6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring chlordane, its metabolites, and other biomarkers of exposure and effect to chlordane. 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 may be 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 may be included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1   BIOLOGICAL MATERIALS

Since technical chlordane is a mixture of organochlorine compounds and since many of these chemicals are metabolized in the body, analytical methods for chlordane are designed to quantitate major components of technical chlordane and their metabolites. The methods commonly used for the quantification of chlordane compounds in human blood, adipose tissue, and milk are given in Table 6- 1. Generally, these methods employ procedures such as digestion, extractions with solvents, or column chromatography to reduce interferences from lipids and other pollutants that may be present in the sample. Recently, supercritical carbon dioxide as the extraction medium has received some attention (Lopez-Avila et al. 1990a; Nam et al. 1989, 1990). Methods using supercritical fluid extraction (SCF) have good recoveries and avoid the use of potentially polluting solvents. Gas chromatography with electron capture detection (GC/ECD) is the method of choice for quantification, because of its high sensitivity for chlordane compounds and its lack of sensitivity for the large excesses of lipids in the sample matrix (Young et al. 1984). The use of capillary columns is likely to show better resolution of the components, although high resolution gas chromatography columns (HRGC) have rarely been used for biological samples for chlordane. Recently, techniques have been developed in which enantomers (optical isomers) of technical chordane components can be determined. This has been accomplished by using chiral high-resolution GC columns containing ß-cyclodextrin derivatives (Buser et al. 1992). Using such techniques, enantiospecific biological processes affecting

TABLE 6-1. Analytical Methods for Determining Chlordane in Biological Materials[a]

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human blood	Sample mixed with water and extracted with acetone-hexane; extract evaporated to dryness; treated with H_2SO_4/HNO_3 at 0°C; product mixed with water subjected to cyclic steam extraction with heptane; extract reduced by iron/acetic acid and concentrated	GC-ECD	No data	96 at 10 µg/kg	Saito et al. 1985
Human blood	Sample mixed with formic acid extracted with hexane; cleaned up by Florisil column chromatography; extract concentrated	GC-ECD	0.01 µg/L	No data	Wariishi et al. 1986
Human blood	Sample mixed with H_2SO_4 extracted with hexane/acetone; concentrate extract	GC-coulometric detector and ECD	15 ng (coulometric) 10 ng (ECD)	75–86 at 50–2000 ng added	Griffith and Blanke 1974
Human adipose tissue	Fat from tissue extracted with solvent; cleaned up by column chromatography and concentrated	GC-ECD	0.1 ppm (oxychlordane)	102 ppm (oxychlordane)	Barquet et al. 1981
Adipose tissue	Fat extracted from tissue with solvent; subjected to GPC and Florisil cleanup; concentrated	HRGC-ECD	1.3 µg/kg (trans-) 1.0 µg/kg (cis-)	87–100 (trans-) at 10–500 ng/g 64–91 (cis-) at 10–500 ng/g	LeBel and Williams 1986; Williams et al 1988a

TABLE 6-1. Analytical Methods for Determining Chlordane in Biological Materials *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Adipose tissue	Dry macerated sample extracted with petroleum ether and aceto-nitrile; concentrated; cleaned by column chromatography	GC-ECD	No data	>80	EPA 1977
Human milk	Potassium oxalate added to sample and extracted with solvent to extract lipid; dissolved in aceto-nitrite and extracted in hexane; cleaned by column chromatography; concentrated	GC-ECD	No data	79 (*trans-*) 97 (*cis-*) 90 (oxychlordane) at 0.2 ng/L	Tojo et al. 1986
Human sebum (skin lipids)	Sampled with gauze defatted and moistened with ethanol. Potassium oxalate added to sample and etracted with solvent, dissolved in acetonitrate, extracted with hexane, and cleaned by column chromatography	GC-ECD	No data	107.8 (*trans*) 116.2 (*cis*)	Wariishi and Nisahiyama 1989
Serum, liver, blood cells,	Extraction with acetone-hexane-diethyl-ether-petroleum ether (blood cells, liver) hexane (serum)	HRGC-MS/SIM	No data	No data	Mussalo-Rauhamaa 1991
Feces	*Solvent extraction*	*GC-ECD and* GC-coulometric detector	*No data*	*No data*	*Aldrich and Holmes* 1969

TABLE 6-1. Analytical Methods for Determining Chlordane in Biological Materials *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Solvent extraction	GC-ECD and GC-coulometric detector	No data	No data	Aldrich and Holmes 1969

[a]Methods are suitable for *cis*- and *trans*-chlordane, oxychlordane, *trans*-nonachlor, and other chlordane compounds.

ECD = electron capture detector; GC = gas chromatography; GPC = gel permeation chromatography; H_2SO_4 = sulfuric acid; HNO_3 = nitric acid; HRGC = high resolution gas chromatography; MS = mass spectrocopy; SIM = selective ion monitoring

biological behavior and biodegradation may be better understood and readily differentiated from nonenantiospecific abiotic processes.

6.2 ENVIRONMENTAL SAMPLES

The analytical methods used for the quantification of chlordane in environmental samples are given in Table 6-2. The collection efficiencies for chlordane from air by five solid sorbents (C-18, Carbowax GC, Chromosorb 102, polyurethane foam, and Tenax GC) have been compared, and both Chromosorb 102 and polyurethane foam are preferable. Though Chromosorb 102 is more efficient than polyurethane foam at low sampling rates, it has the disadvantage of giving more interference during gas chromatography analysis than polyurethane foam (Roper and Wright 1984). A more recent study compared the trapping efficiencies of chlordane on four absorbents used by government and laboratories to collect airborne pesticides, chromosorb 102, ORB0 44, polyurethane foam (PUF), and Tenax GC (Leidy and Weight 1991). The efficiencies of all absorbents were comparable and ranged from 87-92%. Environmental samples are prepared and interferences removed by a variety of methods such as solvent extraction, column chromatography, gel permeation chromatography (GPC), sulfuric acid treatment, and activated copper treatment (Alford-Stevens et al. 1988; EPA 1986a, Hopper 1982; Young et al. 1984). The different methods for extraction and cleanup of chlordane in soil and sediment samples have been compared; it was concluded that mechanical shaking with Florisil clean up provided the best precision, but sonication with gel permeation chromatography (GPC) was less time consuming and may be applicable to a wide variety of pollutants (Alford-Stevens et al. 1988). Treatment with sulfuric acid was found to reduce interferences from polychlorinated biphenyls and p,p-DDT in waste water samples without altering the concentration of chlordane; sample treatment with ethanolic potassium hydroxide may partially destroy chlordane (Hemandez et al. 1987).

The preferred method for the quantification of chlordane in environmental samples is gas chromatography (GC) with electron capture detector (ECD) and the subsequent confirmation by mass spectrometry (MS). The ECD discriminates large excesses of lipids from the matrix, that otherwise interfere with MS (Sperling et al. 1985). Interferences by phthalate esters can pose a serious problem in chlordane determinations using ECD. These can be avoided by using a microcoulometric or electrolytic conductivity detector (EPA 1982a). Otherwise, this interference is minimized by avoiding contact with plastics and by scrupulous cleanup of glassware and reagents. The use of capillary columns will provide better separation of components (Sperling et al. 1985).

TABLE 6-2. Analytical Methods for Determining Chlordane in Environmental Samples[a]

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Ambient air	Sample passed through porous polyurethane foam or Tenax; desorbed by soxhlet extraction; extract cleaned by column chromatography and fuming H_2SO_4 and fraction concentrated	GC-ECD	No data	75-95	Billings and Bidleman 1980
Ambient air	Sample passed through polyurethane foam; soxhlet extracted with solvent and concentrated	HRGC-ECD and HRGC-MS	$0.15\ \mu g/m^3$	No data	Hsu et al. 1988
Ambient air	Sample passed through filter and Chromosorb 102 absorbent; extracted with toluene	GC-ECD	$0.1\ \mu g$ per sample	96-100	NIOSH 1989b
Indoor air	Sample passed through chromosorb 102; extracted ultrasonically with solvent and concentrated	GC-ECD	No data	95-108 at 0.0021– $0.0185\ mg/m^3$	Thomas and Nishioka 1985
Water, waste water	Extract sample with solvent; clean up with Florisil and/or activated copper and concentrate extract	GC-ECD (EPA method 608)	$0.014\ \mu g/L$ (waste water)	93 at $20\ \mu g/L$ (waste water)	EPA 1982a; Reding 1987
Water, waste water	Extract sample with solvent at basic pH; concentrate extract.	GC-MS (EPA method 625)	No data	No data	EPA 1982a

TABLE 6-2. Analytical Methods for Determining Chlordane in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water, waste water	Extract with solvent at pH 7; concentrate extract.	HRGC-MS (EPA method 625.1 and 680)	1-10 µg/L 46 at 3 µg/L	93 at 20 µg/L	Eichelberger et al. 1983; Alford-Stevens et al. 1988
Drinking water	Extract with solvent; column chromatographic cleanup; concentrate.	GC-ECD	23 ng/L (*trans-*) 28 ng/L (*cis-*)	79 (*trans-*) 94 (*cis-*)	Barquet et al. 1981
Drinking water	Extract with solvent and concentrate.	HRGC-MS (EPA method 508)	0.002 µg/L (*trans-*) 0.004 µg/L (*cis-*)	mean recovery recovery at 0.7 µg/L 87.6 (*trans-*); 87.2 (*cis-*)	Reding 1987; Lopez-Avila et al. 1990b
Waste water	Extract with solvent; clean with concentrated H$_2$SO$_4$; concentrate extract	GC-ECD	No data	114 at 0.16 mg/L	Hernandez et al. 1987
Groundwater/ leachate	Extract in solvent; clean up by column chromatography; concentrate extract	GC-ECD (EPA-CLP method)	0.5 µg/L	No data	EPA 1987a
Water, soil, sediment and solid waste	Extract in solvent; clean up by column chromatography and/or activated copper if required; concentrate	GC-ECD (EPA method 8080)	0.14 µg/L	0.82c to 0.04 where c is the actual concentration	EPA 1986a

TABLE 6-2. Analytical Methods for Determining Chlordane in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil and sediment	Sample extracted with solvent in a shaker or by sonication and cleaned up by Florisil on GPC	HRGC-MS	No data	No data	Alford-Stevens et al. 1988
Soil and sediment	Extract in solvent ultrasonically; clean up by column chromatography; concentrate	GC-ECD	80 µg/kg	No data	EPA 1987a
Soil, sludges, and solid wastes	Extract in solvent ultrasonically; clean up by column chromatography; and activated copper if required; concentrate.	GC-ECD	9.4 µg/kg (soil) 140 µg/kg (sludge) 1.4 mg/kg (solid waste)	0.82c - 0.04 where c is the actual concentration	EPA 1986a
Herring fat	Extract fat by solvent; clean up by H_2SO_4 treatment and silica gel column chromatography; concentrate	GC-NCI-MS	No data	No data	Jansson and Wideqvist 1983
Vegetable oil	Dissolve sample in a solvent/ solvent mixture; filter if necessary clean up by GPC; Florisil column chromatography and alkali; concentrate extract	GC-ECD	0.3 ppm	No data	Young et al. 1984
Cod liver oil	Sample dissolved in a solvent is partitioned in dimethyl formamide; subjected to reversed phase partitioning; and extract concentrated	HRGC-MS	No data	No data	Sperling et al. 1985

TABLE 6-2. Analytical Methods for Determining Chlordane in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Poultry, beef, and swine fat	Dissolve fat in solvent mixture; clean up by GPC; concentrate extract.	GC-ECD	No data	85-110 at 0.11-0.56 ppm	Ault and Spurgeon 1984; Ault et al. 1985
Cod liver oil, corn oil, butter fat, fat extracts from FDA adult total diet	Sample dissolved in solvent subjected to GPC and Florisil clean-up; extract concentrated	GC-ECD	No data	86-98	Hopper 1982

[a]Methods are suitable for *cis-* and *trans-*chlordane, oxychlordane, *trans-*nonachlor and other chlordane compounds.

CLP = contract laboratory program; ECD = electron capture detector; GC = gas chromatography; GPC = gel permeation chromatography; H_2SO_4 = sulfuric acid; HRGC = high resolution gas chromatography; MS = mass spectrometry; NCI-MS = negative chemical ionization-mass spectrometry

An enzyme immunoassay has been developed for screening soil samples for chlordane (U.S. EPA 1988d). While the method is potentially inaccurate, due to cross reactivity of the enzyme with other cyclodiene pesticides, it is rapid, simple, sensitive, and inexpensive and is therefore useful for screening purposes and field applications.

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 chlordane 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 chlordane.

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.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Levels of various components of technical chlordane (principally *cis-* and *trans-*chlordane, *cis-* and *trans-*nonachlor) and its metabolites (principally oxychlordane) in body tissue and fluids are elevated in individuals exposed to chlordane. While analytical methods are available (Aldrich and Holmes 1969; Barquet et al. 1981; EPA 1977; Griffith and Blanke 1974; LeBel and Williams 1986; Mussalo-Rauhamaa 1991; Saito et al. 1985; Tojo et al. 1986; Wariishi et al. 1986) for the quantification of chlordane compounds and their metabolites in biological matrices, and these methods have good sensitivity and specificity, there are no data correlating these levels with environmental chlordane concentrations to which a person was exposed.

There are no biomarkers of effect for chlordane.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. The levels of chlordane in different environmental media can be used to indicate exposure of humans to mixture compounds through the inhalation of air, ingestion of drinking water and foods, and exposure to soils containing chlordane. If a correlation with human tissue or body fluid levels is available, the intake levels from different environmental sources can be used to estimate the body burden of the chemical in humans. Although the products of biotic and abiotic processes of chlordane in the environment are known, few systematic studies are available in which the concentrations of its reaction products were measured in the environment. In instances where the product(s) of an environmental reaction is (are) more toxic than the parent compound, it is important that the level of the degradation products in the environment be known. Analytical methods for the determination of chlordane compounds and their degradation products in air, water, soil, sediment, and food are available, and these methods have good sensitivity and specificity. The methods for determining degradation products of chlordane compounds are similar to those for the parent compounds.

6.3.2 On-going Studies

No significant on-going studies are in progress for the development of new analytical methodologies for analysis of this compound in environmental or biological samples.