An Investigation of Multiple Biomarkers among Workers Exposed to Styrene and Styrene-7,8-oxide

Stephen M. Rappaport, Karen Yeowell-O'Connell, William Bodell, Janice W. Yager, and Elaine Symanski

School of Public Health, University of North Carolina, Chapel Hill, North Carolina 27599-7400 [S. M. R., K. Y.-O., E. S.]; Brain Tumor Research Center of the Department of Neurological Surgery, University of California, San Francisco, California 94143-0806 [W. B.]; and Environmental Division, Electric Power Research Institute, Palo Alto, California 94303 [J. W. Y.]

ABSTRACT

Investigations of cancer and cytogenetic damage among reinforced-plastics workers have produced contradictory results. In all studies, the focus has been on styrene rather than the carcinogen, styrene-7,8-oxide (SO), and the potential for coexposure to SO has not been considered. This study investigated the relative contributions of airborne styrene and SO and of smoking toward several SO-specific biomarkers (DNA and albumin adducts) in the blood of 48 reinforced-plastics workers. Among individual subjects, albumin and DNA adducts as well as sister chromatid exchanges were significantly correlated with styrene exposure. However, among the 20 subjects with measurements to both styrene and SO, albumin adducts were significantly correlated with exposure to SO but not to styrene. Finally, among the 10 job groups, surprisingly strong correlations (0.709 ≤ r ≤ 0.966) were found between all SO biomarkers and exposure to SO but not to styrene. Calculations suggest that SO was about 2000 times more effective than styrene in producing SO biomarkers. After accounting for the disparate exposures to the two chemicals, a typical worker received 71% of the systemic dose of SO via inhalation; nonetheless, 5 of the 20 subjects received the majority of the SO dose from styrene. Cigarette smoking increased levels of SO-albumin and SO-DNA adducts, suggesting that SO was a constituent of tobacco smoke. We conclude that inhalation of SO should be considered in any interventions to reduce health risks.

INTRODUCTION

Styrene is a prominent industrial chemical used to produce plastics and resins throughout the world. More than 6 million metric tons of styrene are consumed annually in the U.S. alone (1), where about 30,000 workers are exposed to the substance and an additional 300,000 are potentially exposed in 20,000 factories (2). Occupational exposures to styrene are modest (<10 mg/m³) in most segments of industry, except those involving production of reinforced plastics, where air levels between 40 and 400 mg/m³ are common (1).

About 94% of inhaled styrene is retained in humans (3) and then metabolized via hepatic cytochrome P-450 isozymes [CYP2B6 is most effective, followed by CYP1A2, CYP2E1, and CYP2C8 (4)] and epoxide hydrolase enzymes to styrene glycol and its oxidation products, which are excreted in the urine (reviewed in Refs. 1, 5–6). Since this metabolism proceeds almost exclusively through the reactive epoxide hydrolase enzymes to styrene glycol and its oxidation products, which are excreted in the urine (reviewed in Refs. 1, 5–6).

SO was a constituent of tobacco smoke. We conclude that inhalation of SO should be considered in any interventions to reduce health risks.

Received 5/6/96; accepted 9/26/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1. This work was supported by Grant R01OH02221 from the National Institute of Occupational Safety and Health of the Centers of Disease Control.

2. To whom requests for reprints should be addressed, at School of Public Health, C.B.

3. The abbreviations used are: SO, styrene-7,8-oxide; SCE, sister chromatid exchange; SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

4. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

5. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

6. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

7. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

8. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

9. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

10. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

11. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

12. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

13. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

14. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

15. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

16. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

17. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

18. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

19. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

20. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

21. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

22. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.

23. SO-Alb, SO-DNA, SO-Hb, adducts resulting from reaction of SO with albumin, DNA, and hemoglobin, respectively.
The overall aim of the research was to correlate the levels of each SO biomarker with the corresponding exposures to styrene. Airborne SO was also measured among 20 of the 48 workers to gauge the potential importance of coexposure to SO (25), and information concerning cigarette consumption was obtained from all subjects to control for the effect of smoking upon SCE induction (36).

Using subject-specific averages of exposure and biomarker levels among these workers, we observed significant correlations between styrene exposure and SCEs (after adjustment for cigarette consumption; Ref. 36), DNA adducts (23), and SO-Alb (but not SO-Hb; Ref. 25). This tends to support the earlier positive studies linking SO biomarkers and styrene exposure among reinforced-plastics workers. However, we also observed that SO-Alb was significantly correlated with exposure to SO, but not to styrene, among the 20 subjects for whom air concentrations of both substances had been measured (25).

To shed light upon the relative importance of exposures to styrene and SO, we conducted further analyses of our data, after aggregation by job group and smoking status, so that the few measurements of SO exposure could be extended to the maximum numbers of subjects and that the effects of smoking upon all SO biomarkers could be carefully examined. Here we provide evidence that, indeed, the systemic dose of SO arising from styrene was so small that coexposure to SO was a more important contributor to the SO biomarkers among most of our subjects. Based upon measurements of DNA and protein adducts, we offer additional evidence that cigarette smoke contains SO.

**MATERIALS AND METHODS**

**Subjects.** The cohort consisted of 48 healthy workers of both sexes employed during 1987–1988 in a factory where boats were manufactured. Subjects were recruited with informed consent from jobs where airborne styrene was known to be present without regard for the intensity of exposure or of smoking status, provided that they had been in their current jobs for at least 1 year. Job titles were obtained from company records and confirmed by the subjects. Cigarette consumption, as well as levels of factors that might affect SCE frequency, were determined by questionnaire. Detailed descriptions of the cohort, the questionnaire data, and the randomization of all subjects and measurements were given by Yager et al. (36). This investigation was performed after approval by the institutional review boards of the University of California, Berkeley and the University of North Carolina, Chapel Hill with assurances filed with and approved by the U.S. Department of Health and Human Services.

**Sampling of Air, Exhaled Air, and Blood.** Multiple samples of air and biological specimens were obtained from all subjects. Briefly, air, mixed-exhaled air, and blood samples were collected during seven surveys over 1 year. Full-shift personal exposures to styrene were measured among all available subjects during each survey. Likewise, the levels of styrene in mixed-exhaled air were measured among all available subjects three times during each survey. However, the measurement of full-shift exposure to SO was restricted to five subjects per survey (for six of the seven surveys) who had been chosen at random from jobs involving use of styrene-containing resins. Blood was collected by venipuncture during four of the surveys at intervals of about 3 months. Details of collection and storage of samples prior to the various assays were given by Horvath et al. (23), Yeowell-O’Connell et al. (25), and Yager et al. (36). Average exposures to styrene and SO for all subjects varied among the surveys (styrene, 47.2–76.1 mg/m³ for seven surveys; SO, 21.1–261 µg/m³ for six surveys) but displayed no indications of a trend over the course of the investigation.

**Assays of SO Biomarkers.** DNA adducts were quantified using P1-nuclease-enhanced 32P-postlabeling; the first [designated SO-DNA(1)] was identified as N2-(2-hydroxy-1-phenylethyl)-2′-deoxyguanosine-3,5-biphosphate, and the second [SO-DNA(2)] was unidentified (23). Two cysteine adducts of albumin (designated SO-Alb(α) and SO-Alb(β), representing electrophilic attack of SO by either the α or β carbon, respectively) were cleaved from the protein by Raney nickel, derivatized, and analyzed by gas chromatography-mass spectrometry in the negative ion/chemical ionization mode (25). Table 1 shows, by survey, the numbers of subjects assessed for each of the SO biomarkers as well as for exhaled styrene.

**Statistical Analyses.** Data were analyzed using the SAS statistical system. Since repeated measurements of airborne exposures and biomarker levels were collected over 1 year, raw data from all surveys were first averaged by subject, except for exhaled styrene, which was averaged first by subject within surveys (typically, three measurements per survey per subject) and then (by subject) over all surveys. Unweighted subject-specific averages of each variable were used in correlation analyses to investigate the relations between exposures and biomarker levels among all subjects (n = 48), among smokers (n = 26), and among nonsmokers (n = 22). (Note that nonsmokers included former smokers.) These correlation analyses were repeated using subject-specific averages of the natural logarithms of exposures and biomarker levels with essentially the same results (data not shown). In an additional analysis, mean exposures and biomarker levels for workers sharing the same job title (n = 10 job groups) were computed. Details of the job groups, including numbers of subjects, smokers, and SO measurements, and the mean exposures to styrene and SO are given in Table 2. Note that 6 of the 10 job groups, containing 40 of the 48 subjects, included at least one measurement of SO. Using the job-specific averages, correlation coefficients between airborne exposures and all biomarker levels were also calculated. Pearson correlation coefficients and two-tailed significance levels were used.

Multiple linear regression analyses were performed on the job-specific averages to investigate the relative influence of styrene and SO on biomarker levels, using the following model:

\[
\text{Biomarker} = \beta_{\text{so}} + \beta_{\text{styrene}}(\text{Styrene}) + \beta_{\text{SO-SO}}(\text{SO}) + \beta_{\text{SO-Styrene}}(\text{SO} \cdot \text{Styrene}) + \text{Error} (A)
\]

where exposures to both styrene and SO (in µmol agent/m³) and an interaction term were evaluated as independent variables. Variances of the ratios of the estimated coefficients for styrene and SO exposure, i.e., \(\beta_{\text{SO}}/\beta_{\text{styrene}}\), were estimated by Taylor series approximations.

The effect of smoking on biomarker levels was evaluated by ANOVA after categorizing exposures to styrene and SO based upon subject-specific averages. Three categories of styrene exposure were used [low, 3.70 mg/m³ (SE, 0.86; range, 1.74–74.4 mg/m³); medium, 15.6 mg/m³ (SE, 3.14; range, 7.40–80.5 mg/m³); and high, 156 mg/m³ (SE, 6.58; range, 7.40–80.5 mg/m³)]; and SO exposure was evaluated as a continuous variable (25). Table 1 shows, by survey, the numbers of subjects assayed for each of the SO biomarkers as well as for exhaled styrene.

**Table 1** Number of subjects from each survey with measurements of biomarkers

<table>
<thead>
<tr>
<th>Survey</th>
<th>Month of sample collection</th>
<th>Exhaled styrene</th>
<th>SO-Alb</th>
<th>SO-DNA</th>
<th>SCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>July 1987</td>
<td>31</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>October 1987</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>November 1987</td>
<td>46</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>December 1987</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>February 1988</td>
<td>24</td>
<td>38</td>
<td>30</td>
<td>41</td>
</tr>
<tr>
<td>6</td>
<td>April 1988</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>June 1988</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>39</td>
</tr>
</tbody>
</table>

**Table 2** Characteristics of job groups evaluated in the pooled analyses

<table>
<thead>
<tr>
<th>Job title</th>
<th>No. of subjects (smokers)</th>
<th>No. subjects with SO measurements</th>
<th>Mean styrene exposure (µg/m³)</th>
<th>Mean SO exposure (µg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminator</td>
<td>16 (10)</td>
<td>11</td>
<td>130</td>
<td>182</td>
</tr>
<tr>
<td>Service</td>
<td>6 (3)</td>
<td>2</td>
<td>27.9</td>
<td>77.6</td>
</tr>
<tr>
<td>Mold repair</td>
<td>3 (3)</td>
<td>3</td>
<td>117</td>
<td>198</td>
</tr>
<tr>
<td>Patching</td>
<td>8 (3)</td>
<td>3</td>
<td>13.6</td>
<td>96.0</td>
</tr>
<tr>
<td>Painter</td>
<td>6 (4)</td>
<td>1</td>
<td>27.7</td>
<td>158</td>
</tr>
<tr>
<td>Spray operator</td>
<td>1 (1)</td>
<td>1</td>
<td>141</td>
<td>74.4</td>
</tr>
<tr>
<td>Mechanic</td>
<td>4 (1)</td>
<td>1</td>
<td>8.30</td>
<td></td>
</tr>
<tr>
<td>Deck rigger</td>
<td>2 (1)</td>
<td>1</td>
<td>4.21</td>
<td></td>
</tr>
<tr>
<td>Assembly</td>
<td>1 (1)</td>
<td>1</td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>Supervisor</td>
<td>1 (0)</td>
<td>0</td>
<td>25.1</td>
<td></td>
</tr>
</tbody>
</table>

*Includes laminator supervisors.

Downloaded from cancerres.aacrjournals.org on April 13, 2017. © 1996 American Association for Cancer Research.
it impractical to control for exposures to both chemicals in the same analyses. Current smoking status was dichotomized (nonsmokers versus smokers).

RESULTS

Summary Statistics. Table 3 summarizes the mean exposures and SO-DNA levels for all study subjects combined and is stratified by smoking status or job group. The ranges of variables were between ~2-fold (SCes) and 500-fold (exhaled styrene). Sample means of exposures and biomarker levels were higher among smokers than among nonsmokers; however, only the levels of SO-DNA(1), SO-DNA(2), and SCes were significantly elevated among smokers (P < 0.05, t test with unequal variances). Aggregation by job substantially reduced the ranges of all variables, particularly for SO exposure, where the range decreased from about 200-fold to about 3-fold.

Correlation of Variables. Pearson’s correlation coefficients of the subject- and job-specific averages are shown in Table 4 for all bivariate relationships between biomarker levels and exposure to styrene or to SO. Styrene exposure was highly correlated with the level of exhaled styrene among all subjects (r = 0.910), nonsmokers (r = 0.948), and smokers (r = 0.900), suggesting that inhalation was the primary route of exposure to styrene. Although not shown in Table 4, exposures to styrene and to SO were marginally correlated among all subjects (r = 0.327; P = 0.160; n = 20) and among nonsmokers (r = 0.575; P = 0.136; n = 8) but not among either smokers or job groups (P ≥ 0.399).

Among all subjects, the correlation coefficients between styrene exposure and the levels of SO-Alb, SO-DNA, and SCes, although modest in magnitude (0.224 ≤ r ≤ 0.387), were at or near a 0.05 level of statistical significance (0.008 ≤ P ≤ 0.126). After dividing subjects by smoking status, styrene exposure was more highly correlated with SO-DNA among nonsmokers than among smokers, suggesting that cigarette smoking increased the variability of SO-DNA among subjects. On the other hand, styrene exposure and SO-Alb appeared to be moderately correlated among smokers, whereas very little correlation was detected among nonsmokers. The subject-specific correlations also point to several significant relationships between SO exposure and the SO biomarkers. SO-Alb was correlated with SO exposure with unequal variances. Aggregation by job substantially reduced the ranges of all variables, particularly for SO exposure, where the range decreased from about 200-fold to about 3-fold. Correlation of Variables. Pearson’s correlation coefficients of the subject- and job-specific averages are shown in Table 4 for all bivariate relationships between biomarker levels and exposure to styrene or to SO. Styrene exposure was highly correlated with the level of exhaled styrene among all subjects (r = 0.910), nonsmokers (r = 0.948), and smokers (r = 0.900), suggesting that inhalation was the primary route of exposure to styrene. Although not shown in Table 4, exposures to styrene and to SO were marginally correlated among all subjects (r = 0.327; P = 0.160; n = 20) and among nonsmokers (r = 0.575; P = 0.136; n = 8) but not among either smokers or job groups (P ≥ 0.399).

Among all subjects, the correlation coefficients between styrene exposure and the levels of SO-Alb, SO-DNA, and SCes, although modest in magnitude (0.224 ≤ r ≤ 0.387), were at or near a 0.05 level of statistical significance (0.008 ≤ P ≤ 0.126). After dividing subjects by smoking status, styrene exposure was more highly correlated with SO-DNA among nonsmokers than among smokers, suggesting that cigarette smoking increased the variability of SO-DNA among subjects. On the other hand, styrene exposure and SO-Alb appeared to be moderately correlated among smokers, whereas very little correlation was detected among nonsmokers. The subject-specific correlations also point to several significant relationships between SO exposure and the SO biomarkers. SO-Alb was correlated with SO exposure with unequal variances. Aggregation by job substantially reduced the ranges of all variables, particularly for SO exposure, where the range decreased from about 200-fold to about 3-fold. Correlation of Variables. Pearson’s correlation coefficients of the subject- and job-specific averages are shown in Table 4 for all bivariate relationships between biomarker levels and exposure to styrene or to SO. Styrene exposure was highly correlated with the level of exhaled styrene among all subjects (r = 0.910), nonsmokers (r = 0.948), and smokers (r = 0.900), suggesting that inhalation was the primary route of exposure to styrene. Although not shown in Table 4, exposures to styrene and to SO were marginally correlated among all subjects (r = 0.327; P = 0.160; n = 20) and among nonsmokers (r = 0.575; P = 0.136; n = 8) but not among either smokers or job groups (P ≥ 0.399).
were investigated. The main effect of exposure to SO was generally significant at a level of 0.05 (SO-Alb(a), P = 0.034; SO-Alb(b), P = 0.180; SO-DNA(1), P = 0.010; SO-DNA(2), P = 0.009; SCEs, P = 0.003), whereas that of styrene was not (P > 0.315). The estimated main effects were: \( \beta_\text{so} = 0.750 \text{ nmol SO} / \text{g Alb} \), \( \beta_\text{sty} = 0.596 \text{ nmol SO} / \text{Alb} / \mu \text{mol SO/m}^3 \), and \( \beta_\text{sty} = 2.77 \times 10^{-4} \text{ nmol SO} / \text{Alb} / \mu \text{mol styrene/m}^3 \) (average: 1.29 \times 10^{-4}, P = 0.122). The \( \beta_\text{sty}/\beta_\text{so} \) ratio was 4.65 \times 10^{-4} (\mu \text{mol SO/\mu mol styrene}) with an estimated variance of 8.08 \times 10^{-8} (\mu \text{mol SO/\mu mol styrene})².

The above multiple regression analysis of the job-specific averages assumes that the estimated coefficients \( \hat{\beta}_\text{sty} \) and \( \hat{\beta}_\text{so} \) for each independent variable were not influenced by differences in the proportions of smokers among the job groups (Table 2). To evaluate this possibility, a multiple regression model was applied to the subject-specific averages. The main effect of exposure to SO was generally significant at a level of 0.05 (SO-Alb(a), P = 0.034; SO-Alb(b), P = 0.180; SO-DNA(1), P = 0.010; SO-DNA(2), P = 0.009; SCEs, P = 0.003). The overall results indicate that cigarette smoking produced effects that were at or near a 0.05 level of significance for at least one comparison involving each type of SO biomarker (SO-Alb(a)/SO exposure, P = 0.006; SO-DNA(2)/styrene exposure, P = 0.097; SCEs/styrene exposure, P = 0.040).

### DISCUSSION

As noted in the introduction, virtually all of the inhaled dose of styrene is transformed to SO in the human liver by cytochrome P-450 isozymes (CYP2B6, CYP1A2, CYP2E1, and CYP2C8). Immediately after formation, however, SO is efficiently metabolized to styrene glycol by hepatic epoxide hydrolases. Thus, only a small fraction of the hepatic dose of styrene reaches the blood in the form of SO, which can react with macromolecules, including DNA. Inhalation of SO, on the other hand, results in direct absorption of SO into the blood, where reactions can take place prior to metabolic deactivation of SO in the liver. Thus, whereas only a small portion of the styrene dose can produce SO biomarkers in the blood, a potentially much larger portion of the dose of inhaled SO can do so. The proportion of inhaled SO reaching the blood has not been measured; however, some losses of the dose of inhaled SO can do so. The proportion of inhaled SO reaching the blood has not been measured; however, some losses of the dose of inhaled SO can do so. The proportion of inhaled SO reaching the blood has not been measured; however, some losses of the dose of inhaled SO can do so.

#### Table 5 Effects of exposure category and smoking status upon SO biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Styrene exposure</th>
<th>Smoking status</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>SO exposure</th>
<th>Smoking status</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO-Alb(a)</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>1.67</td>
<td>0.506</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>1.02</td>
<td>0.304</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>1.17</td>
<td>0.616</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>1.82</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>1.32</td>
<td>0.568</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1.65</td>
<td>0.372</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>1.53</td>
<td>0.441</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>2.89</td>
<td>0.592</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>11</td>
<td>2.26</td>
<td>0.850</td>
<td>P = 0.019</td>
<td>0</td>
<td>4</td>
<td>1.02</td>
<td>0.304</td>
</tr>
<tr>
<td>SO-Alb(b)</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0.278</td>
<td>0.118</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0.147</td>
<td>0.086</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>0.171</td>
<td>0.100</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>0.210</td>
<td>0.157</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>0.218</td>
<td>0.133</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0.295</td>
<td>0.096</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>0.279</td>
<td>0.167</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>0.672</td>
<td>0.556</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>0.259</td>
<td>0.121</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>2.38</td>
<td>0.467</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>11</td>
<td>0.440</td>
<td>0.476</td>
<td>P = 0.577</td>
<td>0</td>
<td>4</td>
<td>6.62</td>
<td>5.13</td>
</tr>
<tr>
<td>DNA(1)</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>2.22</td>
<td>0.282</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>25.8</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>11.1</td>
<td>1.1</td>
<td>0</td>
<td>4</td>
<td>13.1</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>13.0</td>
<td>12.7</td>
<td>1</td>
<td>6</td>
<td>23.8</td>
<td>29.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>11.8</td>
<td>9.97</td>
<td>P = 0.863</td>
<td>0</td>
<td>4</td>
<td>13.1</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>11</td>
<td>27.5</td>
<td>33.9</td>
<td>0.097</td>
<td>0</td>
<td>4</td>
<td>6.62</td>
<td>5.13</td>
</tr>
<tr>
<td>DNA(2)</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>5.63</td>
<td>2.22</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>7.07</td>
<td>9.40</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>12.2</td>
<td>13.3</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>23.4</td>
<td>28.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>16.7</td>
<td>13.6</td>
<td>1</td>
<td>6</td>
<td>22.7</td>
<td>21.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>12.6</td>
<td>10.3</td>
<td>P = 0.618</td>
<td>0</td>
<td>4</td>
<td>23.4</td>
<td>28.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>11</td>
<td>23.7</td>
<td>24.4</td>
<td>0.095</td>
<td>0</td>
<td>4</td>
<td>7.07</td>
<td>9.40</td>
</tr>
<tr>
<td>SCEs</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0.738</td>
<td>0.415</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>5.96</td>
<td>0.415</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>0.843</td>
<td>0.147</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>7.14</td>
<td>0.147</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>0.586</td>
<td>0.501</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>6.65</td>
<td>0.501</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>0.593</td>
<td>1.12</td>
<td>0</td>
<td>5</td>
<td>7.20</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>0.589</td>
<td>1.02</td>
<td>0</td>
<td>5</td>
<td>7.20</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>9</td>
<td>7.27</td>
<td>1.38</td>
<td>0.040</td>
<td>0</td>
<td>4</td>
<td>6.62</td>
<td>5.13</td>
</tr>
</tbody>
</table>

*a* Low; 1, medium; 2, high.

*b* Nonsmokers; 1, smokers.

0, low; 1, high.
to know the fraction of the hepatic dose of styrene which reaches the blood as SO.

Since SO-adducts of blood proteins should be proportional to the integrated blood concentration of SO over some interval of time (39), it is possible to estimate the fraction of the hepatic dose of styrene that reaches the blood as SO via measurements of protein adducts in animals to which styrene and SO had been administered. We had previously used measurements of SO-Hb and SO-Alb from such experiments in rats to estimate that this fraction was about 2% (40). However, due to differences in affinities of the hepatic cytochrome P-450 and epoxide hydrolase enzymes between humans and rats (20, 35, 41), it is reasonable to expect that the corresponding fraction would be substantially less than 2% in humans. For example, Korn et al. (20) indicated that, at a given dose of styrene, human blood levels of SO should be 5—10% of those in rats and mice, due to the slower metabolism and more rapid elimination of SO in humans compared to rodents.

The fraction of the hepatic dose of styrene reaching the blood of humans as SO was estimated in the current investigation via the regression of SO-Alb(a) on exposures to styrene and SO in Eq. A (based upon job-specific averages). Assuming that the rates of uptake of SO and styrene were equal, that both chemicals entered the blood from the lungs without loss, that SO-Alb(a) was produced exclusively in the blood, and that the overall rate of reaction of SO in the blood was pseudo-first order, then the \( \beta_{SO} / \beta_{styrene} \) ratio (\( \mu \text{mol SO/}\mu \text{mol styrene} \)) gives a point estimate of this fraction. In this case, \( \beta_{SO} / \beta_{styrene} \) ratio = 4.65 \( \times \) \( 10^{-4} \) %, with an approximate 95% confidence interval of 0.01% to 0.10%. This indicates that only about 1/2000 of the styrene dose was released from the human liver into the blood under the above assumptions. By applying this value of 4.65 \( \times \) \( 10^{-4} \) (\( \mu \text{mol SO/}\mu \text{mol styrene} \)) styrene to the 20 subjects in our study with measurements of SO, we estimate that the proportions of the systemic SO dose associated with styrene exposure ranged from 10 to 76%, with a median value of 29%. Thus, a typical worker in our cohort obtained more than two-thirds of his or her SO dose from exposure to SO. Nonetheless, 5 of the 20 subjects had an estimated fraction of at least 50%, suggesting that styrene exposure was the major contributor to the SO dose in some situations.

The above calculations indicate that SO exposure should have been the primary determinant of increased levels of SO biomarkers among most of the workers in our study. Such behavior is illustrated among the various job groups in Fig. 1. In each case, the expected upward trend in the level of biomarker with increasing SO exposure is readily apparent. Furthermore, at least one-half of the variation in the average biomarker level of each job group can be attributed to SO exposure (Table 4; 0.502 \( \leq r^2 \leq 0.933 \)). Such large proportions of explainable variability of macromolecular adducts and SCEs are remarkable in our experience.

Since nonspecific cytogenetic end points are sensitive to the effect of cigarette smoking, we had previously analyzed our data to demonstrate that levels of SCEs were higher among smokers than nonsmokers in categories of low, medium, and high exposure to styrene (36). However, no evidence has been presented heretofore, suggesting that smoking also increased levels of macromolecular adducts of SO. Our results, summarized in Table 5, indicate that cigarette smoking also affected the levels of both SO-Alb and SO-DNA. This is illustrated in Fig. 2 for the two strongest effects, i.e., SO-Alb(a) versus SO exposure and for SO-DNA(2) versus styrene exposure. Thus, it appears that smoking shows effects on SO-Alb and SO-DNA that are independent of the level of exposure to styrene or SO.

Although our results suggest that cigarette smoking increased the levels of SO biomarkers in the blood, the origin of such an effect remains a matter of conjecture. The most likely explanation is that cigarette smoke contains SO. Although we can find no confirmation of this in the literature, we note that SO has been identified in cured tobacco (42) and could thus be volatilized during the smoking process. It is also reasonable to speculate that SO could be produced in situ in cigarette smoke, possibly from oxidation of styrene, which is a known constituent of tobacco smoke (43) and would certainly be present in the airways of persons exposed to styrene. It is also conceivable that SO-adducts were higher among smokers because of differences in metabolism (depletion of epoxide hydrolases, for example) associated with coexposures to the many xenobiotic compounds in cigarette smoke. For example, it has been shown in human lung tissues that recent smoking inhibited the activity of cytosolic (but not microsomal) epoxide hydrolase in a dose-dependent fashion, i.e., activity decreased with increasing cigarette consumption (44).

We noted in the introduction that two large studies of reinforced plastics workers (14—15) failed to detect a significant trend between cumulative exposure to styrene and cancer. However, since neither of these studies considered coexposure to SO, inferences regarding a lack of causality can neither be extended to airborne SO nor to a...
combination of exposures to styrene and SO. Indeed, the study of Wong et al. (14) pointed to excesses of cancers at many sites, including the lung. [Note also that two earlier studies also reported increases in lung cancer in the reinforced-plastics industry (11–12)]. Since SO is directly mutagenic and carcinogenic, we speculate that the lung, being the site of entry of airborne SO, might be at particular risk to injury by this substance. We also find it interesting that the only animal bioassays of SO were carried out by gavage and that the site of cancers in those studies was the forestomach, the site of entry (8–10).

Although our findings that inhalation of SO and smoking contributed significantly to SO biomarkers are interesting, we recognize that our study was small (48 subjects overall and only 20 with measurements of both styrene and SO) and was not designed to test for these particular effects. Nonetheless, the results are sufficiently provocative to motivate larger investigations of exposed populations. We encourage others to consider the potential significance of inhalation of SO, either as an airborne contaminant or as a component of cigarette smoke. We also note that efforts to control exposures within the reinforced-plastics industry have focused upon styrene, with only limited effectiveness (30). Given the marginal correlation of exposures to styrene and SO that we observed, interventions directed at reductions of SO exposure could well be more important in reducing health risks in this industry.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Leonore Dionne, who supervised collection of the field data; David Ting and Jeff Woodlee, who analyzed samples of air and exhaled air; Eva Horvath, who measured the DNA adducts; William Paradisin, who measured the SCEs; and Lawrence Kupper and R. C. Yu, who offered advice on statistical analyses.

REFERENCES

BIOMARKERS IN WORKERS EXPOSED TO STYRENE AND SO


An Investigation of Multiple Biomarkers among Workers Exposed to Styrene and Styrene-7,8-oxide


Cancer Res 1996;56:5410-5416.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/23/5410

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.