7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring styrene, its metabolites, and other biomarkers of exposure and effect to styrene. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

As a volatile material, styrene is readily determined by gas chromatographic (GC) analysis. As a hydrocarbon, styrene is detected very sensitively by flame ionization detection (FID); its aromatic nature enables some selectivity by photoionization detection (PID); and it can be specifically identified by mass spectrometry (MS). Styrene is usually collected from the gas phase or from vapor evolved from the sample matrix on a column of solid sorbent, such as Tenax®. Cryogenic (low temperature) collection and sorption in organic liquids are also possible.

Capillary gas chromatography, also known broadly as high-resolution gas chromatography (HRGC), has greatly facilitated the analysis of compounds such as styrene that can be measured by gas chromatography and has resulted in vast improvements in resolution and sensitivity. It has made the choice of a stationary phase less important than is the case with the use of packed columns. The instrumental capability to separate volatile analytes by HRGC is, for the most part, no longer the limiting factor in their analysis.

The specific analytical methods used to quantify styrene in biological and environmental media samples are summarized below.

7.1 BIOLOGICAL MATERIALS

Methods have been described for the determination of styrene in expired air (Kneip and Crable 1988a; Stewart et al. 1968; Wallace and Pellizzari 1995; Wallace et al. 1996), blood (Antoine et al. 1986; Ashley et al. 1992; Bartolucci et al. 1986; Guillemin and Berode 1988; Withey and Collins 1977), urine (Dolara et al. 1984; Ghittori et al. 1987; Pezzagno et al. 1985), adipose tissue (Engstrom et al. 1978a), and other

tissues (heart, lungs, liver, spleen, kidney, brain) (Withey and Collins 1977) (Table 7-1). These methods generally require styrene release from the sample matrix and collection on a column of solid sorbent or collection as headspace gas; more recent methods permit the exhaled breath to be delivered directly, via a breath interface, to the mass spectrometer (Wallace et al. 1996). Cryogenic collection is also possible (Romieu et al. 1999). Of the available methods for detecting styrene, FID is the most sensitive, and MS is the most specific, the latter of which can be made more sensitive by tandem MS approaches (MS/MS) (Wallace et al. 1996). Levels of detection are in the low parts per billion range for breath samples (Stewart et al. 1968; Wallace et al. 1996) and parts per trillion range for blood samples (Ashley et al. 1992).

The major metabolites of styrene in humans are mandelic acid (MA) and phenylgloxylic acid (PGA). Detection of these metabolites in urine is the most commonly performed procedure as an indicator of exposure to styrene. Procedures have been described for their measurement in urine (Baselt 1988a; Dolara et al. 1984; Engstrom et al. 1976; Kneip and Crable 1988b, 1988c; Korn et al. 1984; Pezzagno et al. 1985; Sedivec et al. 1984; Sollenberg et al. 1988). Generally, these styrene metabolites are converted to volatile derivatives and measured gas chromatographically or determined directly by high performance liquid chromatography (HPLC). Two other styrene metabolites that may result from exposure to styrene are 4-vinylphenol (Pfaffli et al. 1981) and styrene glycol (phenyl ethylene glycol) (Guillemin and Berode 1988), but methods for the detection of these metabolites in biological materials have not been worked out in detail. Sensitive methods are also available for measuring styrene oxide in blood (Kessler et al. 1990; Langvardt and Nolan 1991), although these techniques are probably more useful in research on styrene toxicity than in detecting or quantifying styrene exposure.

Methods for detection of styrene and its metabolites in biological materials are summarized in Table 7-1.

7.2 ENVIRONMENTAL SAMPLES

Styrene determined in environmental samples is usually collected on solid sorbents (from air; Zielinska et al. 1996) or on solid sorbents after purging in a gas stream (water, soil, solid waste samples; Miermans et al. 2000)). Styrene from such samples is measured very sensitively by GC/FID and very specifically by GC/MS. Methods for the determination of styrene in environmental samples have been standardized by the American Society for Testing and Materials (ASTM 1988a, 1988b), EPA (1986a, 1986b, 1989c, 1989d, 2003), and NIOSH (1984). Relatively low detection limits can be achieved for the determination of styrene in environmental samples and the accuracy appears to be acceptable for those limited cases in

Table 7-1. Analytical Methods for Determining Styrene in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Styrene analyte					
Adipose tissue	Evaporation into nitrogen, collection as vapor	GC	No data	No data	Engstrom et al. 1978a
Breath[a]	Collection in Saran bag	GC/FID	0.05 ppm	No data	Stewart et al. 1968
Breath	Sorption onto silicagel, desorption into headspace	GC	0.1 ppm	No data	Kneip and Crable 1988a
Breath	Collection in Tedlar bag, followed by sorption of VOCs (including styrene) onto Tenax	GC/MS	Low ppb	No data	Wallace et al. 1996
Breath	Collection onto personal monitoring badge, followed by solvent extraction	GC/FID	0.9 ppb[b]	No data	Romieu et al. 1999
Blood	Purge at 40–50 °C with helium, collection on Tenax-GC/silica	GC/MS	No data	CV<5%	Antoine et al. 1986
Blood	Headspace analysis	GC/FID	No data	No data	Bartolucci et al. 1986
Blood	Collection in vacutainer with EDTA as anticoagulant, headspace analysis	GC	No data	No data	Guillemin and Berode 1988
Blood	Headspace analysis	GC	0.02 µg/mL	No data	Withey and Collins 1977
Blood	Vacutainer collection, followed by purge and trap	GC/MS	9 ppt (95% CI: 4–21 ppt)	95–121%	Ashley et al. 1992
Heart, lungs, liver, spleen, kidney, brain	Hemogenate prepared for headspace analysis	GC	0.01 µg/g	No data	Withey and Collins 1977
Urine	Headspace from sample maintained and 37 °C for 2 hours	GC/MS	No data	No data	Ghitori et al. 1987
Urine	Sorption on XAD-2, elution with n-hexane	HPLC/UV	<0.7 µg/L	72±10%	Dolara et al. 1984
Urine	Headspace analysis	GS/MS	No data	No data	Pezzagno et al. 1985
Styrene metabolite analyte					
Urine for MA	Extraction with ethyl acetate, derivatization to isopropyl ester	GC/FID	No data	No data	Korn et al. 1984

Table 7-1. Analytical Methods for Determining Styrene in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine for MA	Extraction with diethyl ether, silylation	GC	No data	No data	Engstron et al. 1976
Urine for MA and PGA metabolites	Extraction and derivatization	GC/FID	0.05 ppm	94% MA, 98% PGA	Bartolucci et al. 1986
Urine for MA metabolite	Acidification, extraction, derivatization	HRGC/FID	10 mg/L	No data	Kneip and Crable 1988b
Urine for PGA metabolite	Reduction, acidification, extraction, derivatization	HRGC/FID	10 mg/L	No data	Kneip and Crable 1988c
Urine for MA and PGA metabolites	Extraction with ethyl acetate, derivatization to methyl esters with diazomethane	GC	10 mg/L	No data	Sedivec et al. 1984
Urine for MA and PGA metabolites	Extraction with ethyl acetate, evaporation derivatization	GC/FID	No data	97–99% relative recovery	Baselt 1988a
Urine for MA, PGA, and hippuric acid metabolites	Direct injection	HPLC/UV	<1 µg/mL[c]	<3% deviation from true value at 5 µg/mL	Regnaud et al. 1987
Urine for MA and PGA (stereo-selective)	Extraction and derivatization	HRGC/FID	No data	No data	Korn et al. 1987
Blood for styrene oxide	Extraction with n-hexanone, concentration by evaporation	GC/FID	1 ng/mL	72±8%	Kessler et al. 1990
Blood for styrene oxide	Extraction with benzene	GC/MS	10 ng/g	92±21%	Langvardt and Nolan 1991

[a]Unless otherwise designated, analyses are for styrene.
[b]Values were reported for benzene; authors indicated that other VOCs had similar levels of detection
[c]Detection limits were 0.63 µg/mL for mandelic acid, 0.78 µg/mL for phenylglyoxylic acid, and 0.52 µg/mL for hippuric acid.

CV = coefficient of variation; CI = confidence interval; EDTA = ethylene diamine tetra acetic acid; FID = flame ionization detector; GC = gas chromatography; HPLC = high-performance liquid chromatography; HRGC = high-resolution gas chromatography; MA = mandelic acid; MS = mass spectrometry; PGA = phenylglyoxylic acid; UV = ultraviolet; VOC = volatile organic compound

which accuracy data are available. For example, the most sensitive for styrene detection limits were 0.002 μg/L in water, 4 μg/kg in soil, and 500 μg/kg in solid waste. No significant reports were found pertaining to styrene degradation products in environmental samples. Information on methods for the determination of styrene in environmental samples is summarized in Table 7-2.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of styrene is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of styrene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Styrene and its primary metabolites, MA and PGA, can be detected in several human tissues (blood, urine, adipose tissue, and in several organs [Table 7-1]), as well as in exhaled breath. The detection limits range in the parts per billion to parts per trillion range (Ashley et al. 1992). Approaches have been developed to provide more efficient and rapid assessment of exposure, such as where the subject breathes for short periods of time into different types of sample collectors (tedlar bags, evacuated canisters, even directly into a mass spectrometer interface [Wallace et al. 1996]), allowing samples to be collected efficiently for subsequent or immediate analysis. Personal monitoring badges containing charcoal have also been developed for longer-term assessment of exposure to styrene and other volatiles, which have been calibrated for comparison to levels detected in the subjects' blood (Romieu et al. 1999). There are no data needs at this time.

Table 7-2. Analytical Methods for Determining Styrene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Food	Homogenization, headspace sampling	GC/MS	1 µg/kg	No data	Gilbert and Startin 1983
Air	Retention by activated carbon	GC[a]	No data	No data	ASTM 1988a
Air	Retention by activated carbon, elution with carbon disulfide	GC[b]	No data	No data	ASTM 1988b
Air	Retention by activated carbon, elution with carbon disulfide	HRGC/FID	0.01 mg/sample	No data	NIOSH 1984
Wastewater	Stable isotope dilution method, followed by solvent extraction	GC/MS	10 µg/L	No data	EPA 2001
Water	Purge by helium, collection on activated charcoal/silica gel/Tenax®	GC/PID	0.01 µg/L	96–104%	EPA 1989f
Water	Purge by helium, collection on activated charcoal/silica gel/Tenax®	GC/PID	0.008 µg/L	No data	EPA 1989g
Water	Purge by helium, collection on activated charcoal/silica gel/Tenax®	HRGC/MS	0.20 µg/L	120% (at 1 µg/L)	EPA 1989h
Water	Purge by helium, collection on activated charcoal/silica gel/Tenax®	HRGC/MS	0.04 µg/L	102%	EPA 1989i
Water	Purge and trap with nitrogen or helium	GC/PID	0.01 µg/L	104%	EPA 1995
Water	Purge and trap	GC/PID	0.01 µg/L	104%	EPA 1996
Water	Purge by helium, and trap at low temperature	GC/FID	0.002 µg/L	No data	Miermans et al. 2000
Water	Purge and trap on VOCARB or equivalent	GC/MS	0.039 µg/L	94–110%	USGS 1998
Soil, low level	Purge by helium, collection on solid, thermal desorption	GC/MS	4 µg/kg	No data	EPA 1986c
Solid waste, nonwater miscible	Purge by helium, collection on solid, thermal desorption	GC/MS	500 µg/kg[c]	No data	EPA 1986c

Table 7-2. Analytical Methods for Determining Styrene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Solid waste	Purge by helium, collection on solid, thermal desorption	GC/MS	500 µg/kg[c]	No data	EPA 1986b

[a]Absorption characteristics for sampling atmospheric vapor with activated carbon for subsequent analysis by GC.
[b]Generic method for the determination of organics.
[c]Estimation from detection limits in water.

FID = flame ionization detector; GC = gas chromatography; HRGC = high-resolution gas chromatography; MS = mass spectrometry; PID = photoionization detection; UV = ultraviolet

Methods for Determining Biomarkers of Exposure and Effect. Biological monitoring of styrene exposure has been reviewed (Guillemin and Berode 1988). Sensitive and selective methods are available for the qualitative and quantitative measurement of styrene and its two major metabolites, MA and PGA, in samples from exposed individuals after the analytes are separated from their biological sample matrix. The concentration of these metabolites in urine has been found to correlate with average exposure levels in air (Härkönen et al. 1978), and so may be used as a biomarker of exposure. However, measurements of MA and PGA are not specific for this purpose (Bartolucci et al. 1986) and these metabolites can result from the metabolism of other organic substances, particularly ethylbenzene (Baselt 1988b). As noted in Chapter 6 for studies of the general population, styrene has been identified in adipose tissue at concentrations of 8–350 ng/g (EPA 1986d), in blood at a mean concentration of 0.4 μg/L (Antoine et al. 1986) and in exhaled breath at mean concentrations of 0.7–1.6 μg/L. Levels of MA and PGA in biological samples from the general population probably are below the detection limits of methods that are currently used (Baselt 1988a). However, it is likely that normal background levels of these metabolites in unexposed individuals are too low to be of any significance. Although new and improved methods for the determination of styrene and its metabolites in biological samples need not have a high priority, additional work on standardization of these methods for use in biological samples accompanied by additional studies involving interlaboratory comparisons of recovery, accuracy, and precision data would be useful.

Clinical means have been proposed to indicate exposure to styrene. In general, these are not sufficiently sensitive, specific, or well characterized. The most common symptom of exposure, impairment of central nervous system function, is not unique to styrene. Neither cytogenic monitoring of peripheral lymphocytes nor unscheduled DNA synthesis have been sufficiently well characterized as biomarkers of exposure to styrene.

There is currently some information that can be used to correlate levels of biomarkers of exposure to styrene in biological media with adverse health effects. Central nervous system depression has been correlated with a urinary MA concentration of ≥800 mg/L and a decrement in psychomotor performance in association with a concentration of ≥1,200 mg/L (Härkönen et al. 1978). The styrene concentrations in air producing these effects and urinary MA levels were relatively high. Studies to determine if effects at lower levels of exposure could also be correlated to metabolite levels in urine would be valuable. However, the design of studies involving controlled inhalation exposures in humans is precluded by the potential carcinogenicity of styrene.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. In an occupational setting, the medium that is of most concern for human exposure to styrene is air, although at Superfund sites, contaminated groundwater may pose a danger. Methods are well developed for the determination of styrene in water and air with excellent selectivity and sensitivity (ASTM 1988a, 1988b; EPA 1989f, 1989g, 1989i, 1995, 1996; NIOSH 1984; USGS 1998). Methods for the determination of styrene in soil and waste samples are not as well developed and may require additional testing and validation (EPA 1986b, 1986c, 2001).

The detection limits for styrene in environmental media cited in Table 7-2 (0.01 mg/sample, typically 10 L, NIOSH 1984; 0.002 µg/L in water, Miermans et al. 2000; and 4 µg/kg in soil, EPA 1986c) are low enough to enable the determination of styrene in any environmental medium likely to pose a hazard to health based upon information currently available in the literature. These detection limits are probably below most ambient background levels of styrene.

Sampling methodologies for compounds such as styrene pose typical collection problems that include the collection of samples that are nonrepresentative, may be of insufficient sample volume, may contain interfering materials that result in low sample recovery, or may contain interfering contaminating chemicals. Other sampling methods may be labor-intensive, or require tedious extraction and purification procedures (Green and Le Pape 1987; Miermans et al. 2000). Methods that measure organic compounds such as styrene *in situ* in water and other environmental media without the need for sampling and extraction procedures to isolate the analyte prior to analysis are desirable. One such method has been patented, but no commercial products have been identified (see below).

In regard to methods for determining parent styrene and degradation products in environmental media, the following conclusions may be drawn: Because styrene can be detected instrumentally and determined in air and normal water samples with totally adequate selectivity and sensitivity, no additional data are needed at this time. A moderate need exists to improve methodologies to determine styrene in soil, sludges, and solid wastes. Styrene degradation products are a different matter in that little information is available on their determination in environmental samples. In air, these compounds should consist predominantly of photochemical oxidation products, whereas in water and soil samples, they are expected to be biodegradation products. Additional research is needed on the determination of these materials.

7.3.2 Ongoing Studies

No current ongoing studies were found in the Federal Research in Progress database (FEDRIP 2007) regarding the development of analytical methods for the detection of styrene or its metabolites.

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, is developing methods for the analysis of styrene and other volatile organic compounds in blood. These methods use purge and trap methodology, high-resolution gas chromatography, and magnetic sector mass spectrometry, which give detection limits in the low parts per trillion (ppt) range.

In addition to the detection and identification of styrene using chromatographic means, where current detection limits approach analytical thresholds, other technologies may offer additional routes for detection of styrene in samples, especially for aquatic environmental samples. Immunoassay-based methods of analysis offer rapid analysis times, high sensitivity, and often high selectivity for various organic pollutants and other chemicals of concern. While styrene and its metabolites are considered important chemicals to monitor using such techniques, no known assays have been commercialized, even though one patent has been issued for a method that claims to detect styrene and other VOCs by immunoassay (patent 5,358,851, issued in 1994 [Peck 1994]). No additional information was found regarding the continued development (or use or application) of this technology.