Toxicology Letters 192 (2010) 40–44

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Assessing variability and comparing short-term biomarkers of styrene exposure using a repeated measurements approach

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ARTICLE INFO

Article history:
Available online 11 February 2009

Keywords:
Variability
Styrene
Biomarkers

ABSTRACT

The aim of this work is to compare several short-term biomarkers of styrene exposure, namely urinary styrene (StyU), mercapturic acids (M1 + M2), mandelic acid (MA), phenylglyoxylic acid (PGA), phenylglycine (PHG), and 4-vinylphenol conjugates (VP), for use as biomarkers of exposure in epidemiologic studies. A repeated measurements protocol (typically 4 measurements per worker over 6 weeks) was applied to measure airborne styrene (StyA) and urinary biomarkers in 10 varnish and 8 fiberglass reinforced plastic workers. Estimated geometric mean personal exposures to StyA were 2.96 mg/m^3 in varnish workers and 15.7 mg/m^3 in plastic workers. The corresponding levels of StyU, M1 + M2, MA, PGA, MA + PGA, PHG and VP were 5.13 μg/L, 0.111, 38.2, 22.7, 62.6, 0.978, and 3.97 mg/g creatinine in varnish workers and 8.38 μg/L, 0.303, 146, 83.4, 232, 2.85 and 3.97 mg/g creatinine in plastic workers. Within-worker (σ^2_{wY}) and between-worker (σ^2_{bY}) variance components were estimated from the log-transformed data as were the corresponding fold ranges containing 95% of the respective lognormal distributions of daily levels ($_wR_{0.95}$) and subject-specific mean levels ($_bR_{0.95}$). Estimates of $_wR_{0.95}$ (range: 4–26) were generally smaller than those of $_bR_{0.95}$ (range: 5–790) for both environmental and biological markers; this indicates that exposures varied much more between workers than within workers in these groups. Since attenuation bias in an estimated exposure–response relationship increases with the variance ratio $\lambda = \sigma^2_{wY}/\sigma^2_{bY}$, we estimated values of λ for all exposure measures in our study. Values of λ were typically much less than one (median = 0.220) and ranged from 0.089 for M1 + M2 in plastic workers to 1.38 for PHG in varnish workers. Since values of λ were 0.147 and 0.271 for StyA in varnish workers and plastic workers, respectively, compared to 0.178 and 0.210 for MA in the same groups, our results suggest that either air measurements or conventional biomarker measurements (urinary MA) would be comparable surrogates for individual exposures in epidemiologic studies.

1. Introduction

Styrene is an important industrial chemical primarily used in the production of plastics (Miller et al., 1994; IARC, 1994). Due to its toxicological properties styrene has been classified as a possible carcinogen to humans (group 2B) (IARC, 1994). Its occupational exposure is regulated in many countries with an airborne concentration of 85 mg/m^3 (20 ppm) currently recommended as an exposure limit by both the American Conference of Governmental Industrial Hygienists (ACGIH, 2007) and the Deutsche Forschungsgemeinschaft (DFG, 2007).

The metabolites mandelic acid (MA) and/or phenylglyoxylic acid (PGA) in end-shift urine have generally been the biomarkers of choice for styrene exposure [Biological Exposure Index = 400 mg (MA + PGA)/g creatinine (ACGIH, 2007) and Biological Tolerance Value = 600 mg (MA + PGA)/g creatinine (DFG, 2007)], although blood styrene in end-shift samples has also been recommended [Biological Exposure Index = 0.2 mg/L (ACGIH, 2007)]. Recently, other metabolites of styrene, including a mixture of diastereomeric mercapturic acids, [(R,R)- and (S,R)-N-acetyl-S-(1-phenyl-2-hydroxyethyl)-L-cysteine and (R,R)- and (S,R)-N-acetyl-S-(2-phenyl-2-hydroxyethyl)-L-cysteine] designated as M1 + M2, 4-vinylphenol (VP), excreted as a glucuronide and sulfate conjugates, phenylglycine (PHG) and urinary styrene (StyU) have also been proposed as biomarkers of styrene exposure (Manini et al., 2003; Haufroid et al., 2001; De Palma et al., 2001; Ghittori et al., 1987).

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doi:10.1016/j.toxlet.2009.02.004

It has been shown that air and biological measures of chemical exposure vary both within and between workers in a given observational group (Liljelind et al., 2003; Kromhout et al., 1993). Since these sources of variability have profound implications on our ability to make valid and precise inferences about the levels of occupational exposure in investigations of either health effects or for the control of occupational hazards, it is important that the within-worker and between-worker variance components, designated as σ^2_{wY} and σ^2_{bY}, respectively, be characterized (Rappaport and Kupper, 2008). These variance components can be estimated by applying analysis of variance (ANOVA) or linear mixed-effects models to data containing repeated measurements of air and/or biomarker measurements from representative workers in each observational group. Of particular interest is the variance ratio $\lambda = \sigma^2_{wY}/\sigma^2_{bY}$ which provides an index of the potential for a given exposure surrogate, i.e., a particular type of air measurement or biomarker, to introduce attenuation bias into an exposure–response relationship (Lin et al., 2005; Rappaport and Kupper, 2008). Thus, by selecting from the many possible measures of exposure to styrene, i.e., StyA and the various urinary biomarkers, the exposure measure with the smallest variance ratio λ should minimize the potential bias in an exposure–response relationship due to exposure measurement error.

In a recently published study we focused on an integrated approach to investigate urinary analytes and haemoglobin and albumin adducts as biomarkers of exposure to airborne styrene (StyA) and styrene-(7,8)-oxide (StyOX), and to evaluate the influence of smoking habit and genetic polymorphism of metabolic enzymes *GSTM1* and *GSTT1* on these biomarkers (Fustinoni et al., 2008). To accomplish this, we employed a repeated measurement sampling design in which StyA, as well as urine specimens, were repeatedly collected over a 6-week period in groups of varnish workers and reinforced plastic workers exposed to styrene. In the present study a subset of these data were further analyzed to estimate within-worker and between-worker variance components and the corresponding values of λ for several potential surrogates of styrene exposure, namely, StyU, M1 + M2, MA, PGA, PHG, and VP.

2. Materials and methods

2.1. Study population, design, air and biological sampling

Two groups of male subjects employed in a varnish production plant and in a fiberglass reinforced plastic industry located in Northern Italy were involved in the study. The study was conducted during the periodical health surveillance program and subjects were recruited with the help of the plant's occupational physician. They were informed about the aims and the protocol of the study and provided written consent to be included as human subjects.

All the subjects working in the production department entered the study for a total of 13 varnish workers and 8 plastic workers (Fustinoni et al., 2008). The study design aimed to collect 4 repeated personal air samples and end-shift urine samples over a period of 6 consecutive weeks. Since 3 varnish workers were absent during most of investigated days, they were excluded from the present analysis that finally included 10 varnish workers and 8 plastic workers.

Information about work activities, demographic and lifestyle factors, and medical histories were obtained using a questionnaire administered by an occupational physician (Table 1). Subjects were healthy, and none of them had history of liver or metabolic dysfunction, nor were chronically submitted to pharmacological treatment.

Varnish workers were involved in the production of styrene-containing varnishes as a continue process, while fiberglass reinforced plastic workers were involved in boat production using open mould in a discontinuous process. In both activities individual tasks were the same all over the investigated period. Workers in the same plant were investigated on the same day, and for each day information on job task was collected.

Information about job title, sampling day, frequency of sampling, number of sampling for worker and interval between the repeated measurements is reported in Table 1.

Finally, following this design 38 air and urine samples from the varnish workers and 30 from the plastic workers for the determination of StyA and StyU, M1 + M2, MA, PGA, PHG and VP were collected.

Table 1
Selected characteristics of investigated subjects and details on sampling protocol.

	Varnish workers	Fiberglass reinforced plastic workers
Number of subjects	10	8
Age*	30 ± 5	41 ± 9
Smokers (%)	38	25
Number of cigarette/day*	11 ± 2	20
Job title	6 producers 1 warehouse man 2 quality control technicians 1 supervisor	2 laminators 4 assemblers 2 finishers
Sampling day	Wednesday/Thursday	Tuesday/Wednesday
Number of samplings (sampling/subject)*	3.8 ± 0.4	3.7 ± 0.5
Sampling frequency (time/week)	0.7	0.7
Interval between the repeated measurement* (days)	12 ± 3.6	12 ± 3.5

* = mean ± SD

2.2. Analysis of air and biological samples

2.2.1. Airborne styrene

The air concentrations of StyA were determined as described by Tornero-Velez et al. (2000). The detection limit was 0.3 mg/m^3.

2.2.2. Unmetabolized styrene in urine

Levels of StyU were determined by headspace solid-phase microextraction (SPME) followed by GC–MS analysis as previously described by Fustinoni et al. (2008). The detection limit was 0.2 μg/L.

2.2.3. Styrene metabolites in urine

Urinary metabolites were assayed by liquid chromatography with *tandem* mass spectrometry (LC–MS/MS), as previously described (Manini et al., 2002). Limits of detection were 0.1 mg/L for both MA and PGA, 0.01 mg/L for PHG and 0.0004 mg/L for each mercapturic acid, 0.015 mg/L for VP-G and 0.005 mg/L for VP-S. Concentrations of metabolites in urine samples were expressed as a function of creatinine concentration, measured by the method of Jaffe. Creatinine levels ranging between 0.3 and 3.0 g/L were considered acceptable (WHO, 1996).

2.3. Estimation of variance components

Within-worker and between-worker variance components (σ^2_{wY} and σ^2_{bY}) were estimated by applying a one-way random effects (ANOVA) model to the data from each group of workers separately. Analyses were applied after natural logarithmic transformation of air or urinary measurements to achieve approximate normality and homogeneity of variance (Rappaport and Kupper, 2008). The following model was used:

$$Y_{ij} = \ln(X_{ij}) = \mu_y + b_i + e_{ij} \quad (1)$$

for $i = 1,2,\cdots, k$ persons and $j = 1, \cdots, n$ days, where X_{ij} represents the exposure level or the urinary biomarker for the ith person on the jth day. The mean and variance of Y_{ij} are designated as μ_Y and σ^2_Y, respectively. Under Model (1), μ_Y represents the true fixed mean (logged) exposure or biomarker level for the group; b_i represents the random effect of the ith person and e_{ij} represents the random deviation of the observed logged exposure level Y_{ij} on the jth day for person i from the subject-specific mean (logged) level μ_{Yi}. It is assumed that b_i and eij are mutually independent and normally distributed random variables, with means of zero and variance σ^2_{bY} and σ^2_{wY}, respectively. Thus, the total variability in the logged exposure levels experienced by a group is given by $\sigma^2_Y = \sigma^2_{bY} + \sigma^2_{wY}$. Also, $Y_{ij} = \ln(X_{ij})$ is normally distributed with mean μ_Y and variance σ^2_{bY}.

StyA and urinary biomarker data from the 10 varnish workers and the 8 plastic workers, with 4 nominal measurements per worker were fit in Model (1). Following Rappaport (1991), scale-independent measures of within-worker and between-worker variability, i.e., ${}_wR_{0.95} = \exp(3.92\sigma_{wY})$ and ${}_bR_{0.95} = \exp(3.92\sigma_{bY})$, were also estimated. Note that ${}_wR_{0.95}$ represents the fold range containing 95% of the X_{ij} values for the ith worker and ${}_bR_{0.95}$ represents the fold range containing 95% of the subject-specific mean exposure levels for the group of workers.

2.4. Bias in estimating exposure–response relationship

The variance ratio ($\lambda = \sigma^2_{wY}/\sigma^2_{bY}$) can be used to evaluate the potential that measurement error in a given exposure metric will lead to attenuation bias in an exposure–response relationship (Rappaport, 1991; Rappaport and Kupper, 2004; Liljelind et al., 2003; Lin et al., 2005). Assume an individual-based study design where the health outcome and exposure levels are measured in each member of a