6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring chlorobenzene 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 chlorobenzene. 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 chlorobenzene in environmental samples are 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

Many of the considerations regarding the analysis of halogenated alkanes and alkenes in biological samples (Fishbein 1985) similarly apply to the determination of chlorobenzene in these samples. Although most environmentally significant halogenated alkanes and alkenes have boiling points below 100°C, chlorobenzene is relatively less volatile with a boiling point of 132°C. The water solubility (25°C) of chlorobenzene is 472 mg/L, which is lower than the water solubilities of most environmentally and toxicologically significant halogenated alkanes and alkenes. Along with many halogenated alkanes and alkenes, chlorobenzene is classified as a purgeable species for purge-and-trap analysis (EPA 1982a, 1982b). Therefore, many of the approaches and methods used for the determination of halogenated alkanes and alkenes in biological samples are applicable to chlorobenzene, although they have not been validated as a sampling method.

Because chlorobenzene is volatile, has limited water solubility, and has a moderate affinity for lipid tissue, chlorobenzene is easily lost from biological samples. Appropriate care must be exercised in handling and storing such samples for analysis of chlorobenzene.

The methods that generally are used to remove volatile organic chemicals (VOCs) from biological samples for analysis are applicable to chlorobenzene. These include headspace analysis, purge-and-trap (gas stripping) collection from aqueous solutions or slurry samples, solvent extraction, and direct collection on resins. Headspace analysis offers speed, simplicity, and good reproducibility for a particular type of

sample. However, partitioning of the analyte between the headspace and the sample matrix is dependent upon the nature of the matrix and must be determined separately for different kinds of matrices (Walters 1986).

Purge-and-trap collection is well suited to biological samples that are soluble in water and is readily adapted to biological samples from techniques that have been developed for the analysis of halocarbons such as chlorobenzene in water and wastewater. For water-insoluble materials, the purge-and-trap approach is complicated by the uncertainty of partitioning the analyte between sample slurry particles and water.

Homogenization of tissue with the extractant and lysing of cells improves extraction efficiency. When multiple analytes are determined using solvent extraction, selective extraction and loss of low-boiling compounds can cause errors. The commercial availability of highly purified solvents has largely eliminated problems with solvent impurities, although high costs, solvent toxicities, and restrictions on spent solvent disposal must be considered. Directly coupled supercritical fluid extraction-gas chromatography has been used for the determination of polychlorinated biphenyls (Hawthorne 1988) and should work well for the determination of chlorobenzene in biological samples.

Analytical methods for the determination of chlorobenzene in biological samples are given in Table 6-1.

6.2 ENVIRONMENTAL SAMPLES

Purgeable organic compounds such as chlorobenzene can be determined in water by the purge-and-trap technique. This method consists of bubbling inert gas through a small volume of the sample and collecting the vapor in a trap packed with sorbent. The analytes are then removed from the trap by heating it and backflushing the analytes onto a gas chromatographic column. The two materials most widely used for adsorption and thermal desorption of volatile organic compounds collected by the purge-and-trap technique are Carbotrap® consisting of graphitized carbon black, and Tenax® a porous polymer of 2,6-diphenyl-pphenylene oxide (Fabbri et al. 1987).

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

Sample Matrix	Sample Preparation	Analytical Method	Sample Detection Limit	Accuracy	Reference
Breath, blood, urine	Breath collected on Tenax, blood and urine subjected to purge-and-trap, concentrated on cryogenic capillary trap, thermally removed to GC.	GC/MS	No data	No data	Barkley et al. 1980
Fish tissue	Grind with sodium sulfate, extract with hexane/acetone	GC/ECD	No data	No data	Oliver and Nicol 1982a, 1982b
Adipose tissue	Extraction, bulk lipid removal, Florisil fractionation	HRGC/MS	0.1 μg/g	No data	Mack and Stanley 1984
Adipose tissue	Heated dynamic headspace purge-and-trap	HRGC/MS	2 ng/g	No data	Stanley 1986
Biofluids[a]	Dilute with water, sealed vial, collection of headspace vapors	GC/ECD	No data	No data	Suitheimer et al. 1982
Blood, tissue	Macerate tissue in water, warm blood or tissue, pass inert gas through, trap on Tenax, thermal desorption	GC/MS	3 ng/mL blood 6 ng/g tissue	No data	Pellizzari et al. 1985

[a] Among the compounds for which this method was used are benzene, m-xylene, carbon tetrachloride and chloroform. The method can be adapted to chlorobenzene although the procedures do not list this compound specifically as an analyte.

GC = gas chromatography; MS = mass spectrometry; ECD = electron capture detector; HRGC = High Resolution Gas Chromatography; μg/g = microgram per gram; ng/g = nanogram per gram.

6. ANALYTICAL METHODS

The introduction of capillary column chromatography has markedly improved both the sensitivity and resolution of gas chromatographic analysis of environmental samples such as chlorobenzene. Because of the very small quantities of sample required, capillary column chromatography has made sample delivery more difficult. One of the more promising approaches to sample introduction using capillary columns with purge-and-trap collection is the use of cryofocussing. Basically, this procedure consists of collecting purged analyte on a short section of the capillary column cooled to a low temperature (e.g., -100°C) temperature, followed by heating and backflushing of the sample onto the analytical column. Chlorobenzene has been determined in water by this method (Washall and Wampler 1988).

Chlorobenzene can be removed from water by adsorption on synthetic polymers contained in cartridges, followed by thermal desorption of analyte (Pankow et al. 1988). Among the products used for this purpose are Tenax-GC® and Tenax-TA®.

Analytical methods for the determination of chlorobenzene 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 chlorobenzene 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 chlorobenzene.

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.

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

Sample Matrix	Sample Preparation	Analytical Method	Sample Detection Limit	Accuracy	References
Air	Collect on Tenax GC, thermal desorption, cryogenic collection on a capillary trap, thermal transfer to GC	GC/MS	0.47 parts per trillion	No data	Krost et al. 1982
Air	Coconut shell charcoal sorption, carbon disulfide desorption	GC/FID	10 μg per sample	No data	NIOSH 1984
Water	Purge-and-trap	GC/HSD	0.25 μg/L	No data	EPA 1982a
Water	Purge-and-trap	GC/MS	0.2 μg/L	No data	EPA 1982b
Water	Purge-and-trap	GC/MS	6.0 μg/L	No data	EPA 1982c
Water	Sorption on small dead volume Tenax cartridges, thermal desorption	HRGC/MS	No data	No data	Pankow et al. 1988
Contaminated soil	Purge-and-trap	GC/HSD	300 μg/kg	No data	EPA 1986a
Wastes (non-water miscible) and soil	Purge-and-trap	GC/MS	250 μg/kg	No data	EPA 1986b
Wastes (water miscible and non-water miscible) and soil	Purge-and-trap	GC/MS	250-2500 μg/kg	No data	EPA 1986c

GC = gas chromatography; MS = mass spectrometry; FID = flame ionization detector; μg = microgram; HSD = halide specific detector; L = liter; HRGC = high resolution gas chromatography; kg = kilogram.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Excellent sensitive and selective methods are available for the qualitative and quantitative measurement of the parent compound, chlorobenzene after it is separated from its sample matrix. Methods need to be validated for chlorobenzene.

Further studies on the transfer analytes that have been purged or extracted from a biological or environmental sample quantitatively and in a narrow band to the capillary GC would better characterize exposure. Improvements in cryofocussing of VOC analytes for capillary GC determination of VOCs (Washall and Wampler 1988) should improve sensitivity for the determination of chlorobenzene.

Metabolites of chlorobenzene in biological materials cannot be determined in routine practice because of the lack of standard methods for measuring these metabolites. Further research on supercritical fluid (SCF) extraction holds great promise for meeting the goals of quantitative, rapid, easily performed, low cost, and safe procedures for the determination of nonpolar organic analytes such as chlorobenzene in biological samples.

Central nervous system, liver, and kidney injuries are characteristic biomarkers for effects of chlorobenzene intoxication. Since the effects are indicative of exposure to many other toxicants, methods are needed for more specific biomarkers.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining the parent compound, chlorobenzene, in water, air, and waste samples with excellent selectivity and sensitivity are highly developed, thus the database in this area is good and undergoing constant improvement.

Means to measure organohalides such as chlorobenzene in situ in water and other environmental media could contribute to environmental studies of this compound.

Degradation products of chlorobenzene in environmental media are difficult to determine. This difficulty is not so much an analytical problem as it is a problem of knowing the fundamental environmental chemistry of these compounds in water, soil, air, and biological systems.

6.3.2 On-going Studies

Research is ongoing to develop a "Master Analytical Scheme" for organic compounds in water (Michael et al. 1988), which includes chlorobenzene as an analyte. 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 semivolatile compounds and some compounds that are only "semi-soluble" in water, as well as volatile compounds (bp < 150°C).

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 chlorobenzene and other volatile organic compounds in blood. These methods use purge and trap and magnetic mass sector spectrometry which gives detection limits in the low parts per trillion range.