V. RECOGNITION OF THE HAZARD

Environmental Sampling and Analytical Methods

The following discussion about the methods of sampling and analysis of styrene briefly describes various methods that have been used or proposed. While the recommended methods of sampling and analysis will usually be the most useful, local factors, e.g., availability of equipment, knowledge of usual concentrations, confounding variables, exposure patterns, etc. may make another method desirable.

(a) Sampling Methods

(1) Absorption Methods

Airborne styrene has been absorbed into liquids in glass impingers or bubblers. The liquid may be inert to styrene [35,236,237,238,239, 240], may react, or may contain solutes that react with styrene to form suitable derivatives [241,242,243,244]. Cooling has sometimes been employed to minimize evaporation losses and increase trapping efficiency [98,107,236,238,242]. Liquids that have been used to collect styrene from air include ethanol [35,236,237,241,245], isopropanol [107], methanol [98], glacial acetic acid [238], isooctane [240], concentrated sulfuric acid [246], dilute sulfuric acid [243], cold carbon tetrachloride [98], and nitric acid in sulfuric acid [242,244]. Some of these liquids are corrosive or otherwise toxic, so their use in a manner that might result in contact with workers (e.g., from broken impingers) is undesirable. Also, liquid impingers should be avoided when possible because they may impose restrictions on a worker's activities to avoid spillage.

(2) Adsorption Methods

Styrene has been adsorbed onto the surface of solid sorbents such as silica gel [107,238,245,247,248], porous polymers [249,250], and activated carbon [249,251,252,253,254,255,256,257].

In 1965, silica gel, as a collection medium for styrene, was evaluated by Van Mourik [107] during industrial hygiene surveys. By collecting duplicate field samples over a 4-year period at airborne styrene concentrations averaging 20 ppm, Van Mourik [107] found that the relative standard deviation of duplicate samples was 4.6%. Styrene was desorbed by isopropyl alcohol. To investigate the stability of adsorbed styrene, duplicate samples were collected in midget impingers containing isopropyl alcohol; results with silica gel were always higher. Van Mourik [107] concluded that silica gel collected styrene efficiently and that the adsorbed styrene was stable. In 1966, Campbell and Ide [245] obtained comparable recoveries of styrene from both silica gel and an ethanol bubbler over the range of 10-200 ppm.

Styrene adsorbed on porous polymers or charcoal can be removed by heating [249] and can be conveyed directly into a gas chromatograph, thereby avoiding dilution of styrene with a solvent. In 1976, Parkes et al. [249] found a relative standard deviation of 4.3% from the analysis of samples collected from air containing 11.8 ppm of styrene; the total amount of styrene collected was 5 µg. By removing styrene from activated charcoal with heat, they found a lower detection limit of 0.2 ppb in 10-liter air samples. Extrapolating this detection limit to styrene dissolved in 1 ml of carbon disulfide, Parkes et al. [249] estimated that the detection limit of styrene would be 40 ppb in a 10-liter air sample. This estimate does not consider possible problems that may be associated with recovery and solvent interference. A disadvantage of using thermal desorption is that the entire sample is usually used for a single analysis.

Charcoal as a collection media for styrene has been extensively studied [249,251,252,253,254,255,256,257,258,259]. In an initial study in 1966, Fraust and Hermann [251] obtained five replicate samples from air containing 100 ppm of styrene by sampling for 10 minutes at 200 ml/min. Each sample was analyzed in triplicate and a relative standard deviation of 7% was found. The investigators [251] found that when an atmosphere containing 100 ppm of styrene was sampled at 1 liter/min, the collection efficiency was 85%, but when the same atmosphere was sampled at 100 ml/min, the collection efficiency was only 30%. This rather unexpected variation in sampling efficiency with respect to sampling rate has not been confirmed by other investigators.

In 1978, using four charcoal tubes in series, each tube containing 350 mg of charcoal, and by sampling at 200 ml/min, Severs et al. [252] recovered 93-100% of styrene under varying relative humidities (23-80%), sample volumes (70-95 liters), and styrene concentrations (0.4-14 ppm). The smallest quantity of styrene collected during this study [252] was equivalent to sampling 10 liters of air at 4 ppm. This study demonstrates that styrene can be recovered in high yields from charcoal tubes if breakthrough can be avoided, indicating that desorption efficiency is adequate.

In 1977, Saalwaechter et al. [258] also studied styrene stability on charcoal tubes under varying conditions: temperature (-13 to 48°C), storage time (1-80 days), and light (19 days of sunlight, light from a sun lamp for 24 hours, or no light). The investigators [258] concluded that the stability of styrene on charcoal was not affected by temperature, light, or storage time. From their studies of the effect of humidity (7-94%) on the adsorption of toluene onto charcoal, it was inferred that high humidity may also adversely affect the adsorption of styrene onto charcoal. Saalwaechter et al. [258] also found that the efficiencies of desorbing styrene from charcoal with carbon disulfide were equivalent (85-89%) for charcoal obtained from three different manufacturers. Using an air flow of 1 liter/min, a relative humidity of 7%, and a styrene concentration of about 150 ppm, 1% breakthrough for the 100 mg front section occurred at 29.1 liters, at which time the front section of the charcoal tube held 18 mg of styrene.

Tubes containing charcoal have been designed for use in industrial hygiene surveys and are commercially available [253]. Such tubes were evaluated in 1976 by Burnett [253] at 50, 100, and 200 ppm of styrene by sampling these atmospheres at 1 liter/min. The mean percent recovery of styrene was 85%. These tubes contained two sections of charcoal; the main collecting section had 100 mg, and the reserve (backup) section, 50 mg.

In 1975, using tubes containing 200 mg of charcoal, Kalliokoski and Pfaffli [254] found that charcoal could collect 70 mg of styrene by sampling at 0.2-0.3 liters/min from a chamber containing 1,120 ppm of styrene. With charcoal tubes loaded with up to 66 mg of styrene, about 95% of the styrene remained on the charcoal after drawing fresh air through the tubes at this flow rate for 3 hours. Styrene was desorbed from the charcoal with dimethyl formamide; the desorption efficiency was 72.3%.

In 1972, after sampling air that contained 49, 494, or 987 ppm of styrene through 200-225 mg of charcoal at 0.2 liters/min for 1 hour, Gotell et al. [35] recovered 96%, 90%, and 87% of the styrene, respectively. Carbon disulfide was used to desorb styrene from the charcoal.

In 1977, NIOSH contractors evaluated data on the collection of styrene by charcoal tubes at 100, 200, and 400 ppm by sampling at 0.2 liters/min for 25 minutes [255]. Desorption efficiencies of 87% at 100 ppm, 88% at 200 ppm, and 93% at 400 ppm were found when carbon disulfide was used to desorb the styrene. At 400 ppm, sampling continued for 111 minutes before a significant amount of styrene (5%) passed through the charcoal, which then contained 36 mg of styrene.

Although the report by Fraust and Hermann [251] indicated that the collection efficiency of charcoal tubes was affected by the sampling rate, data from other studies [252,253,254,255,258] indicate satisfactory collection of styrene at all sampling rates studied. Passive dosimeters, which collect organic vapors through the mechanism of molecular diffusion and adsorption onto charcoal can also be used for monitoring styrene exposures [257].

(3) Cryogenic Methods

In 1967, Klyuzko and Vovyanko [247] used a liquid oxygen trap for sample collection to study styrene and ethylbenzene air pollution caused by the polystyrene industry. The frozen compounds were released directly into a gas chromatograph by heating the trap. This method, also discussed by Parkes et al. [249], did not seem to offer any advantages over other methods of collection for industrial hygiene use, but did create a potential hazard in the transport and use of liquid oxygen.

(4) Methods of Collecting Total Air Samples

The methods discussed above concentrate and remove styrene from air. Total air samples collected in syringes, pipets, bottles, or plastic

bags for analysis by a variety of methods do not concentrate styrene; these methods [69,243,260,261,262] usually sample small volumes of air over short periods of time. Because of the small sampling volumes and short sampling times, such samples may be better able to reflect periodic fluctuations in styrene concentrations than are samples collected by absorption, adsorption, or cryogenic methods. This may be desirable if the sampling times are carefully chosen [263]. Under certain conditions, however, glass surfaces may adsorb styrene [264] and some plastic bags are permeable to styrene [265]. Thus, care must be taken to ensure that all the styrene has been recovered from the collection device. This, however, may not be a problem below 100 ppm of styrene. In 1974, De Gesero [32] reported that styrene concentrations in air samples collected in plastic bags were stable for 4 hours. However, in 1978, Severs et al. [252] found that 20% of the sample in plastic bags had been lost after the same time period. Various materials may be useful as sampling bags, but the user should be aware of possible sample loss and evaluate the integrity of the sample in the bags.

(5) Sampling with Analytical Devices

Air samples may be drawn directly into analytical devices such as infrared (IR) analyzers, indicator solutions, indicator tubes, and combustible gas analyzers. These methods will be discussed in the following section and may be useful when immediate results are needed, automatic continuous monitoring is desired, or precision and accuracy are not critical.

(b) Analytical Methods

(1) Spectrophotometric Methods

Measurement of the absorption of light by styrene is the basis of spectrophotometric methods [98,236,237,239]. Spectrophotometric determinations are subject to interference from compounds of similar structure but can be used when the other components present are known or can be shown not to interfere. In cases where other compounds are present, it is necessary to determine their interference with the styrene absorption spectrum. For example, if styrene were to be analyzed by IR spectrophotometry in the presence of butadiene, a wavelength of 11 μm would not be appropriate since butadiene also absorbs light at this wavelength.

(2) Colorimetric Methods

Although styrene does not absorb visible light, the formation of a colored derivative has been used to permit colorimetric analysis [98,238,242,243,244,246,248]. The usual requirements of such a method (i.e., the color-producing reaction must be quantitative and specific for the compound being analyzed, the color must be stable long enough to permit measurement of color intensity, and the color intensity must be

proportional to the concentration of the material being analyzed) have not been difficult to meet with styrene except that color stability has often been poor, necessitating prompt analysis [242].

(3) Polarographic Methods

Polarography [99] is an electroanalytical method in which the analyte undergoes an oxidation or a reduction reaction at an electrode. The potential at which the reaction occurs is a function of the structure of the analyte and the current produced is generally proportional to its concentration. Styrene cannot be determined in aqueous solution by this method because water is reduced before styrene. However, a derivative formed by the reaction of styrene with nitrous acid has been shown to be reducible in an aqueous medium [99]. The interferences from other unsaturated compounds and the completeness of the derivatization have not been extensively studied.

(4) Chromatographic Methods

Chromatography [35,241,247,249,250,251,254,255,256,260,266,267], including gas, thin layer, and paper methods, can separate a mixture into its component parts in addition to measuring the amounts of each. Gas chromatography is the best of these methods because results are easier to quantify than with the others. Interferences can usually be circumvented by changing the carrier gas flow rate, column temperature, or column packing material. Unequivocal identification of each component can be made by a combination of gas chromatography and mass spectrometry [250]. Portable gas chromatographs are available. Detailed analytical methods for styrene have been developed [249,254,256,259].

(c) Methods that Combine Sampling and Analysis

Instruments that combine sampling and analysis in one operation include combustible gas indicators [35], IR analyzers [69], and direct-reading colorimetric devices [35,243,244,261]. An advantage of these methods is that concentrations are immediately known. The methods are useful for determinations of styrene concentrations before workers enter confined spaces, for detection of leaks, etc.

Combustible gas indicators measure total combustible gases in the air, and may be used to determine styrene where it is known to be the only or the predominant combustible vapor present in the air. IR detectors [69] are capable of some selectivity because they can monitor a selected IR wavelength to measure concentrations of the desired compound in the presence of others that do not absorb in that region. Styrene has its strongest absorbance in the region of 10-15 μm, as, for instance, do butadiene, acrylonitrile, and methyl methacrylate. An analytical wavelength at which interference is minimal must be selected. Direct-reading colorimetric tubes [35,121,261] and colorimetric indicator solutions [243,244,261] have also been used for styrene determination.

(d) Recommendations

It is recommended that the method developed by NIOSH [259] for styrene collection on charcoal with analysis by gas chromatography be used to measure worker exposure to styrene. Under certain circumstances, the use of other methods such as direct-reading colorimetric devices [35,243,244,261] to obtain an indication of styrene concentration before entering a confined space may be useful. Similarly, a combustible gas indicator [35] can be useful, especially where specificity and accuracy are less important and where concentrations based on brief sampling are needed (e.g., peak or ceiling concentrations).

Charcoal is recommended as the adsorbent for collection of styrene because it has been tested and found to collect styrene efficiently, because tubes containing suitable charcoal are commercially available, and because removal of styrene from charcoal can be nearly quantitative. Gas chromatography is recommended as the analytical method for styrene because it is reliable and very sensitive. Although testing has not been extensive at low concentrations, the data indicate that the analytical lower limit may be less than 100 µg per sample [252,259]. This corresponds to approximately 2.5 ppm in a 10-liter air sample. If greater sensitivity is required, substitution of thermal for liquid desorption could be used [249]. Field studies with both silica gel [107] and charcoal (FA Madsen, written communication, December 1976) and laboratory studies with charcoal [254,258] indicate that styrene is stable on solid sorbents.

Styrene can be collected efficiently on charcoal at rates of 1 liter/min or less. Sampling at 1 liter/min for 15 minutes should provide an adequate sample for measuring ceiling concentrations. At a styrene concentration of 25 ppm, sampling at 1 liter/min for more than 4 hours might result in sample loss; sampling at about 250 ml/min is recommended for measuring TWA exposures if long sampling times are used. Appendix I gives specific directions on the recommended methods of sampling and analysis.

In operations where styrene and peroxides may be mixed, sampling for styrene oxide should be conducted as part of a program to investigate the possible role of styrene oxide in the production of mutations, terata, abortions, and cancer. Styrene oxide can be sampled and analyzed by a recently developed NIOSH analytical method [268]; other methods have also been used successfully [233,269].

Biological Monitoring of Exposure

There are circumstances where biological evaluation of worker exposure to styrene is a desirable adjunct to environmental monitoring. Increased styrene absorption may occur with increased respiration [88]. An instance of possible styrene exposure during its use in a nonoccupational situation (i.e., repair work with reinforced plastics at home) has been described in the Case Studies and Miscellaneous Reports Section [66], and there is also

a possibility of ingesting styrene present in foods and beverages because of careless work practices. Biological monitoring in conjunction with environmental monitoring can aid in identifying and eliminating other such sources of styrene absorption. Biological evaluation of exposure may also be a valuable adjunct to environmental monitoring in situations such as RP/C processes where skin contact with styrene-containing resins is common and inhalation exposures to styrene are especially variable.

Several experimental studies and one occupational study, which have been described in Chapter IV, discussed the elimination of styrene in the breath of individuals after exposure [35,69,71,72,88,146]. Only 2-3% of the amount of styrene absorbed is eliminated in the breath as unchanged styrene during several days after removal from exposure. While breath analysis can unequivocally establish that exposure to styrene has occurred [69,70], concentrations in alveolar air have not been well correlated with arterial concentrations. In 1974, Astrand et al. [88] found a poor relationship between alveolar and arterial styrene, but because they believed arterial and capillary styrene concentrations were in good agreement they recommended fingertip blood samples be taken and analyzed for styrene. For biological monitoring, Hake et al. [70] preferred analysis of alveolar air for styrene, with sampling being performed 15 minutes after exposure and analysis performed the same day. Stewart et al. [69] noted a rapid decrease in alveolar styrene concentration such that 4 hours after exposure at 117 ppm only about 0.1 ppm styrene was found in exhaled air. Because of this and the marked slope of the decay curves [35,69,88], breath analysis does not adequately estimate exposures unless conditions, especially time of sampling, are well controlled.

As described in Chapter IV, urinary mandelic acid has been demonstrated to be the major metabolite of styrene in humans [143,144]; the amount excreted in the urine reflects the amount of styrene absorbed from respiration [88,143,144,156] and, to a lesser degree, contact with the skin [151,152,153]. Mandelic acid excretion in the urine has been studied extensively in subjects not exposed [35,79,121,159] and in workers and others exposed to styrene [35,75,79,90,92,109,121,125,156,159,270,271,272, 273]. Phenylglyoxylic acid, which is a metabolite of mandelic acid, has also been found in the urine of workers and experimental subjects exposed to styrene [35,83,90,121,144,156,159,270,271].

Other investigators [68,121,159] have evaluated urinary hippuric acid concentrations after styrene exposure. However, in one study [159] hippuric acid was found to be a poor indicator of styrene exposure unless the styrene concentration was greater than 100 ppm, and, in another study [121], no increase in hippuric acid concentration was found after exposure to styrene at 30 ppm.

Methods that have been developed to measure urinary mandelic and phenylglyoxylic acids include polarography [144], colorimetry [121,270], paper chromatography [122,144], gas chromatography [79,89,122,158,274,275, 276,277], fluorometry [278] and isotachophoresis [279].

In a method described in 1964 by Bardodej [144], mandelic acid was converted to benzaldehyde, which was steam-distilled and analyzed by polarography. Other substances that can be converted to benzaldehyde and thus interfere with this analysis method include phenylglycol and its glucuronide, styrene oxide, aminophenyl acetic acid, phenylalanine, and phenylpyrotartaric acid. In urine samples from unexposed subjects, measurements equivalent to 20 mg mandelic acid/liter were found by Bardodej [144]. In 1967, Huzl et al. [109] used the method of Bardodej [144] and found a relationship between mandelic acid in urine collected during the second half of the workshift and concentrations of styrene measured on single occasions in various workplaces. In 1974, Burkiewicz et al. [272] studied mandelic acid excretion and styrene exposures in workers for 1 week also using the method of Bardodej [144]; no mandelic acid was found in urine samples from 30 unexposed workers. About 436-1,630 mg mandelic acid/liter of urine was found in samples collected during the last 3 hours of work from workers with average styrene exposures of 28-110 ppm. In 1970, Sedivec and Flek [274] used the method of Bardodej [144] to convert mandelic acid to benzaldehyde, but assayed benzaldehyde by gas chromatography rather than by polarography.

Bardodej [144] also described a colorimetric method for analysis of mandelic acid that was based on the reaction of mandelic acid with ferric chloride. Endogenous substances that may interfere with this analysis method are lactic acid, pyrotartaric acid, acetoacetic acid, beta-hydroxybutyric acid, atrolactic acid, and phenylpyrotartaric acid. Thiocyanates, some indole derivatives, and salicylic acid may also interfere. Using the colorimetric method, there were only trace amounts of mandelic acid in urine from healthy unexposed people. This method [144] was studied using workers from various industries who were exposed to styrene at average concentrations of about 20-80 ppm. At the lowest exposure, urinary mandelic acid concentrations were about 200 mg/l and, at the highest exposure concentrations, about 1,600 mg/l.

In 1970, Ohtsuji and Ikeda [121] reported the quantitative reaction of mandelic and phenylglyoxylic acids with a sulfuric acid-formalin reagent. Mandelic acid was measured colorimetrically at 450 nm and phenylglyoxylic acid at 350 nm. When Horiguchi and Teramoto [271] studied this method in 1972, they found that normally occurring substances in urine such as cholesterol, and hydroxyphenylacetic, phenylacetic, vanillic, and ferulic acids would also react with the sulfuric acid-formalin reagent. Because of such interferences, urine samples from unexposed people have indicated concentrations of mandelic acid of 22-698 mg/l [35,79,88,121,159,270,271]. In 1972, the method of Ohtsuji and Ikeda [121] was also used to evaluate industrial exposures by Gotell et al. [35], who found better correlation between 8-hour TWA exposures and urinary mandelic acid concentrations than between the TWA exposures and urinary phenylglyoxylic acid concentrations. However, the data were not linear over the range of the TWA exposure concentrations (17-292 ppm). In 1974, Harkonen et al. [270] also found that urinary mandelic acid concentrations correlated better than phenylglyoxylic acid with TWA exposures to styrene at concentration up to 150 ppm; the data

were also not linear over the range of TWA exposures. Harkonen et al. [270] concluded that because of the variation in the results, the probability of a worker being exposed at less than 50 ppm could not be calculated with precision when using this colorimetric method.

In 1973, Slob [280] used paper chromatography to separate mandelic acid and used gas chromatography of the silyl derivative to measure it. With these techniques, 0.5-10 mg of mandelic acid were found to have been excreted in urine during 8 hours by workers exposed to styrene at about 1 ppm.

In 1974, Buchet et al. [89] and Vivoli and Vecchi [275] described gas chromatographic methods that involved methylation of mandelic and phenylglyoxylic acids. With these methods neither mandelic acid nor phenylglyoxylic acid were found in the urine of unexposed subjects. In styrene-exposed workers the concentrations of both acids using these methods were related to exposure [90,275].

In 1976, Bauer and Guillemin [277] used gas chromatography after methylation of the acids with diazomethane as described by Buchet et al. [89]. They discovered, however, that unless the conditions for methylation were precisely controlled, conversion of phenylglyoxylic acid to its methyl ester was not quantitative (a possible explanation for the relatively poor recoveries of phenylglyoxylic acid found by Saeki [276] in another study).

In 1980, Flek and Sedivec [281] described a method for the simultaneous determination of mandelic and phenylglyoxylic acids in urine. Urine was saturated with ammonium sulfate and acidified, and the two acids were then extracted with ethyl acetate. The mandelic and phenylglyoxylic acids were converted into methyl esters with diazomethane, and then analyzed by gas chromatography. The investigators [281] found no interferences from other urine components. The methyl ester of hippuric acid was found to have a much longer retention time under the specified chromatographic conditions, and thus did not interfere. The lower limits of detection of the two acids were about 10 µg/ml. Flek and Sedivec [281] believed the use of an iodine indicator to show completion of esterification, with the supply of diazomethane then being shut off, corrected the potential of methylation problem described earlier by Bauer and Guillemin [277].

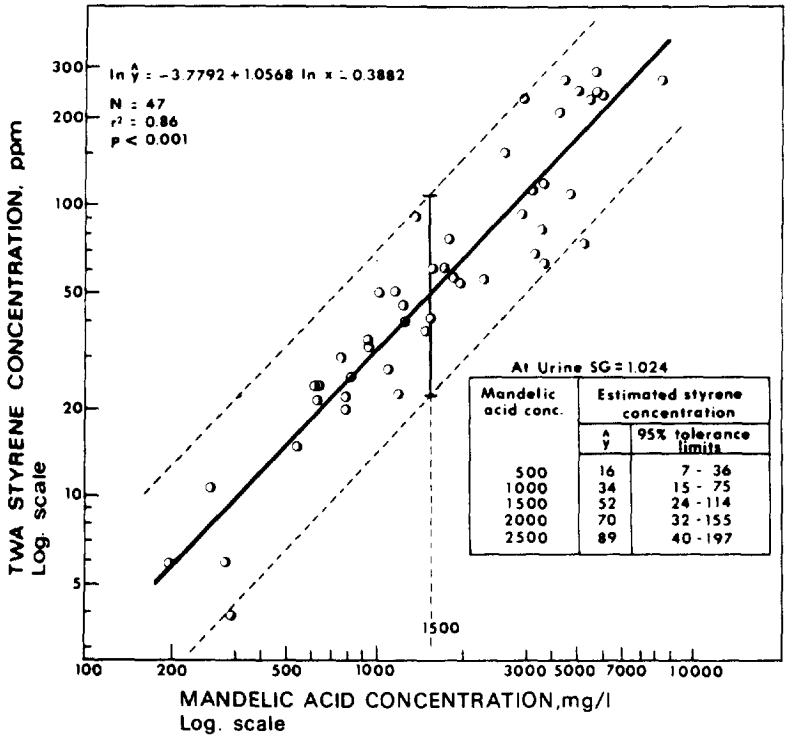
In 1974, a procedure for analysis of the silyl derivative of mandelic acid by gas chromatography was described by Engstrom and Rantanen [79]. They found 94% recovery of mandelic acid that had been added to urine; the coefficient of variation of 10 replicate analyses of the urine of a worker who made plastics was 2%, and the lower limit of detection was 1 mg/l. However, mandelic acid was found in the urine of 10 people who had no history of styrene exposure. The method of Engstrom and Rantanen [79] has been studied extensively in styrene-exposed workers [75,79,92,125], and in control subjects [79,92]. The sensitivity of the method is less than that of the method of Slob [280], but there is a good relationship between

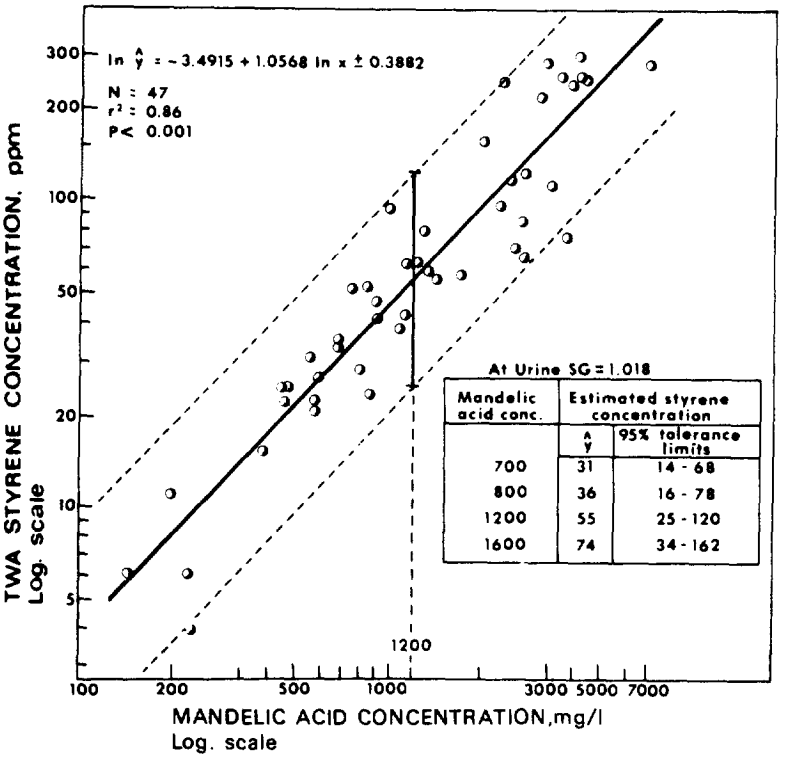
average TWA styrene exposures and urinary mandelic acid concentrations in workers exposed to styrene in the range of about 5-300 ppm [79,92,125], as shown in Figure V-1 on p. 141. The lower figure, representing the regression of mandelic acid with styrene at a urine specific gravity of 1.018, has been copied with permission from the report of Harkonen et al. [125]; the upper figure represents the same regression at a urine specific gravity of 1.024.

In 1976, Saeki [276] also described a method for analyzing the silylated derivatives of mandelic and phenylglyoxylic acids by gas chromatography. By this method, recoveries were 95-100% for mandelic acid and 73-91% for phenylglyoxylic acid. Apparently the method was not used to evaluate exposures of workers.

In 1979, Chakrabarti [278] described a method of analysis of mandelic and phenylglyoxylic acids in urine by fluorometry, after extraction into ether and separation of the acids by thin-layer chromatography. The fluorescent derivatives formed by treatment with concentrated sulfuric acid were described as highly stable. The limits of detection of both acids were 2 μg/ml of urine, with coefficients of variation being less than 15%.

Figure V-1 TWA STYRENE CONCENTRATION VERSUS URINARY MANDELIC ACID CONCENTRATION
(Taken from Harkonen et al [125])





An isotachophoretic method for analysis of urine samples for mandelic, phenylglyoxylic, hippuric, and methylhippuric acids was described in 1977 by Sollenberg and Baldesten [279]. The analytical range was 0.5-35 nmol/0.5 ml sample for each acid. With this method, mandelic acid was not found in the urine of 14 control subjects, but phenylglyoxylic acid at concentrations of 144 and 196 nmol/ml was found in the urine of two subjects. Good agreement was found when some samples were also analyzed by gas chromatography [279].

In a 1979 study of biological monitoring, Fields and Horstman [282] evaluated the styrene exposures of two groups of workers by (1) the sampling and analysis of workers' breathing zones, (2) the sampling and analysis of expired breath at the end of the workshift, and (3) the analysis of urine samples collected just before and just after the workshift, as well as at various times after the shift was over. Both study groups made fibrous glass reinforced boats. The first group consisted of five healthy males, 19-23 years of age, who had been employed making reinforced plastic boats for 2 weeks to 18 months. The second group consisted of 18 healthy males, aged 19-48 years; the authors did not state how long this group had worked. These men in the second group occasionally wore double cartridge organic vapor respirators during some phases of their work.

The investigators [282] found a poor correlation ($r=0.234$) between airborne styrene concentrations and alveolar air concentrations. Urinary mandelic acid was analyzed by the gas chromatographic method of Engstrom and Rantanen [79]; data were categorized by whether or not respirators were worn, and were calculated in terms of three specific gravities, i.e., the two most commonly used literature values of 1.024 and 1.018, and the mean specific gravity of the 62 postshift urine samples, 1.033. For the group not wearing respirators, a correlation coefficient of 0.85 between urinary mandelic acid and airborne styrene exposure was found. With those wearing respirators, there was a poorer correlation ($r=0.57$). These coefficients were the same regardless of which specific gravity adjustment was used. For the group not wearing respirators, a urinary mandelic acid concentration of 1,655 mg/l (adjusted to a specific gravity of 1.024) was equivalent to a styrene exposure of 100 ppm, with 95% confidence limits of 83-117 ppm styrene. Stated another way, an 8-hour TWA exposure of 100 ppm was equivalent to a urine mandelic acid concentration of 1,275-2,063 mg/l. Fields and Horstman [282] also estimated from daily urinary excretion data that mandelic acid concentrations increased slightly through the week, indicating that the amount of mandelic acid excreted in urine sampled at the end of the shift depends largely on that day's absorption and partly on absorption on previous days. In this study [282], there was a markedly better correlation between urinary mandelic acid excretion and styrene exposure than between expired styrene and styrene exposure; thus, mandelic acid excretion would be expected to be a superior method of biological monitoring.

The study of Hake et al. [70] on biological monitoring was reviewed in Chapter IV. These investigators [70] concluded that blood analysis for

styrene, analysis of urinary metabolites, and alveolar breath analysis for styrene were all useful indices of exposure to styrene, though the investigators preferred analysis of alveolar air samples taken 15 minutes after exposure. Hake et al. [70] exposed volunteers at known and, except for one experiment, relatively constant concentrations, whereas the workers studied by Fields and Horstman [282] were performing regular work and exposed at variable concentrations.

As part of the investigation of the workers in a reinforced plastics workshop, Brooks et al. [91] in 1979 and Elia et al. [283] in 1980 described biological monitoring. Post-shift urine samples were collected within 30 minutes of the end of the shift, and analyzed for mandelic acid by the gas chromatographic method of Guillemin and Bauer [158]. Phenylglyoxylic acid was analyzed by reduction with zinc followed by measurement as mandelic acid. Both groups of investigators [91,283] studied correlations of urinary metabolites with airborne styrene in terms of types of coordinates (log-log or linear plots), in terms of mandelic acid alone or the sum of the concentrations of mandelic and phenylglyoxylic acids, and in terms of adjustment for urine dilution either by adjusting for urine specific gravity or by analyzing urine creatinine and expressing results as mass of metabolites per gram of creatinine.

A linear plot of the sum of urinary mandelic and phenylglyoxylic acids vs. airborne styrene concentrations gave a correlation coefficient of only 0.74. All other comparisons gave higher correlation coefficients and involved log-log plots. Log of the sum of the two acids vs. log of the airborne styrene gave r=0.96 when urine dilution was corrected for creatinine, 0.95 when urine dilution was corrected by adjusting urine specific gravity to 1.024, and 0.93 when urine dilution was not corrected. Log of the concentration of mandelic acid only vs. log of the airborne styrene gave r=0.96 when urine dilution was corrected for creatinine, r=0.95 when urine was adjusted to a specific gravity of 1.024, and r=0.94 when urine dilution was not corrected.

These investigators [91,283] concluded that the most effective monitoring of workers exposed to styrene included both biological monitoring and monitoring of airborne exposures; they believed monitoring of post-shift urine samples for metabolites should involve analysis for both acids and that adjustments for urine dilution by correction for either creatinine content or specific gravity should be made.

In 1974, Engstrom and Rantanen [79] compared the results of their gas chromatographic method with the results of the colorimetric method of Ohtsuji and Ikeda [121]. With parallel analyses of urine samples from 10 subjects not exposed to styrene, no mandelic acid was found by gas chromatography whereas 46-698 mg/l were found by the colorimetric method. With parallel analyses of urine samples from 35 plastics workers, the results from the two methods had a correlation coefficient of 0.98 and an average difference of 254 mg/l. It appears that despite the lack of specificity offered by colorimetric methods [121,144], they may be

useful. Application of a colorimetric method may be of particular importance in those situations where appreciable skin contact is possible and where other equipment such as a gas chromatograph is not readily available. With the method of Ohtsuji and Ikeda [121], Harkonen et al. [270] found that the average urinary mandelic acid concentration at a specific gravity of 1.018 associated with an 8-hour TWA exposure of 50 ppm was about 1,660 mg/l. However, the lower 97.5% confidence limit of the mandelic acid concentration for an individual exposed at 50 ppm was below the background of the method, so that the probability of an individual being exposed at less than 50 ppm cannot be calculated with precision from colorimetrically determined mandelic acid concentrations. It is possible that the colorimetric method of Bardodej [144] is at least as accurate and precise as the method of Ohtsuji and Ikeda [121]; however, it has not been as thoroughly evaluated.

When a gas chromatographic method such as that of Engstrom and Rantanen [79] is used to determine mandelic acid concentrations, 500 mg of mandelic acid/liter of urine (corrected to a specific gravity of 1.018) may indicate a recent exposure to at least 10 ppm styrene. From Figure V-1 (see p. 141) a concentration of 1,200 mg mandelic acid/liter of urine (adjusted to a specific gravity of 1.018) corresponds to an average 8-hour TWA styrene exposure estimate of 55 ppm, with 95% confidence limits of about 25-120 ppm. Thus, mandelic acid concentrations in excess of 1,200 mg/l, as determined by the method on Engstrom and Rantanen [79], indicate with 97.5% statistical confidence that styrene absorption was in excess of that from an 8-hour TWA exposure to styrene at 25 ppm. The average mandelic acid concentration (i.e., the point estimate) corresponding to an 8-hour TWA concentration of 50 ppm is about 1,100 mg/l.

Of the various urinary excretion products of styrene, mandelic acid and phenylglyoxylic acid are the most useful for biological monitoring. Although both acids can be analyzed, analysis of just mandelic acid can give useful results [156]. Because the decay curves of urinary metabolites are not as steep as the decay curves of styrene elimination in exhaled air, the time of sampling is less critical. Furthermore, variations in exposure concentrations during the workday should cause fewer variations in urine metabolite elimination than in styrene excretion in exhaled air. The urinary metabolite concentration is more likely to reflect total absorbed dose than is alveolar styrene when workday exposure concentrations have fluctuated.

When evaluating styrene exposure from mandelic acid data, consideration should also be given to pulmonary ventilation, other sources of mandelic acid such as environmental exposure to ethylbenzene [216] or phenylethanol [156], and the metabolism of alpha-hydroxybenzylpenicillin [284]. The suggested analytical method for mandelic acid in urine is described in Appendix II.

Medical Surveillance

Styrene has been shown to have effects on the central nervous system (CNS) and on the skin, eyes, and upper respiratory tract; furthermore, although the evidence is not strong styrene exposure may affect liver function. Thus, preplacement and periodic medical surveillance should include physical examinations and medical and work histories directed to ascertaining the status of the CNS, skin, upper respiratory tract, and the liver. Specific tests, except for serum enzyme assays for assessment of liver status, are not proposed, because other tests are not sufficiently specific or available.

Although the data on teratogenicity and on spontaneous abortions, discussed in Chapter IV, have not been confirmed, they are sufficiently suggestive to warrant, as a prudent preventive measure, advising workers of this information and on the possible risks that could be associated with exposure to styrene during pregnancy.

Biological monitoring of styrene exposure by the analysis of urine for mandelic acid may be a useful adjunct to the environmental monitoring of exposures, especially where breathing zone monitoring does not adequately represent a worker's exposure, for example, where great excursions in airborne concentrations are not detected or where significant percutaneous absorption may occur. Because of the likelihood of both of these occurring in reinforced plastics fabrication (RP/C) operations, biological monitoring may be especially useful there. It may be best to establish the acceptable limit of mandelic acid excretion, in relation to the permissible exposure limit, in each plant, with consideration to the analytical method and, perhaps, sampling circumstances. Pending such a determination, Figure V-1 (see p. 141) can be used for guidance in setting limits of acceptable mandelic acid excretion. Where this biological monitoring indicates excessive styrene absorption, an investigation of the source of the excessive exposure should be initiated. If the cause resulted from failures of engineering controls (leading to excessive exposures) or work practices (leading to ingestion or skin exposure), appropriate corrective action should be taken. If personal habits are the cause (for example, from home activities such as hobbies that lead to additional exposure), the worker should be counseled.

Styrene Oxide in Some Styrene Operations

Two 1979 reports [233,269] described finding styrene oxide at low concentrations in the workroom air in factories making reinforced plastic objects. In the study in Finland by Pfaffli et al. [269], air samples were collected on charcoal, desorbed with dichloromethane, and assayed by capillary gas chromatography with a flame ionization detector. Styrene oxide concentrations were about the same in both personal and general air samples; concentrations were 0.04 ppm in hand lay-up and 0.12-0.17 ppm in spray operations, when styrene concentrations were 59-133 ppm. In the study

in Norway by Fjeldstad et al. [233], personal samples were collected on charcoal, desorbed with carbon disulfide, and analyzed by gas chromatography with a flame ionization detector; confirming analyses were made by high performance liquid chromatography. Styrene oxide concentrations ranged from less than 0.003 ppm to 0.086 ppm; styrene concentrations were 17-289 ppm.

The investigators [233,269] believed that the styrene oxide was formed by the mixing of styrene with peroxides added as curing agents. This seems reasonable, inasmuch as one method of synthesis of epoxides is to react olefins with peroxides.

Thus, it is important to evaluate styrene oxide exposures in work areas where peroxides and styrene are mixed, in view of the toxic problems (i.e., mutagenicity and carcinogenicity) posed by styrene oxide.

In addition to the methods of sampling and analysis for styrene oxide described in the two Scandinavian reports [233,269], a method was developed in 1979 by the Los Alamos Scientific Laboratory under NIOSH sponsorship [285]. Samples were collected in a tube holding a glass fiber filter followed by a sorbent, Tenax-GC. The styrene oxide was desorbed with ethyl acetate and analyzed by gas chromatography with a flame ionization detector. Recoveries of greater than 95% were reported at a relative humidity of 80% with samples of 0.5-44 µg. The limit of detection of the analytical method was 0.1 µg/sample.

VI. DEVELOPMENT OF OTHER OCCUPATIONAL HEALTH STANDARDS

The first recommendation for a worker exposure limit for styrene was by Spencer et al. [53] in 1942. Although they wrote that repeated exposures to styrene at 650 ppm would "probably produce no serious disturbances in man," they recommended 400 ppm as a permissible limit, which Spencer et al. [53] reported had a disagreeable odor but would not cause appreciable eye or nose irritation. In 1943, Mallette [54], in discussing industrial hygiene in synthetic rubber manufacture, pointed out that 200 ppm was a preferable upper limit of styrene exposure because of the eye and nose irritation experienced by workers at 400 ppm.

In 1944, the American Standards Association (ASA) published its American War Standard Allowable Concentration of Styrene Monomer [286]. The recommended maximum allowable limit was 400 ppm for exposures not exceeding a total of 8 hours daily. The report by Spencer et al. [53] was cited, but it was noted that the limit was based on animal experiments and that there had not been sufficient experience with humans to evaluate the response of man.

In 1945, Cook [287] compiled a list of maximum allowable concentrations (MACs) of atmospheric contaminants of several American governmental agencies. Five of the State agencies recommended a MAC of 400 ppm of styrene, but Cook [287] recommended that the value be lowered to 200 ppm because workers had experienced irritation at 400 ppm.

In 1946, the American Conference of Governmental Industrial Hygienists (ACGIH) endorsed the ASA styrene standard of 400 ppm, along with 15 other ASA standards, because they considered that that values had been established and substantiated by the necessary scientific research [288]. In 1947, the ACGIH proposed lowering the MAC to 200 ppm [289]; reasons for the recommendation were not given. In 1956, the ACGIH reported that recommendations for changes in the values of a number of substances had been made [290], among them a change in the value for styrene from 200 to 100 ppm. Subsequent to the 1947 recommendation [289], the terminology for an environmental limit had changed from MAC to Threshold Limit Value (TLV) defined as "the maximum average atmospheric concentration to which workers may be exposed for an eight-hour working day without injury to health" [290].

In 1957, the ACGIH adopted a TLV of 100 ppm [291]. The notation "skin" was added in 1961 by the ACGIH to the TLV of those substances which in liquid form could penetrate the skin and cause systemic effects; styrene was not so noted [292]. In 1962, reports by Spencer et al. [53], Carpenter et al. [68], and an industry bulletin were cited by the ACGIH in support of the styrene TLV of 100 ppm. In 1964, the ACGIH changed the TLV of 100 ppm for styrene from a TWA value to a ceiling value that should not be exceeded [293]; the basis for the change was not given.

In 1967, the ACGIH proposed lowering the TLV for styrene to 50 ppm [294], and it was so listed under "Notice of Intended Changes" in the 1967 and 1968 TLV lists [295,296]. However, the ACGIH decided in 1969 that the TLV for styrene should remain at 100 ppm but as a TWA, i.e., with the removal of the ceiling notation [297]. According to the 1969 ACGIH transactions [298], the recommendation of 100 ppm was based on the experimental human exposure studies of Stewart et al. [69]. The ACGIH [298] stated that, in the study cited [69], none of the subjects exposed at 50 ppm for 1 hour had experienced any symptoms or had abnormal clinical findings; however, exposure at 100 ppm produced mild untoward, but transient, subjective responses in half the subjects exposed. In 1981, the ACGIH [299] decided to change the styrene TLV to 50 ppm with a short-term exposure limit (STEL) of 100 ppm. A TLV of 50 ppm, noted by the ACGIH in their 1980 Documentation of the TLVs [300] as being one-tenth the lowest concentration possibly causing lymphoid or hematopoietic tumors in female rats studied by Jersey et al. [195], and a STEL of 100 ppm were suggested as being reasonable.

Documentation of MAC in Czechoslovakia [301] was published in 1969. The suggested styrene limit was 47 ppm for an average exposure, which was considered to be adequate to prevent chronic poisoning, and 235 ppm for a peak exposure, which was considered adequate to prevent narcotic symptoms. In support of the recommendations, 12 references were cited, including those by Bardodej et al. [97,143], Carpenter et al. [68], and Spencer et al. [53].

In 1970, the American National Standards Institute, Inc., (ANSI) published a standard, Z37.15-1969, for acceptable concentrations of styrene [302]. There were three components, all assuming an 8-hour workday: (1) an acceptable TWA for protection of health; (2) an acceptable ceiling concentration for protection of health; and (3) acceptable maximum peaks above the acceptable ceiling. The ANSI values were a TWA concentration of 100 ppm, a ceiling of 200 ppm (provided the TWA was not exceeded), and maximum peaks of 600 ppm for a duration of not more than 5 minutes once every 3 hours. These recommendations were based on the reports of Spencer et al. [53], Carpenter et al. [68], Wolf et al. [162], and Stewart et al. [69].

The 1969 ANSI Z-37 limit [302] was adopted in 1971 as the Federal OSHA standard (29 CFR 1910.1000, Table Z-2). It consists of a TWA concentration of 100 ppm for an 8-hour day, a ceiling concentration of 200 ppm, and a maximum peak of 600 ppm for no more than 5 minutes in any 3 hours.

A Scandinavian expert group consisting of representatives from Denmark, Finland, Norway, and Sweden, published a review [303] of much of the scientific and technical literature on styrene which included the hygienic limit values in force in various countries. According to their tabulation, 100 ppm was the limit in Australia, Belgium, Finland, Yugoslavia, the Netherlands, Switzerland, Great Britain, and the United States (OSHA); 72 ppm in Italy; 50 ppm in Denmark, Norway, and Japan; 47 ppm in Romania,

Czechoslovakia, and the German Democratic Republic; 24 ppm in Poland; 12 ppm in Hungary; and 1 ppm in Bulgaria and Russia [303]. The MAK value (maximum concentration value in the workplace) for styrene in the Federal Republic of Germany is 100 ppm [304]. In 1981, Sweden [305] adopted a TWA styrene standard of 25 ppm with a 15-minute short-term value of 75 ppm; styrene was designated as being easily absorbed into the body.