6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring pyridine 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 pyridine. 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 pyridine 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 to semivolatile material, pyridine can be determined by gas chromatography (GC) analysis using mass spectrometric (MS) detection. Pyridine is usually collected from the gas phase on a column of solid sorbent, such as Tenax® Pyridine can be removed from aqueous or slurry samples by purge-and-trap techniques or as headspace gas. Cryogenic (low temperature) collection and sorption in organic liquids are also possible.

6.1 BIOLOGICAL MATERIALS

In biological systems in which pyridine may have been metabolized or may itself be a metabolite, consideration should be given to the possible binding of the analyte by endogenous substances in the biological system. However, no information was found in the literature pertaining to such binding or to the release of biologically bound pyridine prior to analysis.

Sensitive and selective methods are available for the qualitative and quantitative measurement of pyridine after it is separated from its sample matrix. Gas chromatography, using either sensitive and highly specific MS or highly sensitive flame ionization detection (FID), is the analytical method most commonly used. Capillary gas chromatography, also known as highresolution gas chromatography (HRGC), has facilitated the analysis of compounds such as pyridine that can be measured by gas chromatography and has resulted in improvements in resolution and sensitivity. It has made the choice of a stationary phase less important than was previously the case with packed columns. The instrumental capability to separate volatile analytes by HRGC is, for the most part, no longer the limiting factor in their analysis. High-performance liquid chromatography (HPLC) has been used to measure isotopically labelled pyridine and its metabolites in urine (Shaker et al. 1982). This method has the advantage of compatibility with the liquid matrix of biological samples.

6. ANALYTICAL METHODS

In biological samples, after pyridine is released from the sample matrix, it is usually determined by gas chromatography. Methods for the detection of pyridine in biological materials are summarized in Table 6-l.

6.2 ENVIRONMENTAL SAMPLES

For the determination of pyridine in air, the analyte is usually trapped and concentrated from a large volume of air on a solid sorbent, such as Tenax® or activated carbon, from which it can be released thermally or eluted with a solvent such as dichloromethane for subsequent measurement. For aqueous samples, pyridine is purged with an inert gas, collected on a solid such as Tenax®, or cryogenically collected, followed by thermal desorption and measurement. Gas chromatography using sensitive and highly specific MS or highly sensitive FID are the analytical methods of choice for the determination of pyridine in environmental samples.

Methods for the determination of pyridine in environmental samples are summarized in Table

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 pyridine 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 pyridine.

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

Sensitive and selective methods are available for the qualitative and quantitative measurement of pyridine after it is separated from its sample matrix.

An area of continuing interest is the ability to transfer analytes that have been isolated from a biological or environmental matrix quantitatively and in a narrow band to the HRGC; therefore this is an area of on-going study.

TABLE 6-1. Analytical Methods for Determining Pyridine in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Extraction through a membrane of Teflon impregnated with n-undecane	GC	1 ppb	3.5-4% repeatability	Audunsson 1988
Urine	Direct injection or injection after protein precipitation using acetonitrile	HPLC	No data	No data	Shaker et al. 1982
Biological liquids	Direct injection or headspace analysis	GC/FID	No data	No data	Dubowski 1975

FID = flame ionization detection; GC = gas chromatography; HPLC = high-performance liquid chromatography

TABLE 6-2. Analytical Methods for Determining Pyridine in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collection on silver-impregnated adsorbent	HPLC	12 ng	No data	Frei et al. 1974
Air	Collection on charcoal, desorption with dichloromethane	GC	0.02 mg/sample	109%± 3.6%	NIOSH 1984
Indoor air	Collection on adsorbent resin, thermal desorption	HRGC/MS	No data	No data	Bayer and Black 1987
Tobacco smoke	Collection by diffusion denuder samplers	GC/MS	No data	No data	Eatough et al. 1989
Coal conversion oil	Extraction with dichloromethane	HPLC/GC	No data	No data	Haugen et al. 1982
Combustion products	Sorption on XAD-2, thermal desorption	GC/MS	0.5 ng	No data	James et al. 1985
River water	Distillation from sodium hydroxide, collection in dilute sulfuric acid, concentration by evaporation	GC	1 µg/L	90%	Sasai and Tsukioka 1981
River water	Distillation from sodium hydroxide, collection in dilute sulfuric acid, concentration by evaporation	GC	10 µg/L	84%	Sasai and Tsukioka 1981
Low level soil, sediment	Purge by helium, collection on solid, thermal desorption	GC/MS	5 µg/L	No data	EPA 1986a
Nonwater miscible waste	Purge by helium, collection on solid, thermal desorption	GC/MS	2.5 mg/kg	No data	EPA 1986a

GC = gas chromatography; HPLC = high-performance liquid chromatography; HRGC = high-resolution gas chromatography; MS = mass spectrometry

Another area of interest is to identify and accurately measure the quantity of compounds in the HRGC peaks. Mass spectrometric detection and Fourier transform infrared spectroscopy (FTIR) have been useful for this purpose.

The metabolites of pyridine in biological materials are difficult to determine in routine practice because of the lack of standardized methods for their measurement. In addition, not all of the pyridine metabolites have been identified and characterized, and this must first be accomplished.

Methods for Determining Biomarkers of Exposure and Effect. As with most xenobiotics, the identification of biomarkers of exposure to pyridine would be helpful in detecting exposure to this compound before adverse morphological or clinical effects occur. There is no available information in the literature that can be used to correlate levels of exposure to pyridine with resulting levels in urine or other biological fluids. Once biomarkers are identified, research on methods to detect them would be useful.

Similarly, no methods have been identified that are sensitive enough to correlate levels in biological media with levels at which biological effects occur. The development of these methods would be useful in the protection of populations exposed to pyridine.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. The media of most concern for human exposure to pyridine are drinking water (primarily from groundwater sources) and air. As illustrated by the data presented in Table 6-2 (e.g., Frei et al. 1974; NIOSH 1984; Sasai and Tsukioka 1981), the methods available for the determination of pyridine in water and air are not adequate to determine natural background levels of this compound. However, these background levels may well be insignificantly low. Methods are certainly adequate to measure the levels of pyridine at which known health effects occur. In general, the precision, accuracy, reliability, and specificity of methods to determine pyridine in water and air are not adequately documented. Additional work in this area would be useful.

Methods for determining pyridine in water, air, and waste samples are undergoing constant improvement. For example, research is on-going to develop a "Master Analytical Scheme" for the determination of organic compounds, including pyridine, in water (Michael et al. 1988). The goal of this project is to detect and quantitatively measure organic compounds at 0.1 μg/L in drinking water, 1 μg/L in surface waters, and 10 μg/L in effluent waters. Analytes are to include numerous nonvolatile compounds and some compounds that are only semisoluble in water, as well as volatile compounds (bp <150°C).

Sampling methodologies for compounds such as pyridine continue to pose problems such as nonrepresentative samples, insufficient sample volumes, contamination, and the need for labor-intensive, tedious extraction and purification procedures (Green and Le Pape 1987). Although HPLC methods have

simplified these procedures, it is desirable to have the means to measure organic compounds such as pyridine in situ in water and in other environmental media without the need for these sampling and extraction procedures to isolate the analyte prior to analysis.

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 pyridine and other volatile organic compounds in blood. These methods use high resolution gas chromatography and magnetic sector mass spectrometry which gives detection limits in the low parts per trillion range.

The Cooperative Institute for Research in Environmental Sciences (CIRES) at the University of Colorado, Boulder, is conducting research to improve methods of analysis for pyridine and related compounds in environmental samples.

Improvements continue to be made in chromatographic separation and detection. Problems associated with the collection of pyridine on a sorbent trap, followed by thermal sorption, may be overcome with direct purging to a capillary column with whole column cryotrapping (Pankow and Rosen 1988) or by trapping on a very thick film (about 100 pm) of cross-linked silicone (Roeraade and Blomberg 1989). Current activities in the areas of supercritical fluid extraction (King 1989) and supercritical fluid chromatography (Smith 1988) include determination of compounds such as pyridine in biological samples and environmental media. Fourier transform infrared flow cell detectors are sensitive and selective for the detection of compounds such as pyridine that have been separated by supercritical fluid chromatography (Wieboldt et al. 1988). Immunoassay methods of analysis are also promising for the determination of various organic substances, and it is reasonable to assume that pyridine and its metabolites are candidates for this type of analysis.