7. ANALYTICAL METHODS

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

7.1 BIOLOGICAL MATERIALS

Analytical methods exist for measuring aldrin, dieldrin, and their metabolites in blood, body tissues, breast milk, urine, food, fish, and feces. The primary method used is gas chromatography (GC) coupled with electron capture detection (ECD). Since aldrin is metabolized rapidly to dieldrin, exposure to aldrin or dieldrin is measured exclusively by determining levels of dieldrin in blood. Exposure is also measured by determining the levels of dieldrin in fat since it is rapidly distributed to adipose tissue. Metabolites of aldrin and dieldrin have been measured in feces and urine; however, they are not routinely used to quantify exposure to aldrin or dieldrin (Klein et al. 1968; Walker et al. 1969). A summary of the methods for various biological media is presented in Table 7-1.

Dieldrin is determined in blood and fat using GC/ECD. Two commonly used preparation methods for determining levels of dieldrin in blood are the acetone extraction procedure and the hexane extraction procedure (EMMI 1997; Robinson et al. 1967). The difference between the two is in the initial step where dieldrin is extracted from blood with either acetone or hexane. Both preparation methods are followed by concentration and extraction with hexane. A comparison of the two methods showed that the concentration of dieldrin in the blood with the hexane extraction method is only 65–70% of the concentration of dieldrin in blood using the acetone extraction method. The authors suggest that the relationship may indicate a partitioning of dieldrin between hexane and whole blood (Robinson et al. 1967). The reproducibility of the acetone technique is better than that of hexane. One preparation

Table 7-1. Analytical Methods for Determining Aldrin/Dieldrin in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood (dieldrin)	Hexane extraction.	GC/ECD	1 ng/mL	100%	MacCuaig 1976
Blood or serum	Samples are extracted using hexane. Concentrate down to 0.5 mL in hexane. Dilution may be necessary.	GC/ECD HERL Method 004	NR	NR	EMMI 1997
Serum (dieldrin)	Denature with methanol, mixed solvent extraction with hexane/ethylether, elute from activated silica gel.	GC/ECD	NR	70–75%	Burse et al. 1983
Adipose tissue	Samples are extracted using petroleum ether and acetonitrile. Filter through sodium chloride. Concentrate to 5 mL in petroleum ether.	GC/ECD HERL Method 001	NR	NR	EMMI 1997
Tissue and human milk	Samples are extracted using acetonitrile and concentrated down using hexane.	GC/ECD HERL Method 003	NR	NR	EMMI 1997
Milk (aldrin and dieldrin)	Milk sample homogenized, fat extraction. Florisil clean-up, elution with hexane and acetonitrile.	GC/ECD	NR	NR	Stacey and Tatum 1985
Milk	Homogenize milk. Multiresidue extraction through microcartridge. Elution with hexane and methanol.	GC/ECD	NR	aldrin 99% dieldrin 70%	Barcarolo et al. 1988

Table 7-1. Analytical Methods for Determining Aldrin/Dieldrin in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Food	Samples are extracted using methyl cyanide. Residues are concentrated in petroleum ether and purified using Florisil.	GC/ECD AOAC Method 970.52	NR	$80%	Helrich 1990
Fatty foods	Samples are extracted using petroleum ether and acetonitrile. Clean-up using Florisil.	GC/ECD FDA Method 211.1	NR	NR	EMMI 1997
Non-fatty foods	Samples are extracted with acetonitrile or water-acetonitrile. Residues are transferred into petroleum ether.	GC/ECD FDA Method 212.1	NR	NR	EMMI 1997
Fish	Blended fish samples are extracted using petroleum ether and acetonitrile. Concentration and cleanup of extrant is done using an alumina or silica column.	GC/ECD USGS Method O9104	NR	NR	EMMI 1997
Feces (9-hydroxy-dieldrin)	Feces homogenized and extracted with acetone, then hexane. Florisil clean-up. Elute with acetone and hexane.	GC/ECD GC/MS	NR	NR	Richardson and Robinson 1971

Table 7-1. Analytical Methods for Determining Aldrin/Dieldrin in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (urinary metabolites of aldrin and dieldrin)	Urine mixed with ethyl ether and petroleum ether. Dried over anhydrous sulfate, concentrated. Florisil clean-up. Elution with ethyl ether/ petroleum ether to remove aldrin and ethyl ether/acetone to remove dieldrin.	GC/ECD	NR	NR	Klein et al. 1968
Fat, liver, brain (dieldrin)	Tissues extracted with hexane/acetone solution. Fats partitioned between hexane and dimethyl formamide. Florisil clean-up. Elution with 10% ether in hexane.	GC/ECD	0.5 ng	95%	Walker et al. 1969

AOAC = Association of Official Analytical Chemists; FDA = Food and Drug Administration; GC/ECD = gas chromatography/electron capture detector; GC/MS = gas chromatography/mass spectrometry; ng = nanogram; NR = not reported; USGS = U.S. Geological Survey

method used for measuring levels of dieldrin in fat includes extraction with hexane/acetone solution, partitioning between hexane and dimethylformamide (DMF), clean-up, and elution in hexane. Recovery and sensitivity of this technique are good. Precision was not reported (Walker et al. 1969).

Aldrin and dieldrin have also been measured in samples of milk using GC/ECD (Barcarolo et al. 1988; EMMI 1997; Stacey and Tatum 1985; Takei et al. 1983). Sample preparation steps for milk involve homogenization, lipid extraction with hexane and acetone, residue extraction with acetonitrile, and partitioning into hexane. Recovery was adequate for dieldrin and good for aldrin. Precision was good. Sensitivity was not reported (Barcarolo et al. 1988).

A method describing the extraction of aldrin and dieldrin from fish samples employs similar procedures (EMMI 1997). This method is only applicable for fish tissue containing at least 0.1 µg/kg of analyte. A specific detection limit, however, was not mentioned for aldrin or dieldrin. Homogenized fish samples are extracted using petroleum ether and concentrated in acetonitrile. Cleanup is performed using an alumina or silica column. A GC/ECD is used to determine the total concentration of aldrin or dieldrin in the sample. Percent recovery was not reported.

7.2 ENVIRONMENTAL SAMPLES

Methods exist for determining aldrin and dieldrin in air, water, municipal effluents, sludge, and soil (Clesceri et al. 1998a; EPA 1986j; NIOSH 1984; OSW 1986a). The most common methods involve separation by GC coupled with ECD, electrolytic conductivity detector, or mass spectrometry (MS). GC has also been used with Fourier transform infrared spectroscopy (FTIR). Table 7-2 summarizes the methods that have been used to analyze for aldrin and dieldrin in environmental samples. The primary methods used for analyzing aldrin and dieldrin in air are GC/ECD and GC/electrolytic conductivity detector. The preparation method recommended by NIOSH for analysis of aldrin in air samples involves trapping the air on a glass fiber filter and extraction in an isooctane gas bubbler (NIOSH 1984). An alternative procedure to this method is the replacement of the gas bubbler with a stainless steel trapping tube packed with Tenax®GC (Wallace and Sherren 1986). Tenax®GC is an efficient absorbent for aldrin. The solvent trapping efficiency for the isooctane procedure ranges from 83 to 94% while the trapping

Table 7-2. Analytical Methods for Determining Aldrin/Dieldrin in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (aldrin)	Adsorption on Tenax®-GC, elution with acetone/ petroleum spirit.	GC/ECD	0.003 ppb	76–110%	Wallace and Sherren 1986
Air (aldrin)	Collection on glass fiber filter; extract in isooctane glass bubbler.	GC/ECD NIOSH Method 5502	2.2 ppm	103%	NIOSH 1984
Water	Samples extracted with methylene chloride. Solvent exchange to hexane prior to GC analysis.	GC/ECD Method OSW 8080A	aldrin 0.004 ppb dieldrin 0.002 ppb	aldrin 81% dieldrin 90%	EPA 1986j
Water	Samples extracted with methylene chloride, dried and concentrated. Solvent exchange to hexane.	GC/MS	1 ppb for aldrin and dieldrin)	aldrin 83–96% in reagent water; 94% river water dieldrin 97–106% in reagent water, 90% in river water	Alford-Stephens et al. 1986
Municipal and industrial effluent	Samples extracted with methylene chloride. Heat solution to 80 EC and add hexane. Concentrate.	GC/ECD APHA Method 6630C	aldrin 0.004 ppb dieldrin 0.002 ppb	aldrin 100% dieldrin 100%	Clesceri et al. 1998a
Municipal and industrial effluent	Sample is extracted with methylene chloride at pH>11 and then at pH<2. Extract is dried, concentrated, and analyzed.	GC/MS APHA Method 6410B	aldrin 1.9 ppb dieldrin 2.5 ppb	aldrin 1–166% dieldrin 29–136%	Clesceri et al. 1998b

Table 7-2. Analytical Methods for Determining Aldrin/Dieldrin in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil	Samples extracted with acetone. Solvent exchange to hexane, dried over sodium sulfate; acetone added.	GC/MS	5 ng (aldrin and dieldrin)	aldrin 76–102% dieldrin 84–101%	Kobayashi et al. 1983
Soil	Soil mixed with acetone, filtered dried, extracted with hexane.	GC/MS	5 ng	aldrin 90% dieldrin 94%	Kobayashi et al. 1983
Solid waste, soils, and groundwater	Sample extraction varies depending on the matrix being tested.	GC/MS OSW Method 8250A	aldrin 1.9 ppb (water) dieldrin 2.5 ppb (water)	aldrin 0.1–166% dieldrin 29–136%	OSW 1986b
Soil/sludge	Samples are extracted with hexane-acetone (1:1) or methylene chloride-acetone (1:1). If necessary, an appropriate clean-up procedure is performed prior to analysis.	Narrow Bore Capillary Column with ECD OSW Method 8081B	aldrin 0.8 ppb (sludge) dieldrin 0.49 ppb (sludge)	aldrin 92% in sludge, 92% in clay dieldrin 89% in sludge, 113% in clay	OSW 1986a

APHA = American Public Health Assoication; EPA = Environmental Protection Agency; GC/ECD = gas chromatography/electron capture detector; GC/MS = gas chromatography/mass spectrometry; ng = nanogram; NIOSH = National Institute for Occupational Safety and Health; OSW = Office of Solid Waste

efficiency for Tenax®GC is >99%. Also, use of Tenax®GC does not require frequent replenishment of the volatile solvent needed for the isooctane bubbler, and the Tenax®GC trapping tube can be transported easily from sampling sites to the laboratory (Wallace and Sherren 1986). The sensitivity of these methods is in the low- to sub-ppb range. Precision is good. Recoveries for these methods are generally good but can range from 76 to 110%, depending on the series of solvents used in the preparation method. The methods most frequently used to analyze water samples containing aldrin and dieldrin are GC/ECD and GC/MS. Interferences by phthalate esters can pose a problem in pesticide determinations when using the ECD. Interferences from phthalates can best be minimized by avoiding contact with any plastic materials. The contamination from phthalate esters can be completely eliminated with an electrolytic conductivity detector (EPA 1986j).

Aldrin and dieldrin are isolated from aqueous media by extraction in methylene chloride followed by drying with sodium sulfate, concentration, and solvent exchange to hexane (Alford-Stevens et al. 1986; EPA 1986j; Marsden et al. 1986). The limit of detection for both aldrin and dieldrin is in the low- to sub-ppb range for GC/ECD and GC/MS, respectively. Accuracy is generally good with the percent recoveries for dieldrin (90–106%) being higher that those for aldrin (81–96%). The precision obtained using GC/MS was better than that obtained using GC/ECD. The majority of analytical laboratories continue to rely on ECD for determination of aldrin and dieldrin. The main reason is that ECD provides a greater degree of sensitivity than MS. The difference in sensitivity has been reported to be as much as 2–3 orders of magnitude. The sensitivity of this method, however, depends on the level of interferences. Samples may require cleanup with a Florisil® column. The ECDs, however, do not provide the molecular structure information that is obtained with an MS detector. The structural information increases the level of confidence that the compound being measured has been correctly identified (Alford-Stevens et al. 1986). GC/FTIR has also been used to measure aldrin and dieldrin in water. However, this is not the recommended method because chlorinated pesticides are weak infrared absorbers (Gomez-Taylor et al. 1978).

Aldrin and dieldrin in solid samples such as soil and sediment are quantified mainly by GC/ECD and GC/MS (EPA 1986j; Kobayashi et al. 1983; Marsden et al. 1986). The soil or sediment samples are prepared for analysis by extraction with a mixture of methylene chloride and acetone, followed by drying with sodium sulfate, and solvent exchange to hexane. Recoveries are generally good, and detection limits are in the low- to sub-ppb range for GC/MS and GC/ECD, respectively. While GC/ECD is highly sensitive, this method requires a complicated clean-up procedure to remove interferences in the sample that produce peaks having the same retention times. The MS detector is a simple, rapid, and selective

method for the determination of aldrin and dieldrin in soil and is free from sample-related interferences (Kobayashi et al. 1983). Aldrin and dieldrin have been measured in fruits and vegetables using GC/ECD. Sample preparation involves boiling in water with a cyclic steam distillation unit with 2,2,4-trimethyl-pentane in the solvent trap. Variations in recoveries were reported. Sensitivity and precision were not reported (Santa Maria et al. 1986).

7.3 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 aldrin and dieldrin are available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (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 aldrin and dieldrin.

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.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Methods exist for determining aldrin and dieldrin in blood (Burse et al. 1983; MacCuaig 1976; Robinson et al. 1967), milk (Barcarolo et al. 1988; Stacey and Tatum 1985; Takei et al. 1983), body tissues (Walker et al. 1969), feces (Richardson and Robinson 1971), and urine (Klein et al. 1968). These methods are sensitive for measuring levels at which health effects might occur, as well as background levels in the population. Methods for determining dieldrin in blood are relatively precise; however, improvements in recovery of dieldrin are needed. These improvements would allow for better evaluation of exposure to aldrin or dieldrin. Sensitive techniques exist for measuring dieldrin in tissues;

however, precision data are lacking. Data on the determination of dieldrin or its metabolites in milk, urine, and feces are limited. More information on the sensitivity and recovery obtained for these methods is needed to evaluate the value of using levels of dieldrin or its metabolites as an indicator of exposure.

Effect. The methods for determining biomarkers of effect are the same as those for exposure, and are subject to the same limitations. Improved methods could allow a better assessment of the relationship between levels of dieldrin in blood, body tissues, and fluids and the known health effects associated with these chemicals.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining levels of aldrin and dieldrin in air (NIOSH 1984; Wallace and Sherren 1986), water (Alford-Stevens et al. 1986; EPA 1986j), and soil (EPA 1986j; Kobayashi et al. 1983; Marsden et al. 1986) are sensitive enough to measure background levels in the environment, as well as levels at which health effects might occur. Analytical procedures for the analysis of aldrin and dieldrin in foods were also located (EMMI 1997). Research investigating the relationship between levels measured in air, water, soil, and foods and observed health effects could increase our confidence in existing methods and/or indicate where improvements are needed.

7.3.2 Ongoing Studies

No ongoing studies regarding new analytical methods for determining aldrin and dieldrin in environmental media or food products were reported in either the CRIS/USDA database or the Federal Research in Progress database (CRIS/USDA 2001; FEDRIP 2001).