased formation of oxidative DNA damage, 8-hydroxydeoxyguanosine, in human livers with chronic hepatitis. Cancer Res. 1994; 54:3171-3172.

[xiii] Farinati F, Cardin R, Degan P, Maria ND, Floyd RA, Thiel DHV, Naccarato R. Oxidative damage in circulating leukocytes occurs as an early event in chronic HCV infection. Free Rad. Biol. Med. 1999; 27:1284-1291.

[xiv] Wong RH, Yeh CY, Hsueh YM, Wang JD, Lei YC, Cheng TJ.

Association of hepatitis virus infection, alcohol consumptionand plasma vitamin A levels with urinary 8-hydroxydeoxyguanosine in chemical workers. Mut. Res. 2003; 535:181-186.