6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring styrene in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify styrene. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect styrene in environmental samples are the methods approved by federal agencies 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 refine 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. 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. Table 6-l lists the applicable analytical methods used for determining styrene in the biological fluids and tissues, and Table 6-2 lists the methods used for determining styrene in environmental samples.

6.1 BIOLOGICAL MATERIALS

Methods have been described for the determination of styrene in expired air (Kneip and Crable 1988a; Stewart et al. 1968), blood (Antoine et al. 1986; 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). These methods generally require styrene release from the sample matrix and collection on a

TABLE 6-1. 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
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
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 at 37°C for 2 hr	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	GC/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
Urine for MA	Extraction with diethyl ether, silylation	GC	No data	No data	Engstrom 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

TABLE 6-1 (Continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
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 dizaomethane	GC	No data	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[b]	<3% deviation from true value at 5 µg/mL	Regnaud et al. 1987
Urine for MA and PGA (stereoselective)	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]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; EDTA = ethylene diaminetetracotic acid; FID = flame ionization detector; GC = gas chromatography; HPLC = high-performance liquid chromatography; HRGC = high-resolution gas chromatography; hr = hour(s); MA = mandelic acid; MS = mass spectrometry; PGA = phenylglyoxylic acid; UV = ultraviolet

TABLE 6-2. 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 1989a
Air	Retention by activated carbon, elution with carbon disulfide	GC[b]	No data	No data	ASTM 1989b
Air	Retention by activated carbon, elution with carbon disulfide	HRGC/FID	0.01 mg/ sample	No data	NIOSH 1984
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
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
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]Estimated 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

column of solid sorbent or collection as headspace gas. Cryogenic collection should also be possible. Of the available methods for detecting styrene, flame ionization detection (FID) is the most sensitive and mass spectrometry (MS) is the most specific.

The major metabolites of styrene in humans are MA and 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; Xneip and Crable 1988b; Kneip and Crable 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. 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 6-l.

6.2 ENVIRONMENTAL SAMPLES

Styrene determined in environmental samples is usually collected on solid sorbents (from air) or on solid sorbents after purging in a gas stream (water, soil, solid waste samples). Styrene from such samples is measured very sensitively by gas chromatography with flame ionization detection (GC/FID) and very specifically by gas chromatography with mass spectrometric detection (GC/MS). Methods for the determination of styrene in environmental samples have been standardized by the American Society for Testing and Materials (ASTM 1989b), U.S. Environmental Protection Agency (EPA 1986a, 1986b; EPA 1989b, 1989c, 1989d), and National Institute for Occupational Safety and Health (NIOSH 1984). As shown by the data in Table 6-1, relatively low detection limits (0.01 mg/sample, 0.10 μg /L in water, 4 μg /kg in soil, 500 μg /kg in solid waste) can be achieved for the determination of styrene in environmental samples and the accuracy appears to be acceptable for those limited cases in which accuracy data are available. No significant reports were found pertaining to styrene degradation products in environmental samples.

Methods for the determination of styrene in environmental samples are summarized in Table 6-2.

6.3 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 the 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 or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Data Needs

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 (Harkonen 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 5 for studies of the general population, styrene has been identified in adipose tissue at concentrations of 8-350 ng/g (Stanley 1986), 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.

As discussed in Section 2.5.2, 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 at all 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 or higher and a decrement in psychomotor performance in association with a concentration of 1,200 mg/L or more (Harkonen 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 greater danger. Methods are well developed for the determination of styrene in water and air with excellent selectivity and sensitivity (ASTM 1989a; EPA 1989f, 1989g, 1989i; NIOSH 1984). Methods for the determination of styrene in soil and waste samples have been available for a shorter length of time and require additional testing and validation (EPA 1986b, 1986c).

The detection limits for styrene in environmental media cited in Table 6-2 (0.01 mg/sample, typically 10 L) (NIOSH 1984), 0.04 μg /L in water (EPA 1989i), 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 continue to pose problems such as nonrepresentative samples, insufficient sample volumes, contamination, and labor-intensive, tedious extraction and purification procedures (Green and LePape 1987). It is desirable to have means to 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.

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.

6.3.2 On-going Studies

The Environmental Health Laboratory Sciences Division of the Center for Environmental Health and Injury Control, Centers for Disease Control, is developing methods for the analysis of styrene and other volatile organic compounds in blood. These methods use purge-and-trap methodology and magnetic mass spectrometry which gives detection limits in the low parts per trillion range.

Research is underway at the Cooperative Institute for Research in Environmental Sciences (CIRES) at the University of Colorado, Boulder, to improve methods for the analysis of styrene and related compounds in environmental samples, particularly atmospheric samples.

Studies are also underway that would improve the means for determining styrene, its metabolites, and related compounds in biological samples and environmental media. Improvements continue to be made in chromatographic separation and detection. Problems associated with the collection of styrene on a sorbent trap, followed by thermal desorption, may be overcome with direct purging to a capillary column with whole column cryotrapping (Pankow and Rosen 1988). Current activities in the areas of supercritical fluid extraction (King 1989) and supercritical fluid chromatography (Smith 1988) include focus on compounds such as styrene and its metabolites in biological samples and environmental media. Fourier transform infrared flow cell detectors are sensitive and selective for the detection of compounds such as styrene that have been separated by supercritical fluid chromatography (Wieboldt et al. 1988). Immunoassay methods of analysis are very promising for the determination of various organic pollutants and toxicants, and it is reasonable to assume that styrene, and particularly its metabolites, are candidates for this type of analysis.