

## Possible metabolic interaction of styrene with organic solvents

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IKEDA, M. and HIRAYAMA, T. Possible metabolic interaction of styrene with organic solvents. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 41—46. Excretion of metabolites of styrene (dose: 2.2 mmol/kg i.p.) in 24-h urine was suppressed when rats were coinjected intraperitoneally with either toluene or trichloroethylene (dose: 2.2—11.0 mmol/kg) in addition to styrene, while a coinjection of n-hexane, ethyl acetate, methylchloroform, or acetone at an equimolar dose was ineffective in all cases. Suppressive effects of toluene and trichloroethylene were further confirmed by 6-h vapor exposure to (a) 0 to 1,300 ppm of toluene in addition to 150—170 ppm of styrene and (b) 0 to 4,000 ppm of trichloroethylene together with 100 or 150 ppm of styrene. The relationship between the amounts of three metabolites (i.e., phenylglyoxylic, mandelic and hippuric acids) in urine and styrene concentration in air was also determined.

**Key words:** coexposure, metabolic interaction, styrene, toluene, trichloroethylene.

Styrene is widely used in the plastics industry. Workers handling styrene may not only be exposed to styrene vapors but also to the vapors of other chemicals simultaneously or sequentially. In the fiber-reinforced plastics shipbuilding industry, for example, coexposure with organic solvents from paint often occurs in addition to exposure to styrene itself.

The purpose of this study was to determine whether organic solvents such as aromatic or chlorinated hydrocarbons affect metabolism, and possibly the toxicity of styrene, when inhaled simultaneously with styrene. A preliminary report on the *in vivo* metabolic interaction between toluene and styrene has already been published (6).

The metabolism of styrene in human subjects (5), as well as in rats (12), has

already been described and may be summarized as follows:

styrene → styrene oxide → styrene glycol  
→ mandelic acid → phenylglyoxylic acid  
    ↘ hippuric acid

A new metabolic pathway to conjugate styrene oxide with glutathione was recently reported (13).

### MATERIALS AND METHODS

Hydroquinone-free styrene was obtained from Koken and Co., Tokyo, Japan.

Male Wistar rats weighing 200—250 g were maintained on commercial laboratory chow and water *ad libitum*. When indicated, urine samples from individual rats were collected separately from feces for 6 h after the intraperitoneal injection or the initiation of vapor exposure, and

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then for the following 18 h to cover a total of 24 h. A preliminary experiment revealed that essentially all the metabolites of styrene and toluene are excreted into urine within 10 h after administration (6).

Phenylglyoxylic and mandelic acids in urine were analyzed according to the method of Ohtsuji and Ikeda (11). Hippuric acid in urine was determined with benzenesulfonyl chloride by the modified method of Ogata et al. (5, 10) with an additional modification that chloroform was replaced with ethanol.

The concentrations of styrene, toluene, and/or trichloroethylene in the exposure chamber were monitored every hour during the exposure (six determinations each were made as the animals were usually exposed for 6 h). A 1-ml air sample was injected into a Hitachi Model 163 gas-liquid chromatograph. The apparatus was equipped with flame ionization detectors (supply of hydrogen and air, 0.8 and 1.6 kg/cm<sup>2</sup>, respectively) and stainless steel columns [2-mm in an inner diameter and 2 m in length, packed with 10 % free fatty acid polyesters on Chromosorb WAW-DMCS, 80–100 mesh (14)]. The temperature of the oven and the injection

port were 100°C and 130°C, respectively, and the flow rate of the carrier nitrogen was 50 ml/min.

## RESULTS AND DISCUSSION

### *Intraperitoneal injection of styrene in combination with various organic solvents*

A screening test was carried out to detect any organic solvent which may modify styrene metabolism when administered simultaneously. Various organic solvents, i.e., n-hexane, ethyl acetate, trichloroethylene, methylchloroform, toluene and acetone at a dose of 2.2 or 11.0 mmol/kg were mixed with styrene (dose; 2.2 mmol/kg) and then diluted with soyabean oil to a total volume of 2 ml/kg. The final preparation was injected intraperitoneally into rats, and the 24-h urine was thereafter analyzed for phenylglyoxylic and mandelic acids. The results were compared with those obtained after an injection of styrene alone in soyabean oil (table 1).

Table 1. Effects of coadministered solvents on styrene metabolism.

| Solvents injected                        | Metabolites <sup>b</sup> |                      |
|------------------------------------------|--------------------------|----------------------|
|                                          | Phenylglyoxylic acid     | Mandelic acid        |
| Styrene                                  | 100 ± 16                 | 100 ± 23             |
| Styrene + n-hexane                       | 102 ± 15                 | 105 ± 44             |
| Styrene + ethyl acetate                  | 105 ± 11                 | 103 ± 11             |
| Styrene + trichloroethylene              | 93 ± 9                   | 88 ± 17              |
| Styrene + trichloroethylene <sup>a</sup> | 51 ± 15 <sup>c</sup>     | 56 ± 16 <sup>c</sup> |
| Styrene + methylchloroform               | 116 ± 25                 | 120 ± 28             |
| Styrene + toluene                        | 94 ± 13                  | 88 ± 19              |
| Styrene + toluene <sup>a</sup>           | 54 ± 15 <sup>c</sup>     | 55 ± 13 <sup>c</sup> |
| Styrene + acetone                        | 105 ± 23                 | 118 ± 41             |

Rats, 6 animals per group, were given 2.2 mmol/kg of styrene intraperitoneally and an equimolar amount of the other chemical unless otherwise specified. Urine samples were collected for 24 h.

<sup>a</sup> Either trichloroethylene (11.0 mmol/kg) or toluene (11.0 mmol/kg) were given together with 2.2 mmol/kg of styrene.

<sup>b</sup> Values are mean ± SD (N = 6), taking the mean values after styrene injection as 100 which was 141 mg/per kilogram per 24 h for phenylglyoxylic acid and 95 mg per kilogram per 24 h for mandelic acid. The counterpart value for hippuric acid was 89 mg per kilogram per 24 h.

<sup>c</sup> Significantly (P < 0.005) lower than the controls (i.e., values for styrene alone).

Most of the solvents were essentially ineffective, including acetone, which is known to enhance aniline hydroxylation *in vitro* but not *p*-nitrotoluene hydroxylation at methyl moiety (7). Both trichloroethylene and toluene at a dose of 2.2 mmol/kg reduced urinary metabolite levels slightly, but the differences were not statistically significant. When the dose of either trichloroethylene or toluene was increased fivefold to 11.0 mmol/kg, the concentration of both phenylglyoxylic and mandelic acid decreased markedly ( $P < 0.005$ ).

These positive findings, together with the negative observations with respect to other solvents, are essentially in accordance with the proposed hypothesis (3) that mutual metabolic suppression may be observed when the two compounds given together undergo microsomal oxidation, while no interaction takes place if one of the two is rather unsusceptible to oxidation.

*Amounts of urinary metabolites in relation to styrene exposure at various concentrations*

For the establishment of the relationship between the intensity of styrene vapor exposure and the amounts of urinary metabolites, rats were exposed to styrene vapor at concentrations of up to 300 ppm for 6 h and urine samples were collected for 24 h. The three urinary metabolites, i.e., phenylglyoxylic, mandelic and hippuric acids, were analyzed and the amounts were plotted against the styrene concentrations (fig. 1).

In contrast to the observations made of factory workers (1, 2), the level of phenylglyoxylic acid was almost linearly related to the styrene concentration up to 300 ppm. The acid level in the urine of rats exposed at the highest styrene concentration was much higher than those of nonexposed rats, an increase of nearly tenfold being recorded. The mandelic acid level was nonlinear to the styrene exposure intensity. It increased markedly as the styrene concentration increased up to 100 ppm, while additional increases were rather small at higher styrene con-

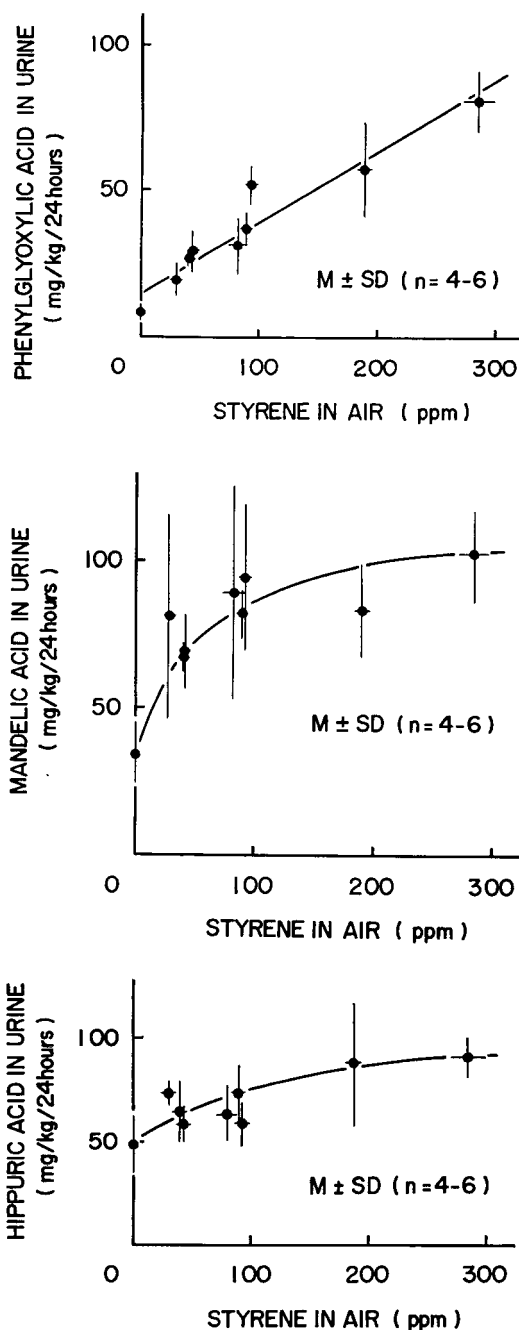


Fig. 1. The amounts of phenylglyoxylic acid, mandelic acid, and hippuric acid in 24-h urine of rats exposed for 6 h to styrene at various concentrations. Each dot in the figure is a mean of four urine determinations and six air determinations. The vertical and horizontal lines indicate standard deviations of the former and the latter, respectively. The thick line in the figure is a calculated regression line in the case of phenylglyoxylic acid and a drawn line for mandelic acid and hippuric acid.

centrations, as observed in workers (1, 2). Because of such relationships, the ratio of mandelic acid over phenylglyoxylic acid may vary depending on the styrene concentration. The variation of the ratio, increasing at higher styrene concentrations up to 30 ppm, has been previously reported for human subjects (11). Hippuric acid is apparently a poor indicator of exposure (fig. 1), as confirmed with factory workers (5, 11).

#### *Coexposure to styrene and toluene vapors*

Suppression of styrene metabolism by co-administered toluene was further confirmed by exposures to a vapor mixture of the two. The styrene concentration was set at three levels, i.e., 50–60 ppm, around 80 ppm, and 150–170 ppm, while toluene concentrations were from 0 up to 1,400 ppm. The urine samples were analyzed for phenylglyoxylic and mandelic acids (table 2). Although the results

with the two lower styrene concentrations were not uniform, those with the highest styrene concentration (experiment 3 in table 2) indicate that less amounts of both phenylglyoxylic and mandelic acids were excreted in urine when the exposure comprised both toluene and styrene at higher concentrations. Such a tendency was more evident in the 6-h urine samples, and this result indicates that suppression is more intensive during the co-exposure period. The fact that the 24-h urine values differed little may show that the suppressed metabolism can be compensated after exposure is terminated and the substrate concentration in the body becomes lower.

#### *Coexposure to styrene and trichloroethylene vapors*

The vapor inhalation experiments with a combination of styrene and trichloroethylene were carried out in the same

**Table 2.** Effects of simultaneous 6-h exposure to styrene and toluene on styrene metabolism.<sup>a</sup>

|                      |                        |                        |                       |                       |
|----------------------|------------------------|------------------------|-----------------------|-----------------------|
| <i>Experiment 1</i>  |                        |                        |                       |                       |
| Styrene (ppm)        | 54 ± 4                 | 59 ± 3                 | 54 ± 4                | 55 ± 5                |
| Toluene (ppm)        | 0                      | 45 ± 3                 | 170 ± 8               | 1,444 ± 87            |
| Phenylglyoxylic acid | 100 ± 36<br>(100 ± 15) | 124 ± 17<br>(102 ± 8)  | 79 ± 9<br>(89 ± 15)   | 73 ± 8<br>(101 ± 8)   |
| Mandelic acid        | 100 ± 25<br>(100 ± 25) | 126 ± 37<br>(87 ± 6)   | 101 ± 21<br>(81 ± 12) | 117 ± 28<br>(89 ± 14) |
| <i>Experiment 2</i>  |                        |                        |                       |                       |
| Styrene (ppm)        | 76 ± 9                 | 87 ± 7                 | 76 ± 6                | 85 ± 4                |
| Toluene (ppm)        | 0                      | 30 ± 5                 | 185 ± 11              | 1,328 ± 38            |
| Phenylglyoxylic acid | 100 ± 28<br>(100 ± 25) | 123 ± 34<br>(105 ± 18) | 93 ± 32<br>(73 ± 17)  | 106 ± 42<br>(97 ± 18) |
| Mandelic acid        | 100 ± 20<br>(100 ± 23) | 104 ± 16<br>(102 ± 22) | 89 ± 20<br>(90 ± 24)  | 86 ± 24<br>(88 ± 21)  |
| <i>Experiment 3</i>  |                        |                        |                       |                       |
| Styrene (ppm)        | 155 ± 18               | 171 ± 7                | 152 ± 16              | 161 ± 23              |
| Toluene (ppm)        | 0                      | 39 ± 4                 | 136 ± 25              | 1,333 ± 62            |
| Phenylglyoxylic acid | 100 ± 34<br>(100 ± 18) | 114 ± 38<br>(114 ± 28) | 79 ± 25<br>(108 ± 20) | 74 ± 18<br>(113 ± 20) |
| Mandelic acid        | 100 ± 19<br>(100 ± 13) | 70 ± 21<br>(102 ± 14)  | 56 ± 12<br>(96 ± 17)  | 57 ± 7<br>(86 ± 10)   |

<sup>a</sup> Vapor concentrations are shown as mean ± SD (N = 6). Metabolite amounts are expressed as mean ± SD (N = 4), taking the mean values after the exposure to styrene alone as 100; the values in the table are 6-h values together with 24-h values in parentheses. For the absolute values, see fig. 1.

Table 3. Effects of simultaneous 6-h exposure to styrene and trichloroethylene on styrene metabolism.<sup>a</sup>

|                         |                        |                        |                      |                       |
|-------------------------|------------------------|------------------------|----------------------|-----------------------|
| <i>Experiment 1</i>     |                        |                        |                      |                       |
| Styrene (ppm)           | 49 ± 2                 | 58 ± 2                 | 52 ± 6               | 53 ± 6                |
| Trichloroethylene (ppm) | 0                      | 86 ± 27                | 471 ± 50             | 4,047 ± 174           |
| Phenylglyoxylic acid    | 100 ± 40<br>(100 ± 19) | 124 ± 30<br>(105 ± 31) | 92 ± 10<br>(73 ± 9)  | 102 ± 23<br>(82 ± 15) |
| Mandelic acid           | 100 ± 23<br>(100 ± 17) | 122 ± 34<br>(100 ± 34) | 98 ± 9<br>(84 ± 10)  | 102 ± 19<br>(83 ± 11) |
| <i>Experiment 2</i>     |                        |                        |                      |                       |
| Styrene (ppm)           | 86 ± 3                 | 106 ± 5                | 95 ± 3               | 101 ± 3               |
| Trichloroethylene (ppm) | 0                      | 33 ± 10                | 437 ± 84             | 4,010 ± 63            |
| Phenylglyoxylic acid    | 100 ± 27<br>(100 ± 19) | 80 ± 16<br>(95 ± 13)   | 39 ± 14<br>(78 ± 20) | 46 ± 8<br>(74 ± 18)   |
| Mandelic acid           | 100 ± 15<br>(100 ± 12) | 98 ± 11<br>(96 ± 9)    | 72 ± 6<br>(85 ± 13)  | 72 ± 4<br>(85 ± 9)    |
| <i>Experiment 3</i>     |                        |                        |                      |                       |
| Styrene (ppm)           | 159 ± 5                | 158 ± 12               | 142 ± 9              | 142 ± 13              |
| Trichloroethylene (ppm) | 0                      | 95 ± 21                | 407 ± 17             | 3,433 ± 268           |
| Phenylglyoxylic acid    | 100 ± 30<br>(100 ± 14) | 68 ± 18<br>(76 ± 25)   | 45 ± 11<br>(66 ± 14) | 47 ± 5<br>(67 ± 14)   |
| Mandelic acid           | 100 ± 15<br>(100 ± 6)  | 89 ± 14<br>(88 ± 13)   | 82 ± 14<br>(94 ± 21) | 72 ± 7<br>(89 ± 4)    |

<sup>a</sup> Vapor concentrations are shown as mean ± SD (N = 6). Metabolite amounts are expressed as mean ± SD (N = 4), taking the mean values after the exposure to styrene alone as 100; the values in the table are 6-h values together with 24-h values in parentheses. For the absolute values, see fig. 1.

manner as the styrene-toluene studies, except that higher trichloroethylene concentrations were employed. The results are summarized in table 3. Essentially no suppressive effect was detected in the experiment with a low styrene concentration of about 50 ppm (experiment 1 in table 3). With higher styrene concentrations (experiments 2 and 3), however, the amounts of urinary metabolites were remarkably reduced when the animals were exposed to both trichloroethylene and styrene at higher concentrations. The reduction was more extensive with phenylglyoxylic acid than with mandelic acid, in accordance with the observation depicted in fig. 1. Suppression could be detected by the analyses of 24-h urine samples as well. Such long-lasting effects may be related to the fact that trichloroethylene has a longer biological half-life than toluene and styrene (4). It should also be noted that the vapor concentrations at which suppressive effects were

observed were not very high. Styrene concentrations over 100 ppm are often reported from various workshops (1, 8, 9). The capacity of rats, and presumably of human subjects as well, to metabolize foreign compounds including styrene may be limited. In fact, the ratio of styrene excreted as urinary metabolites over the styrene dose given intraperitoneally to rats becomes lower when the dose is higher than 250 mg/kg (5), which is roughly equivalent to 6-h exposure at 300 ppm, based on the amounts of urinary metabolites.

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## QUESTIONS AND ANSWERS

Question to Prof. IKEDA

Dr. AXELSON:

Have you tried to feed the animals with hippuric acid, i.e., the metabolite of toluene, to find out if there could be a competitive mechanism in the excretion in the kidney rather than in the metabolism of styrene and toluene?

Prof. IKEDA:

No such studies have been made.