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Inter- and intra-individual sources of variation in levels of urinary styrene metabolites

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Abstract ***Objective***: Given the paucity of studies that have examined variability in biological measures of exposure to workplace contaminants, we quantified the intra- and inter-individual sources of variation in urinary levels of mandelic acid (MA) and phenylglyoxylic acid (PGA) among workers exposed to styrene. A secondary objective was to examine effects of job task and the timing of sampling during the workweek on the variation in workers' urinary styrene metabolite levels. ***Methods***: As part of routine biological monitoring, a total of 1,714 measurements of MA and PGA collected from 331 workers between 1985 and 1999 from eight reinforced-plastics plants were abstracted from laboratory reports. To evaluate sources of variation in levels of urinary styrene metabolites, we applied random-effects models. The influence of job task and day of sampling on metabolite levels was examined using mixed-effects models. ***Results***: PGA levels were characterized by less variation than levels of MA, as were metabolite levels expressed in terms of urinary creatinine concentration. The relative magnitude of the inter-individual to the intra-individual source of variation was generally higher for post-shift urine samples than for pre-shift urine samples. As expected, urinary metabolite levels were highest for laminators and for samples collected at the latter end of the workweek. Owing to the effects of variation from day-to-day, estimates of workers' exposures that rely on single measurements would generally perform poorly in a regression analysis designed to examine effects resulting from chronic exposure. However, the bias in an observed slope coefficient would be diminished if a second or third urine sample were collected. ***Conclusions***: Quantification of the intra- and inter-individual sources of variation provides useful information that can be used to design optimal sampling strategies, which would allow for the collection of sufficient data to estimate workers' exposures reliably when evaluating health risks associated with occupational contaminants.

Key words Exposure assessment · Styrene · Mandelic acid · Phenylglyoxylic acid · Biological monitoring

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Introduction

Styrene is an important chemical used in a broad range of industrial applications including the manufacture of plastics, synthetic rubbers, and resins. Airborne levels of styrene are generally low in most industrial settings except for the reinforced-plastics industry (World Health Organization 1983; IARC 1994), which uses styrene as a solvent for unsaturated resin and as a reactant for polymerization during the production of boats, tanks, and other reinforced-plastics products. The main route of uptake of styrene is through inhalation; studies suggest that percutaneous absorption contributes negligible amounts to total exposure in the reinforced-plastics industry (Sorsa et al. 1991; Limasset et al. 1999). Approximately 60 to 70% of the inhaled styrene is retained in the body and is readily distributed to the blood and fat tissues (Bond 1989; Sumner and Fennell 1994). The major metabolic pathway involves the oxidation of styrene to styrene-7,8-oxide by hepatic cytochrome P-450 isozymes. Styrene oxide is hydrolyzed to styrene glycol, which is subsequently oxidized into mandelic acid (MA) and further to phenylglyoxylic acid (PGA). Excretion of MA appears to be biphasic, with half-times that have been reported to range from 4 to 9 h for the fast elimination phase and 17 to 25 h for the slow elimination phase (Bond 1989). Although there is some indication

that the elimination of PGA is also biphasic, a one-compartment model with a half-life of about 11 h appears sufficient to explain the underlying kinetic processes (Bond 1989). While most of the styrene oxide undergoes hydrolysis to form MA and PGA, a minor pathway involves the conjugation with glutathione to produce mercapturic acids. A small percentage of styrene is eliminated unchanged in expired air or urine or is oxidized to 3,4-styrene oxide, which is converted into 4-vinyl-phenol and excreted in urine as sulphate or glucuronate (Pfäffli et al. 1981).

Biological monitoring of exposure to styrene can be carried out through measurements of styrene or its metabolites in breath, blood, or urine samples (Pezzagno et al. 1985; Guillemin and Berode 1988). Among the biological monitoring methods available, measurements of urinary MA and PGA are the most commonly used biological indices of exposure to styrene. Numerous investigations have focused on the relationship between these metabolites with airborne styrene concentrations or on their relationship with each other (Engström et al. 1976; Guillemin et al. 1982; Ikeda et al. 1982; Apostoli et al. 1983; Franchini et al. 1983; Bartolucci et al. 1986; De Rosa et al. 1988; Imbriani et al. 1990; Brenner et al. 1991; Galassi et al. 1993; Pekari et al. 1993; Marhuenda et al. 1997; Ghittori et al. 1997; Apostoli et al. 1998). Despite the considerable attention that MA and PGA have received in the literature, we are aware of no study that has quantified the sources of variation in levels of these two biomarkers among workers exposed to styrene.

When evaluating exposure variability, important questions relate to the magnitude of the variation from day-to-day (intra-individual source of variation) relative to the variation in exposure levels among individuals (inter-individual source of variation). The extent to which biological measures of exposure vary from day-to-day is dependent upon the variation in external levels of the contaminant, although such fluctuations may be dampened considerably in levels of the contaminant or its metabolite in body fluids (Rappaport 1985). Biological measures of exposure with relatively short half-lives (as with urinary metabolites of styrene) will also be affected by fluctuations in exposure within a workshift (Droz and Guillemin 1983; Droz et al. 1989), which for solvents appears to be heavily influenced by intermittent use (Kumagai and Matsunaga 1999). In addition, assay and physiological variability contribute to fluctuations in biological indices of exposure over time. Inter-individual variation in average exposure levels, which might arise when workers have different tasks or work habits (Rappaport 1991), contributes to differences in biomarker levels among workers. Varying levels of physical activity (Droz and Guillemin 1983; Pezzagno et al. 1988), pharmacokinetic differences due to ethnicity, gender, age, and body mass, and lifestyle factors are also likely to play a role.

It is important to gain knowledge about the magnitude of the sources of variation in exposure indices because such variation induces measurement error, which in turn can cause downward bias in correlation and regression estimates when exposure-response relations are investigated (Thomas et al. 1993). Moreover, the lack of quantitative information on exposure variability makes it difficult to compare the utility of different biomarkers to evaluate occupational exposures in epidemiological investigations. With the exception of two studies evaluating variability in blood and urinary mercury levels among chloralkali plant workers (Symanski et al. 2000) and variability in exhaled-air measurements of styrene and sister-chromatid exchanges (SCEs) in peripheral lymphocytes of boat-manufacturing plant workers (Rappaport et al. 1995), the intra- and inter-individual sources of variation in biomonitoring data have not been quantified.

Given the paucity of studies that have examined intra- and inter-individual sources of variation in biological measures of exposure to workplace contaminants, we undertook this study to characterize variability in urinary levels of MA and PGA among workers exposed to styrene in the reinforced-plastics industry. Our primary goal was to quantify the sources of variation in urinary levels of styrene metabolites in exposed workers and make comparisons across groups of workers from different plants. A secondary objective was to evaluate to what extent job task and day of sampling explained the variability in the biological monitoring data.

Subjects and methods

Study population

The study population consisted of workers employed in factories manufacturing reinforced plastics in the Emilia Romagna region of Northern Italy between 1985 and 1999. Since airborne monitoring of styrene exposure was rarely conducted, information on job titles or the primary work tasks performed by each worker was obtained from work records and from the occupational medicine physicians at each plant. Information about significant changes in the workplace (e.g., modifications to the process and increased or decreased rates of production) that may have resulted in concomitant changes in exposure levels during the period over which the monitoring data were collected was also gathered. Use of personal protective clothing or equipment was not routinely documented and therefore could not be evaluated.

Biological monitoring

As part of routine biological monitoring conducted at each factory, the urinary excretion of the main styrene metabolites, MA and PGA, was evaluated periodically in workers throughout the study period. Spot urine samples were collected at the end of the shift (post-shift samples) or in the morning before the start of work (pre-shift samples). From 1985–1992, styrene metabolites were measured by gas chromatography (GC) with flame ionization detection (Fernandez and Caperos 1979) and by high-performance liquid chromatography (HPLC) (Poggi et al. 1982) thereafter. Urinary creatinine was measured according to the Jaffe method, and used to express concentrations of the styrene metabolites in milligrams per gram creatinine. The same laboratory was used to quantify levels of creatinine, MA, and PGA in urine throughout the study period. Analytical methods have long been checked within the FIOH

Quality Assurance Programme for Organic Solvent Metabolites organized by the Finnish Institute of Occupational Health.

Laboratory reports generated during the period 1985–1999 were accessed to compile a database of biological monitoring measurements. Any unusual values were investigated before the data were entered, but were left intact if no coding errors were identified. Urinary metabolite levels were recorded in units of milligrams per liter (mg/l) and milligrams per gram creatinine (mg/g creatinine). The date the urine samples were collected, the timing of urine collection (pre-shift or post-shift), and the analytical method used to measure levels (GC or HPLC) were also compiled. Measurements recorded without information on the timing of urine collection were excluded. When information was available, the gender and the job title (or primary work task) of each individual were added to the database.

Intra- and inter-individual sources of variation in urinary styrene metabolite levels

The frequency distributions of the untransformed measurements and the natural logarithms of the data were generated to examine the skewness of the data. The logged data were used in subsequent analyses as the transformed values appeared to be more normally distributed. To assess the intra- and inter-individual sources of variation in urinary levels of MA and PGA, a one-way random-effects model (Searle et al. 1992) was applied to data collected at each plant. This model represents the urinary measurement (on the logarithmic scale) for worker i measured on day j as the sum of three terms as specified below:

$$Y_{ij} = \mu + \beta_i + \varepsilon_{ij} \quad (1)$$

where μ, β_i, and ε_{ij} denote the population mean for the group of workers, the random deviation of the i-th worker's logged mean value from the population mean, and the random deviation of the j-th measurement from the i-th worker's mean value. The model assumes that β_i and ε_{ij} are independently and normally distributed with mean zero and variances σ^2_B and σ^2_W, respectively. Thus, the total variation in Y_{ij} is partitioned into two components, a component due to variation in average exposure levels among workers, and a component due to variation in exposure levels from day to day [i.e., $Var(Y_{ij}) = \sigma^2_Y = \sigma^2_B + \sigma^2_W$]. It directly follows that $Cov(Y_{ij}, Y_{ij'}) = \sigma^2_B$ for all i and $j \neq j'$ and that $Cov(Y_{ij}, Y_{ij'}) = 0$ for $i \neq i'$.

Evaluation of systematic changes in metabolite levels over time

Given that plant 1 reported a decrease in production levels from 1992 onwards, a mixed-effects model was applied in preliminary analyses to investigate systematic changes in metabolite levels between the two monitoring periods (1986–1991 and 1992–1999). In these analyses, a fixed effect due to the period of monitoring (for period = 1, 2 as indicated above) was added to the model represented in Eq. 1 (Symanski et al. 1996) and significant differences between monitoring periods were detected ($P < 0.05$). No changes in the workplace were reported at the other facilities during the period over which monitoring data had been collected, and inspection of the time plots revealed no discernible trends or shifts in metabolite levels. Thus, we report herein on the results from the mixed model for plant 1 and on the results from the one-way random-effects model for plants #2–8.

Evaluation of effects related to work tasks and day of sampling

To evaluate effects related to job tasks and day of sampling on urinary metabolite levels of MA and PGA, we applied mixed-effects models. At each plant, job tasks were collapsed into three broad categories: laminators (including workers involved in cutting and spraying, making gel coats, mixing resins or laminating), workers involved in other tasks at the plant (including assembly, finishing, maintenance or managerial positions), and workers whose job tasks were unknown. Due to restrictions associated with small sample sizes, only data sets with at least five workers in each job category were analyzed. When applying the mixed models to our data, we assumed common inter- and intra-individual variances across the three occupational groups. To evaluate the influence of day of sampling as a fixed effect, we broke down the week into an earlier (Mondays and Tuesdays) and a later period (Wednesday through Friday). At least 25% of the measurements at each plant had to be available during each period to be suitable for the analysis of effects due to the timing of sampling during the workweek.

For all models, separate analyses were conducted by plant for the pre- and post-shift urine samples, for raw concentrations of MA, PGA, and the sum of both metabolites, and for concentrations of these metabolites expressed in units of mg/l and mg/g creatinine. Variance components were estimated by the restricted maximum likelihood method, using PROC MIXED available with SAS software (SAS Institute, Cary, N.C., USA). The intra-class correlation coefficient (ICC), defined as the correlation between pairs of measurements collected from the same worker [i.e., $ICC = \sigma^2_B/(\sigma^2_B + \sigma^2_W)$], was computed using estimates of the variance components from each analysis. ICC values close to 1 indicate that single measurements can be used to estimate workers' exposure levels reliably, whereas values near 0 suggest that individual workers' mean levels are extremely difficult to distinguish from one another.

Results

Biological monitoring database

In Table 1, information about the database is broken down by plant. A total of 1,714 measurements of MA and PGA collected from 331 workers from eight reinforced-plastics plants were compiled. Six plants were involved in the production of silos, containers, or tanks, one in the production of carousel parts, and one in the production of polyester resins. There was wide variability across plants as to the period of sampling and the number of years during which measurements were collected, with some plants contributing data for just a few years compared with others where biological monitoring had been conducted for 10 years or longer. Altogether, the data spanned from 1985 through 1999. The majority of the workforce was male except at plant 4, which employed nearly equal numbers of both genders (plant 7 also appeared to be run predominantly by women, but information on gender was missing for approximately one fourth of the workforce). The proportion of workers involved in lamination and other tasks varied widely across plants. Given the changing nature of the workforce and the longitudinal design of the study, it was not possible to summarize information on the median age or length of employment of workers on whom biological measurements had been collected.

Table 2 provides summary statistics for styrene metabolite levels in pre- and post-shift samples at each of the eight plants. For pre-shift urine samples, the mean concentrations ranged across plants from 53 to 245 mg/g creatinine for MA and from 55 to 173 mg/g creatinine for PGA. Compared with all other workplaces, considerably higher and more variable styrene metabolite levels were observed in plant 8. Sampling was more

Table 1 Breakdown of characteristics of the biological monitoring data and the study population exposed to styrene in the reinforced-plastics industry in Emilia Romagna, Italy

	Plant							
	1	2	3	4	5	6	7	8
Product manufactured	Silos and containers	Containers for trucks	Polystyrene	Silos and trucks	Panels for containers	Containers	Carousel parts	Silos
Period of monitoring	1985–1999	1988–1997	1992–1998	1993–1998	1988–1991	1993–1998	1992–1999	1995–1996
Number of years[a]	13	10	7	6	3	5	6	2
N[b]	685	238	305	100	34	65	194	93
Number of workers	90	54	73	18	17	21	30	28
Gender (%)								
Male	54 (60)	40 (74)	52 (71)	8 (44)	15 (88)	17 (81)	9 (30)	28 (100)
Female	28 (31)	4 (7)	5 (7)	9 (50)	2 (12)	–	14 (47)	–
Not known	8 (9)	10 (19)	16 (22)	1 (6)	–	4 (19)	7 (23)	–
Job task (%)[c]								
Assembly	27 (30)	1 (2)	–	–	–	2 (10)	–	–
Lamination	15 (17)	10 (19)	17 (23)	13 (72)	–	2 (10)	15 (50)	22 (79)
Finishing	6 (7)	4 (7)	1 (1)	1 (6)	–	4 (19)	7 (23)	–
Other tasks	10 (11)	6 (11)	36 (49)	1 (6)	–	7 (33)	1 (3)	5 (18)
Missing	32 (36)	33 (61)	19 (26)	3 (17)	17 (100)	6 (29)	7 (23)	1 (4)

[a] Number of years during which biological monitoring was conducted
[b] Total number of measurements
[c] Percentages may not add up to 100 because of rounding

Table 2 Mean values (± 1 SE) of urinary levels of mandelic acid (*MA*) and phenylglyoxylic acid (*PGA*) in workers from the Italian reinforced-plastics industry (*N* total number of measurements, *k* number of workers, n_i number of repeated measurements per worker)

Plant	Pre- or Post-shift	Period	N	k	n_i	Median interval (days)[a]	MA (mg/l)	MA (mg/g creatinine)	PGA (mg/l)	PGA (mg/g creatinine)
1	Pre-shift	1986–1998	489	78	1–21	214	74 ± 4	53 ± 3	79 ± 3	55 ± 2
	Post-shift	1985/1999	196	42	1–9	2	643 ± 39	472 ± 28	219 ± 13	156 ± 8
2	Pre-shift	1988–1997	177	51	1–12	168	80 ± 7	59 ± 4	83 ± 6	61 ± 4
	Post-shift	1989–1997	61	36	1–7	179	163 ± 23	124 ± 15	90 ± 10	73 ± 8
3	Pre-shift	1992–1998	251	66	1–11	189	96 ± 6	70 ± 5	90 ± 5	65 ± 3
	Post-shift	1994–1998	54	33	1–5	231	148 ± 20	111 ± 15	84 ± 11	62 ± 8
4	Pre-shift	1993–1998	100	18	1–16	231	68 ± 8	60 ± 7	94 ± 12	83 ± 11
5	Pre-shift	1988–1991	34	17	1–4	301	113 ± 45	58 ± 16	102 ± 18	58 ± 9
6	Pre-shift	1993–1998	65	21	1–7	398	80 ± 16	59 ± 10	81 ± 11	62 ± 8
7	Pre-shift	1992–1998	42	20	1–7	1,923	122 ± 13	93 ± 12	121 ± 12	89 ± 10
	Post-shift	1995–1999	152	28	1–14	140	410 ± 21	299 ± 14	248 ± 13	180 ± 8
8	Pre-shift	1995–1996	67	28	1–5	164	372 ± 55	245 ± 35	254 ± 30	173 ± 24
	Post-shift	1995–1996	26	16	1–2	253	713 ± 95	632 ± 90	441 ± 60	352 ± 39

[a] Median interval between measurements collected on the same worker

frequently carried out pre-shift rather than post-shift except for at the plant involved in the production of carousel parts (plant 7). As expected, post-shift sampling yielded higher values, on average, than pre-shift measurements. It is important to note, however, that in those plants where both pre-and post-shift samples had been collected, such samples were typically not collected on consecutive days. The median interval between measurements from the same worker ranged from 5 to 64 months, with the exception of post-shift samples from plant 1, which were commonly collected over the course of several days (median interval of 2 days). In evaluating the minimum interval between measurements collected from the same worker, we found that few post-shift samples from plant 2 (10%), even fewer pre-shift samples from plant 1 (< 1%) and none of the pre- or post-shift samples from plants 3–8 or pre-shift samples from plant 2 were separated by intervals of 7 days or fewer (results not shown).

Intra- and inter-individual sources of variation in urinary styrene metabolite levels

Point estimates of the variance components in the log-transformed values for urinary MA, PGA, and the sum of both metabolites are shown in Table 3. In general, PGA levels were characterized by less total variation than levels of MA. With relatively few exceptions, comparisons for each metabolite suggest that the concentrations expressed as a function of creatinine yielded smaller estimates of the intra-individual source of variation. Correspondingly, the estimated values of the intra-class correlation coefficient were typically larger. The

inter-individual variance was generally higher than the intra-individual variance for post-shift urine samples in all plants except for 8. In contrast, the results for styrene metabolite levels measured in pre-shift urine samples suggest greater variation, on average, across workshifts than among workers at the same plant.

Due to restrictions associated with sample size, effects of work tasks on the variation in urinary metabolite levels could be examined only for plants 2, 3, and 7. At each of the three plants, laminators had significantly higher exposure levels ($P < 0.05$) than workers in assembly, finishing, maintenance or managerial positions at the plant ('non-laminators') irrespective of the metabolite analyzed (MA, PGA, or MA + PGA) or the time of sampling (pre- or post-shift) (results not shown). Exposures for workers whose tasks were unknown were also generally higher than non-laminators, but were not significantly different in most cases ($P > 0.05$).

The influence of work tasks on the variation in metabolite levels was further examined by comparing estimates of the inter-individual variance component obtained from the one-way random-effects model ($\hat{\sigma}^2_{B_1}$) with the mixed-effects model ($\hat{\sigma}^2_{B_2}$) with a fixed effect added for job classification (Bryk and Raudenbush 1992). Table 4 shows the proportion of the inter-individual variation explained by job task $\left[\text{i.e.,}\left(\hat{\sigma}^2_{B_1} - \hat{\sigma}^2_{B_2}\right)/\hat{\sigma}^2_{B_1}\right]$ for each metabolite measured in workers from the three different plants. The percent reduction in the inter-individual variance component ranged from 8 to 100%. While this ratio measure

Table 3 Estimated inter- and intra-individual sources of variation ($\hat{\sigma}^2_B$ and $\hat{\sigma}^2_W$) in natural log-transformed levels of urinary mandelic acid (*MA*), phenylglyoxylic acid (PGA), and the sum of both metabolites (MA + PGA) in workers from the Italian reinforced-plastics industry. $\hat{\sigma}^2_Y = \hat{\sigma}^2_B + \hat{\sigma}^2_W$. Intra-class correlation coefficient (*ICC*) estimated by $\hat{\sigma}^2_B/(\hat{\sigma}^2_B + \hat{\sigma}^2_W)$

Plant	Pre-or post-shift	Units	MA				PGA				MA + PGA			
			$\hat{\sigma}^2_W$	$\hat{\sigma}^2_B$	$\hat{\sigma}^2_Y$	ICC[b]	$\hat{\sigma}^2_W$	$\hat{\sigma}^2_B$	$\hat{\sigma}^2_Y$	ICC[b]	$\hat{\sigma}^2_W$	$\hat{\sigma}^2_B$	$\hat{\sigma}^2_Y$	ICC[b]
1	Pre-shift	mg/l	0.69	0.15	0.83	0.18	0.56	0.20	0.75	0.26	0.54	0.18	0.73	0.25
		mg/g creatinine	0.63	0.22	0.84	0.26	0.48	0.21	0.69	0.31	0.47	0.23	0.70	0.33
	Post-shift	mg/l	0.38	0.48	0.86	0.56	0.33	0.28	0.61	0.46	0.33	0.41	0.75	0.55
		mg/g creatinine	0.36	0.52	0.89	0.59	0.25	0.35	0.59	0.59	0.30	0.46	0.76	0.61
2	Pre-shift	mg/l	0.72	0.40	1.12	0.36	0.71	0.40	1.12	0.36	0.58	0.38	0.96	0.40
		mg/g creatinine	0.61	0.46	1.07	0.43	0.64	0.39	1.03	0.38	0.48	0.41	0.89	0.46
	Post-shift	mg/l	0.60	0.64	1.23	0.52	0.46	0.77	1.23	0.62	0.49	0.63	1.13	0.56
		mg/g creatinine	0.49	0.58	1.06	0.54	0.35	0.74	1.09	0.68	0.38	0.58	0.96	0.61
3	Pre-shift	mg/l	0.70	0.36	1.05	0.34	0.60	0.25	0.85	0.29	0.60	0.30	0.90	0.33
		mg/g creatinine	0.52	0.40	0.92	0.44	0.42	0.28	0.70	0.40	0.43	0.33	0.76	0.44
	Post-shift	mg/l	0.37	0.79	1.16	0.68	0.36	0.35	0.71	0.50	0.33	0.54	0.87	0.62
		mg/g creatinine	0.44	0.66	1.10	0.60	0.33	0.35	0.69	0.52	0.35	0.48	0.83	0.57
4	Pre-shift	mg/l	0.67	0.17	0.84	0.20	0.68	0.21	0.89	0.23	0.64	0.18	0.82	0.22
		mg/g creatinine	0.69	0.30	0.99	0.30	0.67	0.30	0.97	0.31	0.64	0.29	0.93	0.31
5	Pre-shift	mg/l	1.24	0	1.24	0	0.93	0.02	0.95	0.02	1.03	0	1.03	0
		mg/g creatinine	1.11	0.05	1.16	0.04	0.67	0.18	0.85	0.21	0.86	0.06	0.91	0.06
6	Pre-shift	mg/l	1.13	0.18	1.30	0.13	0.97	0.06	1.04	0.06	1.02	0.10	1.13	0.09
		mg/g creatinine	1.14	0	1.14	0	0.95	0	0.95	0	1.01	0	1.01	0
7	Pre-shift	mg/l	0.25	0.54	0.80	0.68	0.55	0.16	0.71	0.23	0.36	0.32	0.68	0.47
		mg/g creatinine	0.29	0.84	1.13	0.74	0.44	0.45	0.89	0.50	0.31	0.61	0.93	0.66
	Post-shift	mg/l	0.34	0.50	0.84	0.59	0.33	0.40	0.73	0.54	0.32	0.46	0.78	0.59
		mg/g creatinine	0.26	0.55	0.81	0.68	0.24	0.42	0.67	0.64	0.23	0.51	0.74	0.69
8	Pre-shift	mg/l	0.92	1.64	2.55	0.64	0.60	1.41	2.01	0.70	0.69	1.59	2.29	0.70
		mg/g creatinine	1.89	0.71	2.61	0.38	0.52	1.51	2.03	0.74	0.55	1.78	2.32	0.76
	Post-shift	mg/l	1.11	0	1.11	0	0.53	0.19	0.72	0.27	0.84	0	0.84	0
		mg/g creatinine	1.15	0	1.15	0	0.49	0.03	0.51	0.05	0.72	0	0.72	0

Table 4 Proportional reduction in the inter-individual source of variation among styrene-exposed workers after accounting for job tasks in a mixed-effects model $\left(\hat{\sigma}^2_{B_1} - \hat{\sigma}^2_{B_2}\right)\Big/\left(\hat{\sigma}^2_{B_1}\right)$ where $\hat{\sigma}^2_{B_1}$ and $\hat{\sigma}^2_{B_2}$ are the estimates of the inter-individual variance component under the one-way random effects model and the mixed-effects model, respectively (*MA* mandelic acid, *PGA* phenylglyoxylic acid)

	Plant 2		Plant 3		Plant 7	
	Pre-shift	Post-shift	Pre-shift	Post-shift	Pre-shift	Post-shift
MA (mg/l)	0.18	0.39	0.27	0.17	0.45	0.56
MA (mg/g creatinine)	0.08	0.46	0.25	0.15	0.51	0.44
PGA (mg/l)	0.31	0.44	0.13	0.13	1.00	0.57
PGA (mg/g creatinine)	0.22	0.45	0.13	0.13	1.00	0.44
MA + PGA (mg/l)	0.21	0.42	0.21	0.21	0.72	0.56
MA + PGA (mg/g creatinine)	0.10	0.47	0.20	0.16	0.68	0.44

represents the fraction of the variation among workers that is explained by job classification, careful interpretation is warranted when the point estimate of the inter-individual variance component is small. In that instance, the fixed effect may explain a significant proportion of very little variation. As expected, there were trivial differences in the estimates of the intra-individual variance component under both models (results not shown).

At most workplaces, over 75% of the urine samples were collected during the latter part of the week (Wednesday onwards). However, plants 2 (post-shift samples) and 8 (pre-shift samples) had sufficient data during both periods (early and late in the week) for effects to be examined that were related to the timing of sampling on levels of MA, PGA, and the sum of both metabolites. We found that levels of the sum of both metabolites were higher ($P < 0.05$) for samples collected later in the week than for those collected on Mondays or Tuesdays. In particular, the estimated geometric mean level of MA + PGA in post-shift samples at plant 2 was 84 mg/g creatinine (85 mg/l) for samples collected on Mondays or Tuesdays and 139 mg/g creatinine (197 mg/l) for samples collected later in the week. At plant 8, corresponding results were 102 mg/g creatinine (160 mg/l) and 174 mg/g creatinine (308 mg/l) for pre-shift samples collected early or late in the workweek, respectively. When analyses were conducted for levels of MA and PGA separately, similar patterns emerged with statistically significant differences ($P < 0.05$) detected in all but one case (results not shown).

Discussion

Information on intra- and inter-individual sources of variation in biomarkers of exposure is generally lacking in the literature and we are aware of no studies to date that have quantified the sources of variability in urinary levels of MA and PGA. In our investigation, we found less variability in levels of PGA than in MA, and for concentrations expressed in units of milligrams per gram creatinine. Since the smoothing of variability in airborne exposures depends upon the contaminant's half-life in the body (Rappaport 1985), our results are consistent with the underlying kinetics of both compounds because of the slower rates of elimination for PGA than for MA (Bond 1989). The greater variation of styrene metabolite levels expressed in units of milligrams per liter compared with milligrams per gram creatinine is likely due to fluctuations in urinary flow rate in spot samples, and confirms a similar finding reported in an investigation of biomonitoring data collected from chloralkali-plant workers (Symanski et al. 2000). While expressing metabolite levels in terms of urinary creatinine concentration typically decreased the intra-individual source of variation, it also tended to increase the inter-individual source of variation and thereby increased the total variation in some cases (see Table 3). These results are consistent with those of Alessio et al. (1985) who reported differences in urinary creatinine levels among individuals, and may explain, in part, studies that reported no statistical advantage in evaluating metabolite levels as a function of creatinine (Sollenberg et al. 1988; Imbriani et al. 1990). On the other hand, expressing metabolite levels in terms of creatinine concentration normalizes data not only for urinary flow, but also for body mass and gender. Although this may introduce an additional source of measurement error and also increase inter-individual variation, it is fully justified on pathophysiological grounds. Since the primary aim of biological monitoring (of exposure) is to obtain estimates of internal dose, some components of variation (e.g., errors in sampling and analysis) should be minimized, whereas others, such as inter-individual differences in uptake, metabolism, and excretion, must be taken into account to interpret properly the biomonitoring data for assessing associated health risks (Mutti 1999).

In addition to variation from day-to-day, the intra-day variation in external levels is transmitted to variation in biological levels of the contaminant or its metabolite for compounds with relatively short half-lives. As such, post-shift samples are likely to be more sensitive to peaks in exposures experienced during the day, especially in the latter portion of the work shift. We found, however, that the magnitude of the intra-individual source of variation was generally less for measurements collected at the end, rather than at the beginning, of the workday. Although the greater variation in pre-shift samples may offer partial explanations for the weaker correlation between styrene exposure and urinary metabolite levels observed in morning samples than in end-shift samples (Ong et al. 1994), such results have not been consistent in all studies (De Rosa et al. 1988). Since the pre- and post-shift measurements in our study were not collected on consecutive days, it is also possible that the differences that we observed were due to dissimilarities in the tasks that workers performed from one sampling period to another. Moreover, there are likely to be other factors unrelated to the external exposure that contribute to the intra-individual variation in pre-shift urine samples, perhaps due to the greater analytical variability associated with the very low levels sometimes recorded in such samples. However, more work is warranted before a satisfactory explanation can be provided.

Measurement error operates to bias regression coefficients towards zero when a simple linear model adequately describes an exposure-response relation (Thomas et al. 1993). The slope coefficient in a simple linear regression analysis has been shown (Tielemans et al. 1998) to be attenuated by a factor equal to $\left[\sigma_B^2/\left(\sigma_B^2 + \sigma_W^2/n\right)\right]$ where $\sigma^2{}_B$ and $\sigma^2{}_W$ are the inter- and intra-individual variance components, and n is the number of measurements obtained from each worker. For studies where only one measurement was collected, the attenuation factor is equal to the intra-class corre-

lation coefficient. When making comparisons, we found that pre-shift urine samples were less efficient than post-shift urine samples (i.e., ICC values were smaller for pre-shift than for post-shift samples). This finding opens the question as to whether samples collected prior to the beginning of a work shift are the most reliable measure of styrene uptake as previously suggested (Droz and Guillemin 1983; Bartolucci et al. 1986; Guillemin and Berode 1988; Pekari et al. 1993).

Although our data indicate that a single measurement may be unreliable as a measure of long-term exposure in epidemiological studies, the measurement error that would be introduced would vary by the group of workers under investigation. Figure 1 plots the bias (%) in the regression coefficient as a function of the number of measurements collected on each worker, $\left[1-\hat{\sigma}_B^2/\left(\hat{\sigma}_B^2+\hat{\sigma}_W^2/n\right)\right]\times 100$, for urinary levels of MA + PGA (mg/g creatinine) in pre-shift urine samples. In plants 1, 4, and 5, estimates of workers' exposures that rely on single measurements would perform poorly and would yield observed coefficients that were less than 33% of the true slope. The bias in an observed regression coefficient would be greatly diminished if a second or third urine sample were collected, but the expected benefits lessen thereafter with increasing sample size. In plant 5, however, exposures are characterized by such extreme variability from day-to-day (relative to the variation among workers) that the bias would remain quite substantial even when many measurements are collected from each worker.

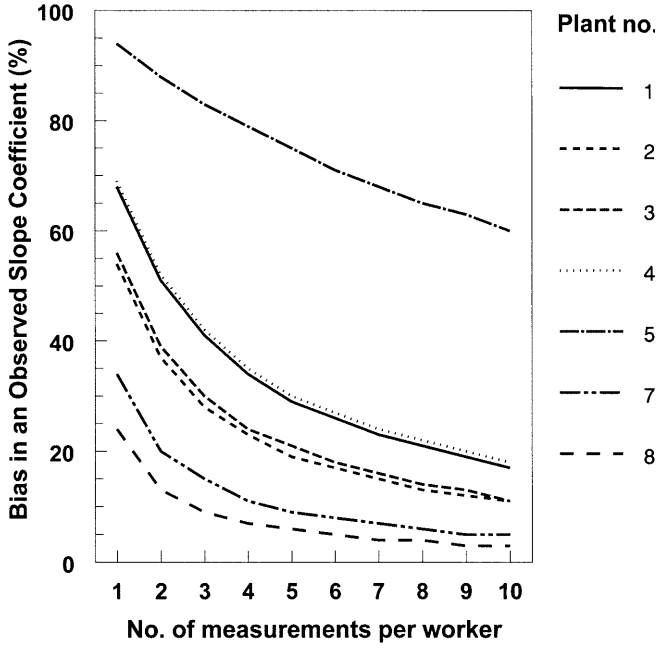

Fig. 1 Bias in the observed slope coefficient $\left[1-\hat{\sigma}_B^2/\left(\hat{\sigma}_B^2+\hat{\sigma}_W^2/n\right)\right]\times 100$ as a function of the number of pre-shift urine samples (n) collected from each worker for mandelic acid + phenylglyoxylic acid (MA + PGA) (mg/g creatinine). Note: $\hat{\sigma}_B^2$ and $\hat{\sigma}_W^2$ represent estimates of the inter- and intra-individual variance components, respectively

While estimates of the total variability in biological measures of exposure are often obtained, lack of information on the magnitude of the inter- and intra-individual sources of variation is highly disadvantageous. For example, there are little differences in the total variation of MA + PGA levels (mg/g creatinine) in plants 4 and 5 ($\hat{\sigma}_Y^2=0.93$ and $\sigma_Y^2=0.91$, respectively). Yet, a similar sampling strategy (in terms of the number of measurements collected from each worker) would produce inconsistent regression results should such data be used to evaluate workers' exposures at both plants (see Fig. 1). The inconsistency would arise because of differences in the relative magnitude of the intra- to inter-individual sources of variation between the two groups of workers, thereby making it difficult to compare the regression results obtained for each group and suggesting that important errors of inference can be made as a result of measurement error.

Our data also confirm previous findings (Sollenberg et al 1988; Imbriani et al. 1990; Marhuenda et al. 1997) of higher styrene metabolite levels towards the end of the workweek. Thus, information about the timing of sampling should be ascertained when evaluating differences in metabolite levels from one time period to another or between different groups of workers, to ensure that valid comparisons are made. Moreover, in analyses that rely on repeated measurements, the assumption that the correlation between measurements collected from the same worker is constant (referred to as compound symmetry) may not be valid. Instead, a more complex covariance pattern might be necessary to account for serial correlation, in which the correlation between measurements from the same worker is a function of the time interval separating them.

The extent to which biological monitoring data will be serially correlated depends, in general, on the half-life of the contaminant and on the frequency of sampling. Overall, there was likely to be little serial correlation among repeated measurements in the current study because the majority of the data were separated by intervals of months or longer, relative to the short elimination half-lives of less than 24 h for both MA and PGA. However, for workers in plant 1 who often provided multiple post-shift urine samples over the course of several days, measurements that were collected closer together may be more highly correlated than those farther apart in time. To explore this possibility, we applied a mixed-effects model with a first-order autoregressive [AR(1)] error structure which assumes that the correlation function decays exponentially as the interval between measurements increases (i.e., $\rho^{\tau}=\alpha^{\tau}$, where τ is the number of days between measurements). Table 5 presents the results from the model with an AR(1) error structure for MA, PGA, and the sum of both metabolites. In all cases, moderate to substantial levels of serial correlation were detected ($\hat{\rho}^{1\ \mathrm{day}}$: 0.49 to 0.76) ($P<0.05$). In the comparison between the results of the two models with different covariance structures (see Table 5), the differences suggest that ignoring serial

Table 5 Comparison in the results from the mixed-model analyses with either a first-order autoregressive [*AR(1)*] or compound symmetry covariance structure applied to post-shift urine samples from plant 1. $\hat{\rho}^{1\ day}$: serial correlation for repeated measurements separated by one day. *MA* mandelic acid, *PGA* phenylglyoxylic acid

	AR (1)			Compound symmetry	
	$> \hat{\sigma}_W^2$	$> \hat{\sigma}_B^2$	$> \hat{\rho}^{1\ day}$	$> \hat{\sigma}_W^2$	$> \hat{\sigma}_B^2$
MA (mg/l)	0.43	0.41	0.64	0.38	0.48
MA (mg/g creatinine)	0.43	0.44	0.76	0.36	0.52
PGA (mg/l)	0.36	0.25	0.49	0.33	0.28
PGA (mg/g creatinine)	0.27	0.31	0.59	0.25	0.35
MA + PGA (mg/l)	0.37	0.36	0.58	0.33	0.41
MA + PGA (mg/g creatinine)	0.35	0.40	0.72	0.30	0.46

correlation results in an underestimation of the intra-individual variance component, which is a well-known consequence of positively autocorrelated processes (Diggle 1990), and an overestimation of the inter-individual variance component. While these differences are modest, the potential for serial correlation in biological monitoring data should be evaluated to avoid problems associated with mis-specified models and possible errors of inference.

Given that the occupational group may distinguish workers based upon what work is performed and where tasks are carried out, it serves as a surrogate for various determinants of exposure, and can easily be evaluated in mixed models to assess the combined effects of 'job' upon exposure (Rappaport et al. 1999). In the current investigation, we classified job titles or the primary work tasks of individuals into three broad occupational groups (laminators, non-laminators, and unknown) to investigate the effect of styrene exposure on levels of urinary metabolites. Consistent with other studies (Galassi et al. 1993), our findings indicate that there are significant differences based on work tasks, which explained moderate to substantial portions of the variation in styrene metabolite levels among workers. While different classification strategies, such as grouping by job title within a plant or grouping by plant alone, may serve to make groups more or less homogeneous, differences in external exposures among workers *within* a group (however classified) clearly contribute to inter-individual variation in internal levels of exogenous contaminants (Droz et al. 1989).

Beyond variation in external exposure levels among workers, certain factors such as gender and age are known to modify body mass and composition, thereby affecting styrene distribution. Others, such as alcohol consumption (Wilson et al. 1983; Berode et al. 1986; Cerný et al. 1990), co-exposure to other solvents (Marhuenda et al. 1997; Apostoli et al. 1998), and workload (Pezzagno et al. 1988) may interfere with styrene metabolism or affect absorption. Unfortunately, data on these variables were not available, so it was not possible to evaluate their roles in explaining the variation in urinary metabolite levels. Notwithstanding this limitation, the utility of random-effects models lies in their ability to represent, through use of a single random term, composite unmeasured effects that contribute to differences between individuals. Given the additional advantage of mixed-models to assess specific determinants of exposure while incorporating random sources of variation, future studies are warranted to apply such models to evaluate the influence of important covariates on urinary levels of MA and PGA.

In conclusion, the information provided in this study, which is not available elsewhere, should prove useful to other investigators when designing prospective sampling strategies to evaluate exposures to styrene in the reinforced-plastics industry. It is important to note, however, that estimates of the variance components may be unique for different worker populations, because the degree of variability in biological measurements of exposure depends on the magnitude of variation in external levels of the contaminant, physiological differences among the workers under investigation, and on sampling and assay variability. Thus, we encourage other investigators to carry out studies to quantify intra- and inter-individual differences in biomarker levels among exposed workers. In instances when routine biological measurements do not provide suitable data for analysis, biological-monitoring strategies should be developed to collect repeated measurements from representative workers so that the sources of variation in exposure can be assessed. Such an assessment would provide useful information for investigators to optimize the design of prospective studies and allow for the collection of sufficient data to estimate reliably workers' exposures, when evaluating health risks associated with occupational contaminants.

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