6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring nitrobenzene 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 nitrobenzene. 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 nitrobenzene 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 a trade association 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.

6.1 BIOLOGICAL MATERIALS

Nitrobenzene is volatile; it has a boiling point of 211°C and a vapor pressure (20°C) of 0.15 mmHg. Its water solubility (20°C) is 1,900 mg/L. It has a log octanol/water partition coefficient value of 1.85, implying a relatively weak affinity for lipids. These properties affect the manner in which biological samples are analyzed for nitrobenzene. Albrecht and Neumann (1985) discussed the difficulty of analysis of nitrobenzene and its metabolite aniline in animals. Excretion of the parent compounds or some metabolites in urine has been determined, but apparently this kind of biological monitoring has so far not produced satisfactory results due to practical and methodological reasons. Nitrobenzene metabolites are bound to blood proteins, both in hemoglobin and in plasma (Albrecht and Neumann 1985). Acute poisoning by nitrobenzene is usually monitored by measuring levels of methemoglobin, which is produced by the metabolic products of nitrobenzene. However, many toxicants produce methemoglobin, and this analysis is not specific enough to be a satisfactory method for monitoring nitrobenzene in animals.

Analytical methods for the determination of nitrobenzene in biological materials are given in Table 6-l.

6.2 ENVIRONMENTAL SAMPLES

Nitrobenzene is determined in environmental samples by collection, extraction with an organic solvent and gas chromatographic analysis (EPA 1982a, 1982b; NIOSH 1984). Flame ionization detection or mass spectrometry may be used for detection.

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

Sample Matrix	Sample Preparation	Analytical Method	Sample Detection Limit	Accuracy	Reference
Urine (spiked with nitrobenzene)	Reduce nitrobenzene, form coupled dye, extract in carbon tetrachloride	Colorimetric at 450 nm	0.8 mg/L	No data	Dangwal and Jethani 1980
Urine	Measure p-nitrophenol metabolite in urine	No data	No data	No data	Ikeda and Kita 1964
Urine	Measure p-nitrophenol metabolite in urine	No data	No data	No data	Lauwerys 1983
Blood	Measure methemoglobin formation in blood	No data	No data	No data	Albrecht and Neumann 1985
Blood	Measure methemoglobin formation in blood	No data	No data	No data	Lauwerys 1983
Blood	Measure methemoglobin formation in blood	Photometric	No data	No data	Dreisbach and Robertson 1987

mg = milligram; L = liter; nm = nanometer.

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Analytical methods for the determination of nitrobenzene in environmental samples are given in Table 6-2.

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, 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 nitrobenzene 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 nitrobenzene.

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 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Sensitive and selective methods are available for the qualitative and quantitative measurement of nitrobenzene after it is separated from its sample matrix. Capillary gas chromatography, also known broadly as high-resolution gas chromatography (HRGC), has greatly facilitated the analysis of compounds such as nitrobenzene 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 much less crucial than was the case with packed columns. The instrumental capability to separate volatile analytes by HRGC is no longer the limiting factor in their analysis. Further development of methods for the transfer of isolated analytes, quantitatively and in a narrow band, to the HRGC, and the identification and accurate measurement of compounds in the HRGC peaks would be useful. Mass spectrometry (MS) has been outstanding for the detection of various organic compounds but other techniques, particularly Fourier transform infrared spectroscopy (FTIR) may be superior for nitrobenzene. Because nitrobenzene is metabolized in biological systems, it is difficult to accurately determine in most biological samples after enough time has elapsed for these metabolic processes to take place. Therefore, although nitrobenzene itself can be easily determined in biological samples, it is rarely found in its unchanged form in these samples. Metabolites of nitrobenzene in biological materials are difficult to determine in

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

Sample Matrix	Sample Preparation	Analytical Method	Sample Detection Limit	Accuracy	Reference
Air at landfill sites	Adsorption on Tenax-GC cartridges, thermal desorption	HRGC/FID	0.05 ppb	No data	Harkov et al. 1985
Air	Adsorption on silica gel, extraction with methanol	GC/FID	0.02 mg/sample	No data	NIOSH 1984
Air	Adsorption on silica gel, extraction with methanol	GC/FID	0.5 mg/m^3	No data	NIOSH 1977
Wastewater	Direct injection of aqueous sample	GC/FID	No data	No data	Patil and Shinde 1988
Wastewater	Extract with dichloromethane, exchange to hexane, concentrate	GC/FID	3.6 µg/L	71± 5.9%[a]	EPA 1982a
Water	Extract with dichloromethane at pH 11 and 2, concentrate	GC/MS	1.9 µg/L	71±31%[a]	EPA 1982b
Soil and solid waste	Extract from sample, cleanup	GC/FID	137 mg/kg[b]	25.7-100%[a]	EPA 1986b
Soil and solid waste	Extract from sample, cleanup	GC/MS	19 mg/kg[b]	No data	EPA 1986c
Soil and solid waste	Extract from sample, cleanup	GC/FID	660 µg/kg[c]	54-158%[a]	EPA 1986d
Soil and solid waste	Extract from sample, cleanup	HRGC/FTIR	12.5 µg/L[d]	No data	EPA 1986e

[a]Relative recovery, percent, ± standard deviation.
[b]Approximate detection limit in high-level soil and sludges.
[c]Approximate detection limit in low-level soil and sediments.
[d]Detection limit in water. Detection limit in solids and wastes is several orders of magnitude higher.

HRGC = high resolution gas chromatography; FID = flame ionization detector; ppb = parts per billion; GC = gas chromatography; mg = milligram; m^3 = cubic meter; µg/L = micrograms/liter; MS = mass spectrometry; kg = kilogram; FTIR = fourier transform infrared spectrometry.

routine practice because of the lack of standardized methods for measuring these compounds and because of the difficulty of correlating the presence or levels of these metabolites directly with exposure to nitrobenzene.

The development of supercritical fluid (SCF) extraction combined with chromatographic analysis will probably be useful for meeting the goals of quantitative, rapid, easily performed, low cost and safe procedures for the determination of poorly volatile organic analytes such as nitrobenzene and its metabolites in biological samples (Hawthorne 1988).

Methods for the determination of biomarkers of effect for nitrobenzene are largely confined to measurement of methemoglobin, which is also produced by numerous toxicants other than nitrobenzene. More specific methods for biomarkers of exposure would be helpful in toxicological studies of nitrobenzene.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining the parent compound, nitrobenzene, in water, air, and waste samples with excellent selectivity and sensitivity are well developed and constantly improving. It is desirable to have the means to measure organic compounds such as nitrobenzene in situ in water and other environmental media without the need for sampling and extraction procedures to isolate the analyte prior to analysis.

6.3.2 On-going Studies

Research is ongoing to develop a Master Analytical Scheme for organic compounds, including nitrobenzene, in water (Michael et al. 1988). The overall goal 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 "semi-soluble" in water, as well as volatile compounds (bp < 150°C). Improvements continue to be made in chromatographic separation and detection, including the areas of supercritical fluid extraction and supercritical fluid chromatography (Smith 1988). An important aspect of supercritical fluid chromatographic analysis of compounds such as nitrobenzene is detection. Fourier transform infrared flow cell detectors are promising for this application (Wieboldt et al. 1988). Immunoassay methods of analysis are promising for the determination of various organic pollutants and toxicants, and nitrobenzene may be a candidate for immunoassay techniques.

6. ANALYTICAL METHODS

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 nitrobenzene and other compounds. These methods use high resolution gas chromatography and magnetic sector mass spectrometry.