ELSEVIER

Journal of Chromatography B, 757 (2001) 59–68

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Determination of styrene and styrene-7,8-oxide in human blood by gas chromatography–mass spectrometry

Rogelio Tornero-Velez[a], Suramya Waidyanatha[a], Hermes Licea Pérez[b], Siv Osterman-Golkar[b], Diana Echeverria[c], Stephen M. Rappaport[a,*]

[a]*Department of Environmental Sciences and Engineering, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7400, USA*

[b]*Department of Molecular Genome Research, Stockholm University, SE-106 91 Stockholm, Sweden*

[c]*Department of Environmental Health, University of Washington, Seattle, WA 98105-6044, USA*

Received 5 September 2000; received in revised form 21 November 2000; accepted 22 January 2001

Abstract

Methods of isotope-dilution gas chromatography–mass spectrometry (GC–MS) are described for the determination of styrene and styrene-7,8-oxide (SO) in blood. Styrene and SO were directly measured in pentane extracts of blood from 35 reinforced plastics workers exposed to 4.7–97 ppm styrene. Using positive ion chemical ionization, styrene could be detected at levels greater than 2.5 μg/l blood and SO at levels greater than 0.05 μg/l blood. An alternative method for measurement of SO employed reaction with valine followed by derivatization with pentafluorophenyl isothiocyanate and analysis via negative ion chemical ionization GC–MS–MS (SO detection limit=0.025 μg/l blood). The detection limits for SO by these two methods were 10–20-fold lower than gas chromatographic assays reported earlier, based upon either electron impact MS or flame ionization detection. Excellent agreement between the two SO methods was observed for standard calibration curves while moderate to good agreement was observed among selected reinforced plastics workers ($n=10$). Levels of styrene in blood were found to be proportional to the corresponding air exposures to styrene, in line with other published relationships. Although levels of SO in blood, measured by the direct method, were significantly correlated with air levels of either styrene or SO among the reinforced plastics workers, blood concentrations were much lower than previously reported at a given exposure to styrene. The two assays for SO in blood appear to be unbiased and to have sufficient sensitivity and specificity for applications involving workers exposed to styrene and SO during the manufacture of reinforced plastics. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Styrene; Styrene-7,8-oxide

1. Introduction

Styrene is a major industrial chemical used in the production of plastics and resins. Once absorbed into the body, styrene is metabolized to styrene-7,8-oxide (SO) via cytochrome P-450 enzymes (reviewed in Refs. [1,2]). SO is genotoxic in mammalian cells in vitro (reviewed in Ref. [3]) and in vivo [4], produces forestomach tumors in rats and mice following administration by gavage [5–7], and has been classified as *probably* carcinogenic to humans by the International Agency for Research on Cancer (2A) [8]. For these reasons, concern has been expressed

*Corresponding author. Tel.: +1-919-9665-017; fax: +1-919-9664-711.

0378-4347/01/$ – see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0378-4347(01)00063-9

about the carcinogenic potential of styrene and SO in humans, particularly in the reinforced plastics industry where exposures are the highest [9]. Although more than 85% of the absorbed dose of styrene is metabolized to SO in humans [1], only a minor fraction of this metabolite reaches the systemic circulation because of efficient intrahepatic hydrolysis of SO to styrene glycol via epoxide hydrolases [9–12]. Styrene oxide is also present at low concentrations in the air of reinforced plastics factories (due to oxidation of styrene) and, upon inhalation, can be directly absorbed into the blood of persons in those facilities (reviewed in Ref. [13]).

Since SO is reactive (the half-life in human blood in vitro is 42 min [14]) and is present at very low concentrations in the systemic circulation, the usual methods for biomonitoring of styrene (e.g., measurement of the urinary metabolites mandelic acid and phenylglyoxylic acid [15–23]) provide no information about the disposition of SO in the body. Furthermore, accurate measurement of SO in blood has been problematic. SO has been measured directly in extracts of blood by gas chromatography (GC) with either flame ionization detection (FID) [24] or electron-impact mass spectrometry (EI-MS) [25–27]. Alternatively, SO has been extracted from blood and hydrolyzed to styrene glycol (SG) followed by derivatization and GC with electron-capture detection [28–30]. While direct measurement of SO is preferable to hydrolysis and derivatization of SG, due to interference from SG arising from metabolism of SO in vivo, the sensitivities of FID and EI-MS are only marginal for this purpose. Indeed, using GC–EI-MS, Langvardt and Nolan [26] and Morgan et al. [27] reported detection limits for SO of 10 μg/l and 3 μg/l, respectively. Interestingly, Kessler et al. [24] reported a lower detection limit of 1 μg/l for SO in blood based upon GC–FID, which was achieved by injecting up to 25 μl of *n*-hexane extracts on-column according to the procedure of Grob, Jr. et al. [31].

Given our interest in estimating blood levels of SO below 1 μg/l among workers exposed to styrene and SO in the reinforced-plastics industry, we developed a direct assay for SO in blood via GC–MS employing positive ion chemical ionization (PCI). We also measured SO in selected blood samples with an alternative assay, based upon reaction of SO with valine followed by derivatization with pentafluorophenyl isothiocyanate and analysis of the products via GC–MS–MS employing negative ion chemical ionization (NCI). This latter assay is a straightforward modification of a procedure for measuring SO-adducts bound to hemoglobin at the N-terminus, which involves a valine residue [30]. In what follows, we will describe both methods and their preliminary application with samples of blood from reinforced-plastics workers. For simplicity, we will henceforth refer to the PCI-MS assay of SO in blood as the ‘‘direct method’’ while the derivatization–GC–MS–MS assay will be referred to as the ‘‘valine method’’.

2. Experimental

2.1. Chemicals

Styrene (99+%), L-valine (99%) and triethylamine (99%) were obtained from Aldrich (Milwaukee, WI, USA). Ethyl acetate (99+%) and pentane were obtained from Fisher Scientific (Pittsburgh, PA, USA). SO (97%), acetophenone (99%) and phenylacetaldehyde (95%, w/w, in diethyl phthalate) were obtained from Fluka (Buchs, Switzerland). $[^2H_8]$SO was kindly provided by Drs. Hendrik Veulemans and Wim Pauwels, synthesized according to the method described in Severi et al. [32]. $[^2H_8]$Styrene (98%) was obtained from Cambridge Isotope Labs. (Andover, MA, USA). Pentafluorophenyl isothiocyanate (PFPITC) (>97%) was obtained from Fluka and purified according to Törnqvist et al. [33].

2.2. Reinforced-plastics workers

Workers were recruited from four reinforced-plastics facilities. The direct method for measuring SO in blood was applied to 35 workers in a single factory manufacturing parts for recreational vehicles and shower stalls. The direct and valine methods were compared by applying both methods to different groups of 10 workers from three facilities involved in the manufacture of boats.

2.3. Exposure to styrene and SO

Styrene and SO were measured by personal sampling according to the method of Tornero-Velez et al. [13].

Briefly, passive monitors (catalog No. 3500; 3M, St Paul, MN, USA) were used to estimate individual shift-long exposures to styrene and SO. After sampling, the devices were capped and stored for up to 24 h, then desorbed with 1.5 ml of ethyl acetate. The solution was decanted into a 4-ml glass vial, which was sealed with a PTFE-lined cap and stored at −20°C for up to 1 month before analysis. Analysis involved injection of 1 μl of the solution into a GC system equipped with either an FID (styrene) or an EI-MS detection system (SO). Analytes were corrected for blanks (at least one unexposed passive monitor per five exposed) and for desorption efficiency as described in Tornero-Velez et al. [13].

2.4. Collection and extraction of blood

Blood sampling was conducted by venous puncture into heparinized tubes among workers at the end of the work-shift (blood was collected within a few minutes of leaving the work area) and among volunteer control subjects as needed. For the workers the mean interval from blood collection to extraction was 4.15 min (SE=0.096 min, n=35). Immediately after collection, a 2-ml aliquot of blood was transferred to an 8-ml vial containing 4 ml of n-pentane and an internal standard consisting of 1 ng of [$^{2}H_{8}$]SO and 2 μg of [$^{2}H_{8}$]styrene. The vial was sealed with a PTFE-lined screw cap and the contents were mixed with a vortex mixer for 30 s. The phases were separated by centrifugation at 800 g for 5 min and the pentane extract was removed for analysis. Pentane extracts were stored in 8-ml vials with PTFE-lined screw caps at −80°C for up to 1 month before analysis by PCI-MS and up to 6 months prior to analysis via the valine method. Analytical standards stored at −80°C showed no apparent decrease in concentration when reanalyzed after several months. Immediately prior to analysis, the extract was concentrated to 25 μl under a gentle stream of nitrogen and transferred to a 200-μl conical vial insert along with 25 μl of ethyl acetate (50 μl final volume) to reduce the volatility.

2.5. Extraction efficiency of SO by n-pentane

The efficiency of the above extraction procedure was evaluated by extracting three 2-ml portions of human blood (pooled from three control subjects) containing 10 ng each of SO and [$^{2}H_{8}$]SO (50 μl of a 200 ng/ml solution of each analyte in ethyl acetate). Following centrifugation, 50 μg of styrene (internal standard) was added to 3.5 ml of the recovered pentane extract. Extraction efficiency was calculated by comparing the peak area ratios (SO:styrene and [$^{2}H_{8}$]SO:styrene) in the recovered extracts to the corresponding peak area ratios of a standard solution, prepared by adding the analytes directly to n-pentane.

2.6. Direct measurement of styrene and SO by GC–PCI-MS

Styrene and SO in pentane extracts of blood were analyzed by GC–PCI-MS (3 μl injected in splitless mode) using a Hewlett-Packard 5890 series II gas chromatograph coupled to a Hewlett-Packard 5989A MS engine. A DB-1, 30 m×0.25 mm fused-silica column (0.25 μm film thickness) (J & W Scientific, Folsom, CA, USA) was used. The carrier gas was He at a flow-rate of 1.5 ml/min. The injection port and source temperatures were 70 and 150°C, respectively. The oven temperature was held at 50°C for 1 min and then increased at 10°C/min to 160°C. Late eluting compounds were removed by increasing the oven temperature at 50°C/min to 250°C where it was held for 5 min. Methane was used as the chemical ionization reagent gas (1.7 Torr, source pressure; 1 Torr=133.322 Pa). The mass spectrometer was operated in selected ion monitoring mode and was focused at m/z 105 (styrene) and m/z 113 [$^{2}H_{8}$]styrene) for the first 7 min of analysis, then focused at m/z 121 (SO) and m/z 129 [$^{2}H_{8}$]SO) for the remainder of the analysis. Approximate retention times of styrene and SO were 5.5 and 9.2 min, respectively, with the isotopically labeled analogs appearing slightly earlier.

Standards were prepared by the addition of SO (0.25 to 1 ng) and styrene (0.05 to 2 μg) to 4 ml n-pentane containing 1 ng of ([$^{2}H_{8}$]SO) and 2 μg of [$^{2}H_{8}$]styrene. Solutions were concentrated under a gentle stream of nitrogen to 25 μl, and transferred to a conical vial insert, to which 25 μl of ethyl acetate was added. The limits of detection (LODs), defined as three times the peak-to-peak background noise, were 0.2 ng for styrene (2.5 μg/ml blood) and 4 pg (0.05 μg/ml blood) for SO.

2.7. Determination of SO by adduction with valine

The valine method was applied to selected samples of the final pentane extracts described above. The 200-μl conical vial insert containing this extract (50 μl) was sealed inside a 2-ml vial (Hewlett-Packard) containing 1 ml of 0.22 *M* valine and 0.22 *M* triethylamine. After inverting the vial to mix the contents, the vial was shaken vigorously for 1 min and maintained at 36°C for 20 h. The contents were then transferred to an 8-ml vial along with 0.5 ml of 1-propanol. To this solution was added 50 μl of PFPITC, and the contents were mechanically shaken for 15 h at 25°C followed by 2 h at 36°C. The reaction mixture was extracted three times with 3 ml of diethyl ether and ether was evaporated under nitrogen at 50°C. The residue was dissolved in 2 ml of toluene, washed once with water (2 ml), twice with 0.1 *M* Na_2CO_3 (2 ml each), and then twice with water (2 ml each). The toluene was evaporated under nitrogen at 50°C and the residue was reconstituted in 1 ml of 80% aqueous methanol and washed with 1 ml of hexane. A 1-ml volume of 40% methanol was added to this solution to bring it to a final concentration of 60% methanol. The final solution of pentafluorophenyl thiohydantoin derivatives was then extracted twice with 2-ml aliquots of hexane, and hexane was evaporated under nitrogen at 50°C. The residue was dissolved in 50 μl of toluene and analyzed by GC–MS–MS as described below.

GC–MS–MS analysis was performed on a ThermoQuest Trace 2000 series GC (San Jose, CA, USA) coupled with a ThermoQuest TSQ-7000 triple stage quadrupole (TSQ) mass spectrometer. Aliquots of 2 μl were injected in splitless mode onto a fused-silica capillary column (EC-5, 30 m×0.32 mm fused-silica column, 1 μm film thickness; Alltech Associates, Deerfield, IL, USA). The column temperature was initially held at 100°C for 1 min, then programmed to 300°C at a rate of 15°C/min, with a final hold time of 4 min. The injector temperature was 275°C, the ion source was 150°C, the transfer line was 250°C and the manifold temperature was 60°C. Helium (99.99% purity) was used as the carrier gas. The mass spectrometer was used in the NCI mode with methane as the ionization gas at a pressure of 3.5–4.0 Torr and an applied collision energy of 12.5 V. The electron multiplier voltage for MS–MS was 1600 V, and the filament emission current was 300 μA. Collision induced dissociation (CID) was used for MS–MS with argon gas pressure at 2.0 mTorr. Reactions monitored by GC–MS–MS were 424→206 and 432→206 (deuterated analogs), where the fragment 206 corresponds to the reagent part (C_6F_5NCS) of the derivative.

Standards were prepared by the addition of SO in pentane (as per Section 2.6) and application of the valine method to the standards. The LOD for SO by the valine method was 2 pg corresponding to 0.025 μg/ml blood.

2.8. Quantification

The concentration of SO in blood was calculated by relating the ratio of peak areas of SO and [$^2H_8$]SO, or their corresponding valine adducts, to the respective ratio of a standard solution (prepared in pentane) or its valine adducts. (Since the valine adducts of SO had been converted to the diastereomers of the pentafluorophenylthiohydantoins of hydroxyphenethylvaline by reaction with pentafluorophenyl isothiocyanate (PFPITC), quantification was based on the sum of peak areas for the diastereomers from samples and standard solutions). Quantification of styrene in blood was similarly based upon the ratio of the peak areas of styrene and [$^2H_8$]styrene.

2.9. Statistical methods

Data were analyzed using the SAS statistical software V6.12 (SAS Institute, Cary, NC, USA). Except where indicated, logarithmic transformation (base e) was applied to the concentrations of styrene and SO in air and blood to remove obvious heteroscedasticity prior to analysis. Values below the LOD were excluded from regression analyses. Levels of SO in blood (direct method) were regressed upon the corresponding levels of SO or styrene in air via linear regression (PROC REG). Linear correlation of levels of SO in blood and the corresponding levels of SO or styrene in air was estimated with the sample correlation coefficient (PROC CORRELATION). The agreement between the direct and valine methods for measuring SO was determined as the intraclass correlation coefficient (ICC, determined as the

ratio of the between-method variance component to the sum of within- and between-method variance components from a one-way analysis of variance via PROC NESTED). The analytical precision for direct measurement of SO and styrene was estimated as the relative standard deviation (RSD), determined from the relationship $RSD=[\exp(s^2)-1]^{1/2}$, where s^2 represents the estimated error term from a one-way analysis of variance (performed with PROC NESTED) of (logged) duplicate injections of SO or styrene in blood from 27 exposed subjects [34]. The analytical precision for measurement of SO by the valine method was determined as the RSD of duplicate assays performed on standards of SO (and fixed amounts of $[^2H_8]$SO) in pentane ($n=16$).

3. Results and discussion

3.1. Extraction efficiency of SO

Traces of SO contamination were detected in all batches of *n*-pentane and *n*-hexane tested in preliminary experiments with the direct method. The presence of SO contamination was subsequently confirmed in selected batches of *n*-pentane by the valine method. Pentane was chosen as the extraction solvent because it contained lower and more consistent background levels of SO than *n*-hexane, yet showed comparable extraction efficiency. We also encountered fewer emulsions of the blood extracts when using *n*-pentane rather than *n*-hexane at a blood–solvent ratio of 1:2. The *n*-pentane extraction efficiencies of SO and $[^2H_8]$SO from blood were 68% (SE=1.6%) and 71% (SE=1.3%), respectively, at a concentration of 2.5 ng/ml, indicating no difference in extraction of the unsubstituted and deuterated analogs ($P=0.23$). Using *n*-hexane to extract SO from blood, Kessler et al. [24] reported extraction efficiencies ranging from 67 to 85%, with a mean of 75% (SE=4.0).

3.2. GC–MS (direct method)

Typical chromatograms for SO obtained by the direct method are shown in Fig. 1. The ion chromatograms depict blood samples from an unexposed subject (A) and an exposed subject (B), the latter containing an estimated 0.28 μg SO/l blood. Relative to the EI spectrum for SO [25] the PCI spectrum is simple, consisting exclusively of the $[M+1]^+$ ion (m/z 121). The detection limit corresponds to 0.1 ng of SO (0.05 μg/l for a 2-ml of blood) compared to 1.0 ng by GC–EI-MS (10 μg/l for 0.1 ml of blood) [26]. Although the lack of fragmentation enhances the sensitivity of PCI-MS relative to EI-MS, specificity suffers if co-eluting peaks share the same $[M+1]^+$ ion. In particular, acetophenone has the same molecular ion and coelutes with SO on some GC columns (e.g., DB-5). Thus, we employed a DB-1 column to resolve the peaks corresponding to SO and acetophenone.

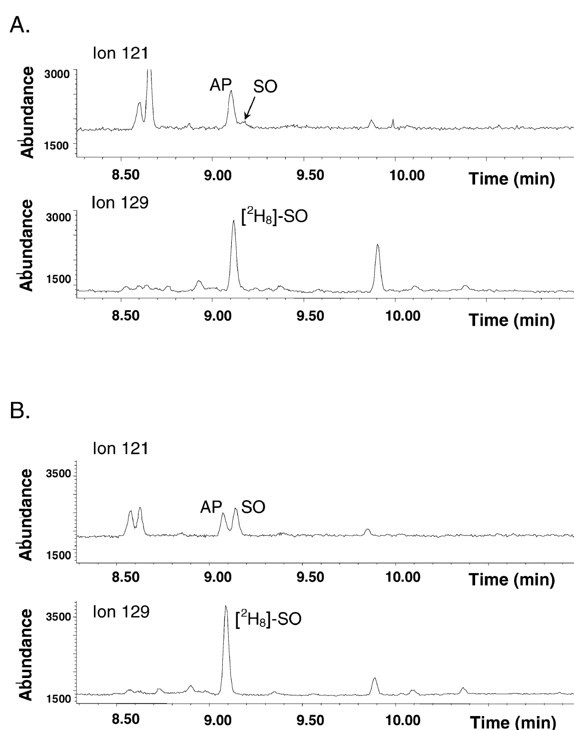

Fig. 1. Selected ion chromatograms (m/z 121 and m/z 129) for the direct determination of styrene-7,8-oxide (SO) in human blood. (A) Blood from a control subject containing a trace level of SO (exposed 9 h to TWA concentrations of <1 ppm styrene and <1 ppb SO; the small amount of SO indicated in this extract was also present in reagent blanks) and (B) blood from an exposed subject containing an estimated 0.28 μg/l SO (exposed 9 h to TWA concentrations of 34 ppm styrene and 14 ppb SO). (Note: AP refers to acetophenone).

3.3. GC–MS–MS (valine method)

The valine method involved the generation of hydroxyphenethylvaline (and its deuterated analog) by the reaction of valine with SO (and $[^2H_8]$SO). Fig. 2 shows the pentafluorophenylthiohydantoin (M_r=444) resulting from reaction of the derivatization reagent, PFPITC, and hydroxyphenethylvaline, and the corresponding deuterated pentafluorophenylthiohydantoin (M_r=452). The MS–MS method utilized a specific parent ion for each pentafluorophenylthiohydantoin showing the greatest abundance in the single-MS spectrum. In the single-MS spectrum these ions were m/z 424 and 432, representing $[M-HF]^-$ arising from the reaction products of valine with SO and its deuterated analog, respectively. The daughter ion used for MS–MS analysis (m/z 206) was the most intense of the CID spectrum. Typical chromatograms for SO obtained by the valine method are shown in Fig. 3. The ion chromatograms obtained in GC–MS–MS depict blood samples from an unexposed subject (A) and an exposed subject (B) containing an estimated 0.25 μg SO/l.

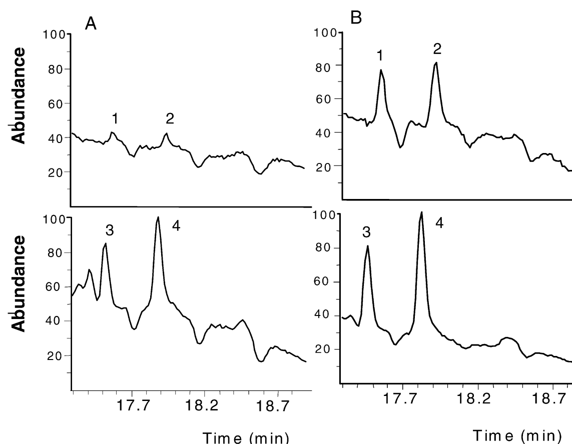

Fig. 3. GC–MS–MS daughter ion chromatograms (m/z 206) of pentafluorophenylthiohydantoins of hydroxyphenethylvaline (diastereomers 1 and 2), and deuterated analogs (diastereomers 3 and 4). (A) Blood from a control subject containing non-detectable levels of SO (exposed 9 h to TWA concentrations of <1 ppm styrene and <1 ppb SO; the small amount of SO indicated in this extract was also present in reagent blanks) and (B) blood from an exposed subject containing an estimated 0.25 μg/l SO (exposed 9 h to TWA concentrations of 57 ppm styrene and 75 ppb SO). [Note: time scales apply to top (1, 2) and bottom (3, 4) ion traces].

3.4. Bias and precision

The use of isotopically labeled analogs as internal standards ($[^2H_8]$SO and $[^2H_8]$styrene) provided an intuitively accurate means for assaying SO and styrene via the direct method. That is, because the chemical and physical properties of SO and $[^2H_8]$SO are nearly identical, any losses occurring during the extraction and concentration procedures (including reactions with blood nucleophiles) should be controlled for.

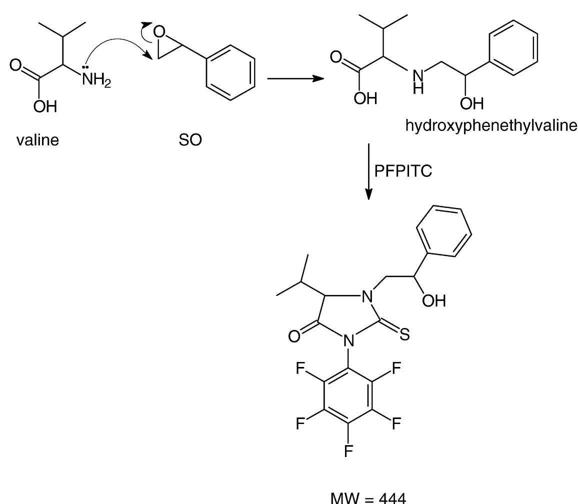

Fig. 2. Reaction scheme to show formation of the pentafluorophenylthiohydantoin of hydroxyphenethylvaline (M_r=444).

Possible bias was also determined by evaluating the agreement of measurements obtained from the direct and valine methods for SO. Fig. 4 shows the relationship between pairs of measurements of SO obtained by the two methods for a series of analytical standards. The agreement is excellent as indicated by the ICC of 0.987 (95% C.I.: 0.912, 0.999). Fig. 5 shows a comparison of these two methods applied to pentane extracts selected from 10 reinforced-plastics workers. Although the mean levels of SO determined by the two methods were not significantly different (P=0.46, paired t-test), the agreement between the methods was marginal (ICC=0.606, 95% C.I.: 0.094, 0.859), primarily due to one highly discordant pair of observations (0.047, 0.317); without this pair (n=9), the agreement would be good (ICC=0.904, 95% C.I.: 0.675, 0.972). The

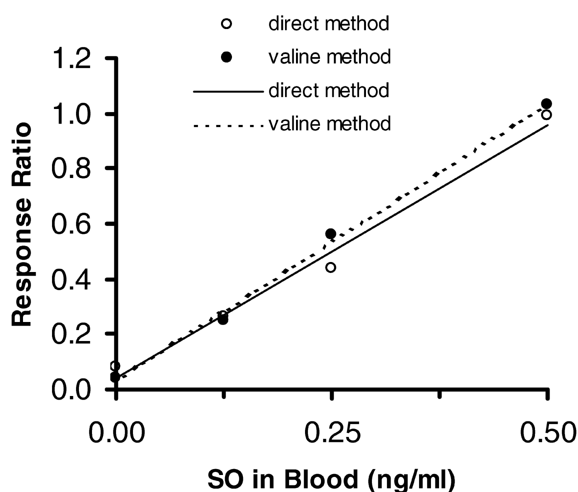

Fig. 4. Relationship between pairs of measurements of SO in blood obtained by the direct and valine methods for a series of analytical standards.

random pattern of the data pairs about the 45° line of strict equality suggests that both methods tend to converge on a true underlying blood concentration.

The precision of the direct method for measurement of SO and styrene was characterized in terms of the RSDs from duplicate injections of the 27 non-zero field samples. These RSDs were 10.4% for SO (0.05 to 0.5 μg/l) and 3.4% for styrene (0.05 to 2.0 mg/l) (n=27). Precision of the valine method was estimated to be 3.8% from duplicate assay of standards of SO (0.00 to 0.5 μg/l), pooled from three sets of standard curves (n=16).

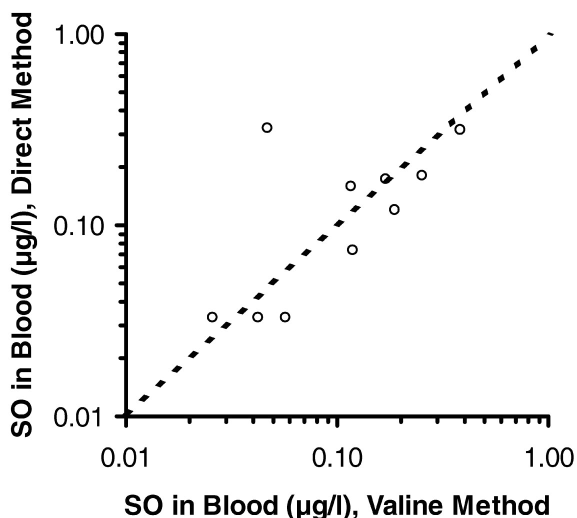

Fig. 5. Application of direct and valine methods to pentane extracts from 10 subjects exposed to a range of air levels of styrene and SO in the reinforced plastics industry.

3.5. Styrene in blood vs. styrene in air

Fig. 6 shows the relationship between blood styrene concentrations found at the end of the work-shift and the full-shift styrene exposures. Linear regression of the logged values yielded the relationship: ln[blood−styrene (mg/l)]=−4.35+ 0.97 ln-[styrene (ppm)] (n=35; r=0.89). The slope (log space) of 0.97 (95% C.I.: 0.79, 1.15) indicates that levels of styrene in blood were virtually proportional to the corresponding air concentrations and that the background levels of styrene were not significant in unexposed subjects. Hence, between 1 ppm and 100 ppm the predicted proportionality constant (natural space, μg/l blood−styrene per ppm−styrene) marginally decreases from 12.9 to 11.2 μg/l per ppm. At an exposure of 50 ppm, the proportionality constant (11.5 μg/l per ppm) agrees well with those reported in several studies (i.e., 11: Ramsey et al. [35]; 20: Bartolucci et al. [36]; 12.5: Zinser et al. [37]; 9.9–14.7 Brugnone et al. [38]). From this proportionality constant we predict that a styrene exposure of 50 ppm would result in 0.57 mg styrene/l of blood at the end of the work shift, consistent with the biological exposure index (BEI) for styrene of

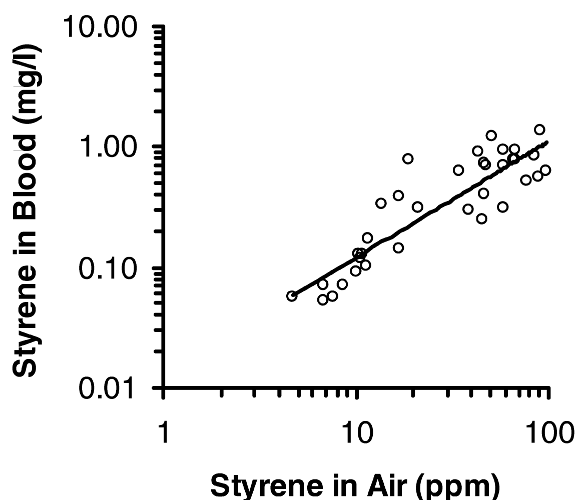

Fig. 6. Relationship between styrene in blood (mg/l) of reinforced plastics workers and daily exposures to styrene (ppm).

0.55 mg/l of the American Conference of Governmental Industrial Hygienist [39].

3.6. SO in blood vs. styrene and SO in air

Fig. 7 shows the relationships between SO in blood (direct method) and the corresponding exposures to styrene (A) and SO (B) in air among the 27 reinforced-plastics workers having SO exposures above the limit of detection (0.05 μg/l). The plot shows that the (logged) SO-blood levels tended to increase linearly with (logged) exposure to either styrene (A), where ln[blood−SO (μg/l)]=−3.23+0.415 ln[styrene (ppm)] (r=0.73, styrene exposures between 4.7 and 97 ppm), or to SO (B), where ln[blood−SO (μg/l)]=−3.27+0.406 ln[SO (ppb)] (r=0.62, SO exposures between 5.7 to 119 ppb). Because the (logged) exposures to styrene and SO were highly correlated in this sample of workers (r=0.93) independent effects of the two agents upon blood levels of SO were not evaluated. However, based upon previous work with albumin adducts of SO among reinforced plastics workers, we suspect that exposure to both styrene and SO contributed significantly to the levels of SO in the blood [9,40]. Although levels of styrene tend to be about 1000 times those of SO in the reinforced plastics industry [13,41,42], the systemic bio-availability of SO arising from inhalation of SO has been estimated to be 2000 times that from styrene [9].

In a similar study, Korn et al. [11] reported the relationship between SO in blood and exposure to styrene (10 to 73 ppm), among 13 reinforced-plastics workers. From least-squares regression (natural space), they reported a slope of 0.058 (SE=0.0011) μg SO/l per ppm styrene (r=0.88). Hence, for a styrene exposure of 50 ppm Korn et al. [11] would predict an end-of-shift concentration of 2.9 μg SO/l blood, which is 14.5 times that predicted from our data (0.2 μg SO/l blood). This disparity in results from the two studies is difficult to explain. However, since Korn et al. [11] did not measure airborne SO, it is possible that extremely high co-exposures to SO among their subjects could have played a role. Certainly, factors related to the particular resin system can significantly affect the amount of SO emitted during production of reinforced plastics [42].

Also, the analytical procedures were quite different in the two investigations since Korn et al. [11] used GC–FID and we employed GC–PCI-MS. The GC–FID procedure was based on the method of Kessler et al. [24] who reported a detection limit of 1 μg/l blood and reasonable precision in assays of SO in rodents when dosed with styrene (SO=20 μg/l;

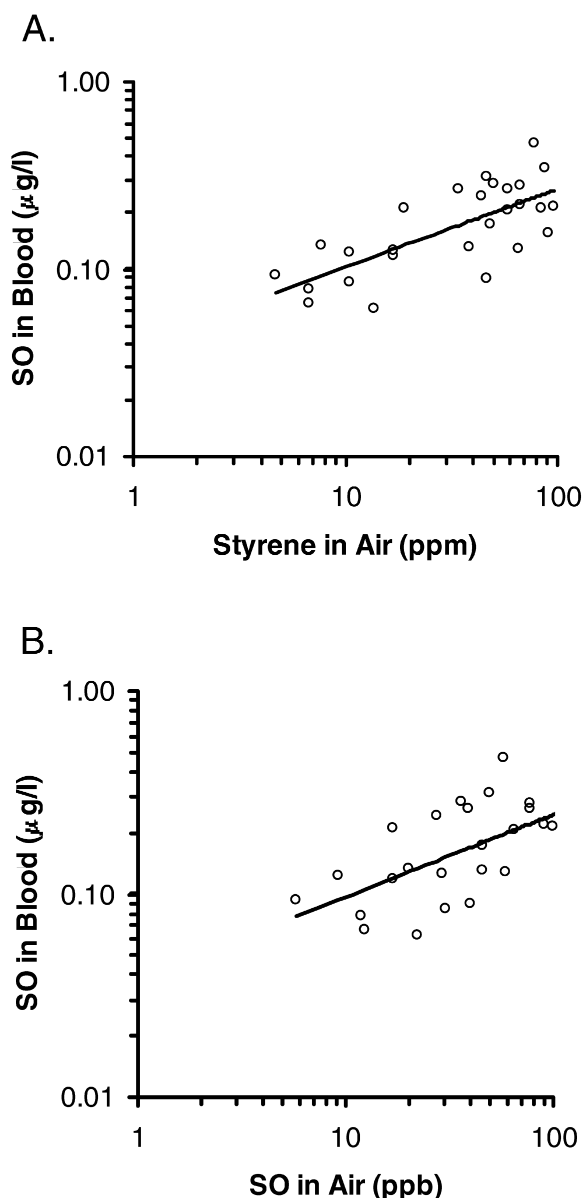

Fig. 7. Relationship between SO in blood of reinforced plastics workers (μg/l) and daily exposure to (A) styrene (ppm) and (B) SO (ppb).

RSD=10.1%, n=8). However, in recent applications of this method Cruzan et al. [43] were unable to detect SO in rat blood below 10 μg/l and reported extremely variable results in measurements of mouse blood below about 3 μg/l (RSD=128–185% for mice with estimated mean blood levels of 2.5 and 1.4 μg/l, respectively) [44]. This suggests marginal sensitivity of the GC–FID method for measurement of SO in blood at levels below about 5 μg/l, where most human samples would be expected.

Finally, since SO is a reactive compound, losses due to reactions with blood nucleophiles prior to extraction should be considered. In our study, the mean interval from blood collection to extraction was 4.15 min. Assuming first-order loss of SO due to reactions in the blood with a half time of 42 min [14] we estimate an average loss of 6.7% from our samples. Obviously, this can account for only a minor portion of the disparity in SO levels between our results and those of Korn et al. [11].

3.7. Direct vs. valine methods

Overall, we regard the measurement of SO via the direct and valine methods to be sufficiently sensitive, precise and unbiased to allow monitoring of levels of SO in the blood of humans and animals exposed to styrene or SO. The two methods provided generally comparable results, although the (NCI-based) valine method demonstrated higher sensitivity (LOD=0.025 μg/l) than the (PCI-based) direct method (LOD=0.050 μg/l). The valine method was also more precise (RSD=3.8%, based upon duplicate standard solutions) than the direct method (RSD=10.4% based upon duplicate injections). For both methods, quantification was limited by adjustment for residual contamination of SO in pentane, which was typically in the range of 0.025 to 0.075 μg/l (see representative chromatograms in Figs. 1 and 3). Since the direct method is simpler and requires less sophisticated instrumentation, it is probably the preferred assay for measurements of SO in blood.

4. Nomenclature

BEI	Biological exposure index
CID	Collision induced dissociation
EI	Electron-impact
FID	Flame ionization detection
GC	Gas chromatography
ICC	Intra-class correlation coefficient
LOD	Limit of detection
MS	Mass spectrometry
NCI	Negative ion chemical ionization
PCI	Positive ion chemical ionization
PFPITC	Pentafluorophenyl isothiocyanate
RSD	Relative standard deviation
SG	Styrene glycol
SO	Styrene-7,8-oxide
TSQ	Triple stage quadrupole
TWA	Time-weighted average

Acknowledgements

We are indebted to Laura Denovan, Erica Swanson and Mark Voightsberger who collected air samples and blood samples from the exposed subjects. We are also grateful to Asoka Ranasinghe for skillful technical assistance with GC–MS–MS. This work was supported by grant R01CA69463 from the National Cancer Institute and through training grant T32ES07018 of the National Institute of Environmental Health Sciences. Financial support for the valine method was obtained from the Swedish Council for Work Life Research.

References

[1] J.A. Bond, Crit. Rev. Toxicol. 19 (1989) 227.
[2] S.J. Sumner, T.R. Fennell, Crit. Rev. Toxicol. Suppl. 24 (1994) 11.
[3] D.H. Phillips, P.B. Farmer, Crit. Rev. Toxicol. Suppl. 24 (1994) 35.
[4] S. Bonassi, F. Montanaro, M. Ceppi, A. Abbondandolo, Biomarkers 1 (1996) 217.
[5] B. Conti, C. Maltoni, G. Perino, A. Ciliberti, Ann. NY Acad. Sci. 534 (1988) 203.
[6] V. Ponomarkov, J.R. Cabral, J. Wahrendorf, D. Galendo, Cancer Lett. 24 (1984) 95.
[7] W. Lijinsky, J. Natl. Cancer Inst. 77 (1986) 471.
[8] International Agency for Research on Cancer, in: Monographs on the Evaluation of Carcinogenic Risks to Humans, Some Industrial Chemicals No. 60, IARC, Lyon, 1994, p. 321.
[9] S.M. Rappaport, K. Yeowell-O'Connell, W. Bodell, J.W. Yager, E. Symanski, Cancer Res. 56 (1996) 5410.

[10] G.A. Csanady, A.L. Mendrala, R.J. Nolan, J.G. Filser, Arch. Toxicol. 68 (1994) 143.
[11] M. Korn, W. Gfrorer, J.G. Filser, W. Kessler, Arch. Toxicol. 68 (1994) 524.
[12] A.L. Mendrala, P.W. Langvardt, K.D. Nitschke, J.F. Quast, R.J. Nolan, Arch. Toxicol. 67 (1993) 18.
[13] R. Tornero-Velez, S. Waidyanatha, D. Echeverria, S.M. Rappaport, J. Environ. Monitor. 2 (2000) 111.
[14] K. Yeowell-O'Connell, W. Pauwels, M. Severi, Z. Jin, M.R. Walker, S.M. Rappaport, H. Veulemans, Chem. Biol. Interact. 106 (1997) 67.
[15] P. Apostoli, F. Brugnone, L. Perbellini, V. Cocheo, M.L. Bellomo, R. Silvestri, Am. J. Ind. Med. 4 (1983) 741.
[16] M. Korn, R. Wodarz, W. Schoknecht, H. Weichardt, E. Bayer, Arch. Toxicol. 55 (1984) 59.
[17] L. Perbellini, L. Romeo, G. Maranelli, G. Zardini, C. Alexopoulos, F. Brugnone, Med. Lav. 81 (1990) 382.
[18] M. Korn, W. Gfrorer, R. Herz, I. Wodarz, R. Wodarz, Int. Arch. Occup. Environ. Health 64 (1992) 75.
[19] E. De Rosa, M. Cellini, G. Sessa, C. Saletti, G. Rausa, G. Marcuzzo, G.B. Bartolucci, Int. Arch. Occup. Environ. Health Suppl. 65 (1993) 107.
[20] D. Marhuenda, M.J. Prieto, J.F. Periago, J. Marti, L. Perbellini, A. Cardona, Int. Arch. Occup. Environ. Health 69 (1997) 455.
[21] P. Apostoli, G. Alessandro, D. Placidi, L. Alessio, Int. Arch. Occup. Environ. Health 71 (1998) 445.
[22] H. Brygiert, J. Adamski, B. Buszewski, Med. Pr. 49 (1998) 439.
[23] S. Kezic, I. Jakasa, M. Wenker, J. Chromatogr. B 738 (2000) 39.
[24] W. Kessler, X.L. Jiang, J.G. Filser, J. Chromatogr. 534 (1990) 67.
[25] F. Bidoli, L. Airoldi, C. Pantarotto, J. Chromatogr. 196 (1980) 314.
[26] P.W. Langvardt, R.J. Nolan, J. Chromatogr. 567 (1991) 93.
[27] D.L. Morgan, J.F. Mahler, J.A. Dill, H.C. Price, R.W. O'Conner, B. Adkins, Fundam. Appl. Toxicol. 21 (1993) 317.
[28] E. Wigaeus, A. Löf, R. Bjurstrom, M.B. Nordqvist, Scand. J. Work Environ. Health 9 (1983) 479.
[29] A. Löf, E. Lundgren, E.M. Nydahl, M.B. Nordqvist, Scand. J. Work Environ. Health 12 (1986) 70.
[30] A. Christakopoulos, E. Bergmark, V. Zorcec, H. Norppa, J. Maki-Paakkanen, S. Osterman-Golkar, Scand. J. Work Environ. Health 19 (1993) 255.
[31] K. Grob Jr., G. Karrer, M.L. Riekkola, J. Chromatogr. 334 (1985) 129.
[32] M. Severi, W. Pauwels, P. Van Hummelen, D. Roosels, M. Kirsch-Volders, H. Veulemans, Scand. J. Work Environ. Health 20 (1994) 451.
[33] M.A. Törnqvist, A. Kautiainen, R.N. Gatz, L. Ehrenberg, J. Appl. Toxicol. 8 (1988) 159.
[34] S.M. Rappaport, Ann. Occup. Hyg. 35 (1991) 61.
[35] J.C. Ramsey, J.D. Young, R.J. Karbowski, M.B. Chenoweth, L.P. McCarty, W.H. Braun, Toxicol. Appl. Pharmacol. 53 (1980) 54.
[36] G.B. Bartolucci, E. De Rosa, G.P. Gori, C. Corona, L. Perbellini, F. Brugnone, Appl. Ind. Hyg. 3 (1986) 125.
[37] D. Zinser, P.M. Bittighofer, T.M. Fliedner, in: E. Baumgartner, W. Brenner, M.P. Dierich, J. Rutenfranz (Eds.), Verhandlungen der Deutschen Gesellschaft für Arbeitsmedizen e.V. Gemeinsam mit der Österreichen Gesellschaft für Arbeitsmedizen, Jahrestagung 1988, Innsbruck, Gentner Verlag, Stuttgart, 1988, p. 473.
[38] F. Brugnone, L. Perbellini, G.Z. Wang, G. Maranelli, E. Raineri, E. De Rosa, C. Saletti, C. Soave, L. Romeo, Int. Arch. Occup. Environ. Health 65 (1993) 125.
[39] ACGIH, Threshold Limit Values and Other Occupational Exposure Values, American Conference of Governmental Industrial Hygienist, Cincinnati, OH, 2000.
[40] K. Yeowell-O'Connell, Z. Jin, S.M. Rappaport, Cancer Epidemiol. Biomarkers Prev. 5 (1996) 205.
[41] P. Pfäffli, A. Säämanen, in: M. Sorsa (Ed.), The Occupational Scene of Styrene in Butadiene and Styrene: Assesment of Health Hazards, IARC Sci. Publication No. 127, IARC, Lyon, 1993, p. 15.
[42] L.A. Nylander-French, L.L. Kupper, S.M. Rappaport, Ann. Occup. Hyg. 43 (1999) 99.
[43] G. Cruzan, J.R. Cushman, L.S. Andrews, G.C. Granville, K.A. Johnson, C.J. Hardy, D.W. Coombs, P.A. Mullins, W.R. Brown, Toxicol. Sci. 46 (1998) 266.
[44] G. Cruzan, J.R. Cushman, L.R. Andrews, G.C. Granville, K.A. Johnson, C. Bevan, C.J. Hardy, D.W. Coombs, P.A. Mullins, W.R. Brown, submitted for publication.