

# Relationship Between Airborne Levels and Urinary Metabolites of Styrene in a Glass-fiber Reinforced Plastic Factory

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**Objectives.** To investigate the relationship between levels of airborne styrene and styrene oxide (SO) and the two main urinary metabolites of styrene, mandelic acid (MA) and phenylglyoxylic acid (PGA) in a glass-fiber reinforced plastic factory.

**Methods.** All 44 workers in the factory participated in the study and were divided into three groups based on exposure to styrene in the workplace (high and low exposure, and control group). Urine samples were collected in the morning and afternoon. Airborne styrene and SO levels were analyzed by gas chromatography. Urinary MA and PGA were measured by HPLC.

**Results.** Airborne styrene concentrations in the glass-fiber reinforced plastic factory did not exceed the 50 ppm exposure limit stipulated by the Taiwan government. The concentration of airborne styrene positively correlated with that of SO ( $r = 0.28, p < 0.05$ ) and urinary PGA ( $r = 0.59, p < 0.01$ ). However, concentration of airborne styrene did not correlate significantly with urinary MA. SO correlated significantly with urinary MA ( $r = 0.25, p < 0.05$ ). Concentrations of airborne styrene, SO, PGA and MA were positively associated with styrene exposure. Based on multiple regression analysis, an increase of 1 ppm styrene corresponded to an increase of 1.33 mg/g cre. in urinary PGA, after adjusting for cigarette smoking, alcohol consumption and use of prescription drugs.

**Conclusions.** Concentrations of styrene and SO in the air significantly correlated with metabolites of styrene in urine. (Mid Taiwan J Med 2005;10:1-7)

## Key words

biological monitoring, mandelic acid, phenylglyoxylic acid, styrene, styrene oxide

## INTRODUCTION

Previous studies [1-3] have confirmed that there is a significant correlation between airborne styrene in the workplace and its metabolites in urine, blood and expired air. Styrene is metabolized by the microsomal cytochrome P-450 system to form styrene-7,8-oxide (SO) (phenyloxirane) [4]. The two main urinary metabolites of styrene are mandelic acid (MA) and phenylglyoxylic acid (PGA). Assessment of

styrene in blood and expired air is difficult because styrene is metabolized rapidly. Because correlations between styrene and its urinary metabolites are largely determined by individual variations in the workplace and physiological factors, research conclusions regarding the relationship between airborne styrene and its urinary metabolites have not been consistent [2,5]. Nylander [6] reported that SO is produced when styrene reacts with either oxygen or oxidants. Previous studies [7,8] have shown that employees working in factories which produce reinforced plastics may be exposed to airborne SO arising from *in situ* oxidation of styrene

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during the polymerization process, although SO concentrations may be relatively low. Rappaport [9] investigated multiple biomarkers (albumin adducts, DNA adducts, sister chromatid exchanges (SCE)) among workers exposed to styrene and SO, and found that they significantly correlated with exposure to SO, but not with exposure to styrene. Therefore, the inhalation of SO should be considered in any intervention to reduce health risks. In workplaces which use styrene, levels of airborne styrene are typically elevated. SO may also be produced in small quantities, but there is little data in the literature to support this hypothesis. For example, average styrene levels in 256 workplaces in Denmark were 172 mg/m<sup>3</sup> (1981 to 1988), but SO levels were not measured [10]. In Taiwan, there is little available data regarding styrene and SO levels in the workplace. The purpose of the current study was to investigate the relationships between levels of airborne styrene and styrene oxide (SO) and the two main urinary metabolites of styrene, MA and PGA in a glass-fiber reinforced plastic factory.

#### **MATERIALS AND METHODS**

All 44 workers in the glass-fiber reinforced plastic factory participated in the study; they were divided into three groups according to a walkthrough assessment of styrene exposure in the workplace: the high exposure group (laminating area, spraying area), the low exposure group (cutting area), and the control group (administrative area). There were no significant differences in gender, education level, and work duration between the three groups. Average duration of employment was approximately 3 years. The factory was established in 1995. Workers were interviewed using a questionnaire to collect demographic data, lifestyle habits (cigarette smoking and alcohol consumption) health status and workload.

#### **Personal and area sampling of styrene and SO [7]**

Personal sampling was conducted by attaching a collar-sampler to each of the 44 workers from 8:00 to 12:00 (morning shift) and

13:00 to 17:00 (afternoon shift). Sampling was performed according to the guidelines stipulated by Taiwan's Institute of Occupational Safety and Health (IOSH). Area sampling was conducted in the three exposure groups, and the 19 sampling points were adjacent to the styrene sources. Samples were taken using a charcoal tube, and immediately stored at 4°C and analyzed by GC/FID (HP-5890) with DB-Wax column (fused silica WCOT (30 m, 0.53 mm ID) within one week. The injector temperature and detector temperature were set up at 200°C. The temperature gradient was from 55°C for 3 min increasing at 30°C/min intervals to 105°C which was kept constant for 6 min. Nitrogen gas at a flow rate of 12 mL/min served as the carrier gas. The calibration curves of styrene and SO ranged from 0.036 to 1.818 mg/mL, and that of SO ranged from 0.6 to 2.4 µg/mL. The correlation coefficients of both calibration curves exceeded 0.995. Relative prediction deviation (RPD) was 3.47% for styrene, and 5.13% for SO. The stability of the charcoal tube was tested at three storage temperatures (-20°C, 4°C and 21°C) at two concentrations (1 PEL (permission exposure level) and 0.5 PEL) over 14 days. Recovery efficiency of styrene and SO was over 90% at day 14 for each temperature. The detection limit of styrene was 1.864 ng and that of SO was 1.625 ng. Desorption efficiency (DE) was assessed by spiking charcoal tubes with three concentrations (0.5 PEL, 1 PEL and 2 PEL) of styrene and SO. Mean desorption efficiency was 86.73% for styrene and 88.15% for SO.

#### **Biological monitoring**

Urine samples from all 44 workers were taken at the end of each shift on the same day personal samples were collected. Mandelic acid (MA) and phenylglyoxylic acid (PGA) concentrations were measured by HPLC. The mobile phase consisted of a mixture of 10% acetonitrile and 90% distilled water (flow rate of 0.6 mL/min). The UV/VIS detector detected PGA at 254 nm, and MA was detected at 220 nm. The calibration curves ranged from 6.0 to 60.0 µg/mL for MA, and 30.0 to 300.0 µg/mL for PGA.

**Table 1. Styrene and styrene-oxide concentrations for personal and area samplings in the three exposure groups**

	Styrene (ppm)	Styrene-oxide (ppb)
Personal sampling (n)		
Control group (12)	0.63 ± 0.84	ND
Low exposure group (16)	2.70 ± 1.29	ND
High exposure group (16)	9.13 ± 5.65	2.0 ± 10.0
Area sampling (n)		
Control group (3)	ND	ND
Low exposure group (7)	1.46 ± 1.04	ND
High exposure group (9)	4.72 ± 3.23	1.0 ± 3.0

ND = no detection.

**Table 2. Correlations between personal sampling for styrene and styrene-oxide and levels of urinary mandelic acid (MA) and phenylglyoxylic acid (PGA) (n = 44)**

	Styrene	Styrene	Styrene	Styrene
Styrene	1	0.276*	0.079	0.587*
Styrene-oxide		1	0.252*	0.118
MA			1	0.231*
PGA				1

\* $p < 0.05$ .

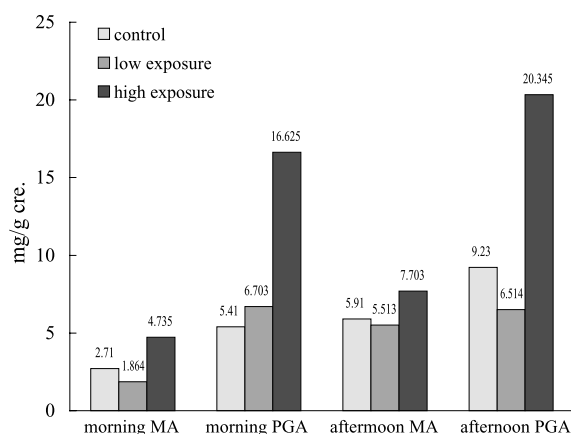


Figure. A comparison of urinary MA and PGA levels (mg/g cre.) between the three groups in the morning and afternoon using multiple regression models, after adjusting for smoking, alcohol consumption, and prescribed medication.

Correlation coefficients for both biomarkers were over 0.999. Stability of MA and PGA in urine was assessed by storing the biomarkers at  $-20^{\circ}\text{C}$  for 14 days. Recovery efficiency for both MA and PGA were approximately 100% on the 14<sup>th</sup> day. Detection limits for MA and PGA were 4.3  $\mu\text{g}$  and 2.1  $\mu\text{g}$ , respectively. Desorption efficiency was assessed by spiking the urine samples with four concentrations (12.04 to 241.6  $\mu\text{g}/\text{mL}$ ) of MA and PGA followed by extraction with desorbed solvent. Mean desorption efficiencies

were 101.1% for MA and 99.8% for PGA.

## RESULTS

Data from personal sampling indicated that styrene concentrations were highest in the high exposure group (9.30 ppm), followed by the low exposure group (2.7 ppm), and the control group (0.63 ppm). SO concentrations were only detected in the high exposure group (2.0 ppb). Findings of area sampling revealed a similar trend, although concentrations were consistently higher in personal sampling.

Concentrations of styrene were highest in the high exposure group and lowest in the control group. SO concentrations were only detected in the high exposure group (1 ppb) (Table 1).

There were significant correlations between airborne SO and styrene ( $r = 0.28$ ,  $p < 0.05$ ), airborne SO and urinary MA ( $r = 0.25$ ,  $p < 0.05$ ), airborne styrene and urinary PGA ( $r = 0.59$ ,  $p < 0.01$ ), and urinary PGA and MA ( $r = 0.23$ ,  $p < 0.05$ ). However, there were no significant correlations between airborne styrene and urinary MA ( $r = 0.08$ ), or airborne SO and urinary PGA ( $r = 0.12$ ) (Table 2).

Afternoon urinary MA and PGA levels were consistently higher than MA and PGA levels

in the morning. Concentrations of urinary PGA and MA were higher in the high exposure group than in the other groups. Multiple regression analysis revealed that urinary PGA levels were significantly affected by airborne styrene and prescription medication. Exposure to 1 ppm airborne styrene caused an increase of 1.33 mg/g cre. of urinary PGA. Workers who took prescribed medication had significantly higher urinary PGA levels than workers who did not. Smoking and alcohol consumption did not significantly affect urinary PGA levels. No factors could significantly explain the variance in urinary MA levels (Figure).

### DISCUSSION

Styrene is a widely used chemical in a variety of industrial processes, particularly in the production of polystyrene plastics, protective coatings, styrenated polyesters, copolymer resins with acrylonitrile and butadiene. Following publication of studies which have shown that styrene is carcinogenic and has a harmful effect on reproductive and developmental processes in animals, levels of exposure to styrene have fallen considerably over the past decade [11,12]. Also, numerous studies [13,14] have suggested that exposure to styrene may have a genotoxic effect on humans. SO is an intermediate metabolite in the biotransformation of styrene, and has been shown to induce adducts in DNA and hemoglobin [9,15]. Phillips and Farmer [16] demonstrated that exposure to styrene resulted in the formation of DNA adducts in human mononuclear cells. However, DNA adducts were not shown to correlate with SCEs. SO is not normally present in the air, but Pfaffli et al [7] reported that SO can be produced during the polymerization process when styrene is oxidized. SO was present in very low concentrations in both of the above studies. However, these data were not validated and the methods for sampling and analysis were not standardized. In the current study, sampling of SO in the air was performed using charcoal tubes and also indicated a strict quality control of analytical method for SO and styrene. Sampling method was conducted in a glass-fiber reinforced plastics

factory to simultaneously collect airborne SO and styrene.

Although airborne SO concentrations were very low, the results showed that concentration of airborne SO correlated positively with styrene concentration ( $r = 0.28$ ,  $p < 0.05$ ). There is little data in the literature on this relationship. One of the reasons for this is that SO levels are typically very low. Also, there are no established standardized sampling and analytical procedures for SO. Pfaffli et al [7] reported that the highest concentration of styrene in personal samples in a reinforced plastic industrial plant was 350 ppm. However, the average concentration of SO alone was 0.1 ppm. Compared with styrene, concentrations of SO and its derivatives were much lower (about 0.5% of the total). Yeowell et al [8] measured SO and styrene levels in a boat manufacturing plant over the course of one year and found that the mean SO concentration ( $159 \mu\text{g}/\text{m}^3$ ) was much lower than the mean styrene concentration ( $64.3 \text{ mg}/\text{m}^3$ ). The above-mentioned studies did not correlate levels of airborne SO with styrene. The presence of SO in the air may be due to the reaction of styrene with either oxygen or oxidants during the polymerization process [6]. Reetz et al [17] showed that airborne styrene and SO were produced by photolysis of chain-chlorinated polystyrene. D'Auria et al [18] reported that the photochemical reaction of styrene with nitrobenzene produced several compounds, such as nitron. They postulated that airborne SO may be produced by a photochemical reaction of styrene. Rappaport et al [19] investigated protein adducts in boat manufacturing workers exposed to airborne styrene and SO, and reported that although SO levels were very low, the dose of protein adducts in the blood arising from inhalation of SO was almost 2000 times that of styrene. Future research should simultaneously measure levels of airborne styrene and SO, and determine the mechanism by which SO is formed.

Urinary PGA significantly correlated with airborne styrene ( $r = 0.59$ ,  $p < 0.01$ ). However, urinary MA did not correlate significantly with airborne styrene. Gobba et al [20] investigated

214 workers environmentally exposed to styrene and reported that airborne styrene correlated with both urinary MA ( $r = 0.82$ ) and PGA ( $r = 0.78$ ). Marhuenda et al [5] showed that there were significant correlations between styrene and urinary MA ( $r = 0.47$ ), urinary PGA ( $r = 0.34$ ) and urinary MA + PGA ( $r = 0.54$ ). The short half-life of urinary MA (about 2 hours) and PGA (about 8 hours) has resulted in a wide variation of correlations in the literature. Shi et al [21] reported that better results were obtained using half-day time-weighted average (TWA) concentrations, instead of whole-day TWA concentrations, when assessing levels of urinary MA and PGA. In addition, levels of urinary MA and PGA may be readily influenced by coexposure to other compounds such as, ethylbenzene, SO, styrene glycol and phenylglyoxylic acid. Workers' alcohol intake has also been shown to affect levels of urinary MA and PGA. In the current study, personal sampling was conducted in two consecutive sessions, in the morning and in the afternoon. The styrene concentrations in the morning session were consistently higher than those in the afternoon session for both area and personal sampling. Levels of urinary PGA and MA were consistently higher in the afternoon, perhaps due to accumulation of styrene in the body. Workers in our study were only exposed to styrene. Few studies have correlated airborne SO levels with urinary MA and PGA. About 85% of styrene absorbed during an 8-hour exposure is excreted in urine as MA. Our results showed that MA correlated significantly with SO ( $r = 0.25$ ,  $p < 0.05$ ), but did not correlate with styrene, possibly due to the low exposure level of styrene (mean exposure in the high exposure group was 11 ppm). Multiple linear regression analysis revealed that styrene and prescribed medication but not alcohol consumption and smoking were significant factors that affected urinary PGA levels. As such, it is important that future research which analyzes urinary PGA as a biomarker takes into account the use of prescription medication as a potential confounder. The results from our study showed that 1 ppm of styrene produced 1.3 mg/g

cre. of urinary PGA ( $\text{PGA (mg/g cre.)} = 5.847 + 1.332 \text{ styrene (ppm)} + 7.234 \text{ drug use}$ ). Eight-hour exposure limit for styrene in Taiwan is currently 50 ppm which corresponds to 66.5 mg/g cre. of urinary PGA, and is lower than levels (118 mg/g cre.) reported in previous studies [22]. The low levels of styrene found in our study may have been due to the narrow range of styrene levels, small sample size, and may also have been affected by genetic factors, which could affect the metabolism of styrene.

In conclusion, there were low levels of styrene and SO in the glass-fiber reinforced plastics factory. Airborne styrene concentration significantly correlated with SO and urinary PGA levels, but not with urinary MA levels. There was a significant correlation between airborne SO and urinary MA levels. Multiple regression analysis showed that an increase of 1 ppm styrene corresponded to an increase of 1.33  $\mu\text{g/g cre.}$  of urinary PGA, after adjusting for smoking, alcohol consumption and use of prescription drugs. It is necessary to conduct further studies to establish the relationship between airborne styrene, SO and their metabolites in Taiwan.

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# 強化塑膠工廠空氣中苯乙烯濃度與其生物偵測指標之相關性

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**目的** 本研究之目的在測量強化塑膠工廠空氣中苯乙烯及苯乙烯氧化物(SO)之濃度，同時亦探討與苯乙烯尿中代謝物苯乙醇酸(MA)及苯乙醛酸(PGA)在濃度之相關性。

**方法** 收集44位強化塑膠工廠勞工依其工作環境暴露苯乙烯分為高、低暴露及對照組，測定勞工在上午及下午空氣中苯乙烯與苯乙烯氧化物濃度及其尿中代謝物濃度。

**結果** 強化塑膠工廠空氣中苯乙烯濃度低於我國勞工法令容許標準(50 ppm)，空氣中苯乙烯濃度與尿中代謝物苯乙醛酸( $r = 0.59$ )及苯乙烯氧化物( $r = 0.28$ ,  $p < 0.05$ )具有顯著性相關性，但與尿中代謝物MA沒有相關性。而苯乙烯氧化物與尿中MA有相關性( $r = 0.25$ ,  $p < 0.05$ )。隨著工作環境空氣中苯乙烯暴露量增加，其相對空氣中苯乙烯氧化物及其尿中代謝物苯乙醛酸濃度均有正相關性。依多變項迴歸分析得知經調整吸菸、喝酒及藥物使用後，每增加空氣中1 ppm 苯乙烯暴露量相當於會增加工作者其尿中1.33 mg/g cre. PGA之量。

**結論** 空氣中苯乙烯、苯乙烯氧化物及其尿中代謝物濃度均呈正相關。(中台灣醫誌 2005;10:1-7)

## 關鍵詞

生物偵測，苯乙醇酸，苯乙醛酸，苯乙烯，苯乙烯氧化物

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