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 endrin, its metabolites, and other biomarkers of exposure and effect to endrin. 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.

6.1 BIOLOGICAL SAMPLES

Endrin is relatively nonvolatile with a vapor pressure (25 (C) of only $2x10^{-7}$ torr. Its water solubility (25 ºC) is 0.20 mg/L, which is much less than the water solubilities of most environmentally and toxicologically significant halogenated alkanes and alkenes, though similar to values for some other common organochlorine pesticides of similar structure. It has a high octanol/water partition coefficient (log K_{ow} = 5.6), implying a strong affinity for lipids. Endrin aldehyde also has a low vapor pressure ($2x10^{-7}$ torr at 25 ºC). Its water solubility (25 ºC) is 50 mg/L and its octanol/water partition coefficient value is approximately the same as that of endrin. There are few corresponding data for endrin ketone; based on its chemical structure, log K_{ow} was calculated to be 4.99 (SRC 1995b) and this is in line with those calculated or measured for endrin aldehyde. These properties affect the manner in which biological samples are analyzed for endrin, endrin aldehyde, and endrin ketone. Specifically, the low volatilities of these compounds preclude their removal from biological samples by purging, and their lipophilicity complicates their removal from lipid fractions of such samples.

Several basic steps are involved in determining high-boiling lipophilic analytes such as endrin and its oxidized derivatives (aldehyde and ketone) in biological materials. The purpose of most of these steps is to remove the analyte(s) from the biological matrix into a concentrated, interference-free form suitable for analysis. Several major steps may be involved.

Tissue may have to be prepared (dried and homogenized) by grinding with sodium sulfate or materials such as reverse phase column packing. Prepared tissue or biological fluids are extracted with an organic solvent to remove analyte, usually with significant amounts of biological matter. The extract may be solvent-exchanged to a solvent more suitable for analysis.

A clean-up step may be employed using gel permeation chromatography, Florisil, silica gel or alumina column fractionation, or solid phase extraction (SPE).

Solvent evaporation/analyte concentration steps may be necessary. Diethyl ether has been a widely used solvent for the extraction of lipophilic organic analytes such as endrin and endrin aldehyde from biological fluids (Zlatkis and Kim 1976). Homogenization of tissue with the extractant, and lysing of cells improves extraction efficiency. When, as is often the case, multiple analytes are determined using solvent extraction, selective extraction and loss of low-boiling compounds can cause errors. The loss of volatile internal standards often results in a recovery of >l00% (Tang et al. 1993). 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. Extraction, the first step in the overall clean-up process, places the analyte in a form and matrix suitable for introduction into the instrument used for analysis. Clean-up of biological samples may often be complex and involve a number of steps (Walters 1986).

In favorable cases, clean-up of biological samples containing endrin and/or endrin aldehyde can be simplified and made faster by using SPE (Marble and Delfino 1988). With this technique, solvent and sample are passed over disposable, prepacked, bonded-phase columns which either retain analyte (for subsequent elution) or retain interfering substances, while allowing for the passage of analyte. Tissue samples ground with reverse phase packing material, placed in a column over activated Florisil, and eluted using acetonitrile resulted in high extraction efficiency and clean-up in one step for crayfish and catfish samples (Long et al. 1991b; Lott and Barker 1993). Long et al. (1991a) also applied this technique to screening of chlorinated pesticides (including endrin) in beef fat. Di Muccio et al. (1990) have shown that sulfuric acid-impregnated Kieselguhr (diatomaceous earth) in SPE columns can improve the chromatography of organochlorine pesticides when the matrix contains fatty materials.

A recent study (Viana et al. 1994) was conducted to examine the impact on recoveries of endrin, endrin aldehyde, and endrin ketone as a result of treatment of extracts with sulfuric acid, potassium

hydroxide, or chromium (VI) oxide. The treatments are sometimes used in clean-up procedures or have been impregnated into adsorbents. Their data showed that endrin is unstable to acid and oxidizing conditions, but is stable in alkali. Endrin aldehyde is stable under acidic conditions, shows some loss with alkali, and is completely decomposed by oxidative conditions. Endrin ketone is stable under acidic and oxidizing conditions, but not to alkaline conditions. It is extremely important that the performance of any preparation scheme be validated with standard compounds before use. An apparently poor recovery for endrin, for example, could be related to its transformation as a result of the procedure.

Procedures for the measurement of endrin and endrin aldehyde in biological samples are the same as those used for other organochlorine pesticides in similar samples. Endrin, endrin aldehyde and endrin ketone are measured by gas chromatography (GC) with electron capture detection (ECD) (EPA 1982a) or GC/MS detection (EPA 1982b). All recently reported work (approximately the past 10 years) used one or both of these techniques. GC/MS should be used to confirm any positive GU/ECD results, since organochlorine pesticides other than endrin respond to the ECD. As already stated, any extraction or processing steps that involve alkaline or acidic conditions may result in the decomposition of endrin or its transformation products (EPA 1982b; Viana et al. 1994). Analytical methods for the determination of endrin, endrin aldehyde, or endrin ketone in human biological samples are given in Table 6-1.

6.2 ENVIRONMENTAL SAMPLES

Endrin and endrin aldehyde are determined in environmental samples by extraction with an organic solvent and GC analysis (e.g., EPA 1982a, 1982b). Various extractions procedures have been evaluated including separatory funnel shake-out and continuous extraction at different pH values (Valkenburg et al. 1989), use of a one-step extractor/concentrator (Harrington-Fowler 1991), and use of a device (“Soxtec”) that has advantages over traditional Soxhlet extraction (Lopez-Avila et al. 1993). Interferences may be eliminated by using silica, Florisil, or alumina column clean-up procedures (e.g., Cruz et al. 1993; Harrington-Fowler 1991). The clean-up of fatty materials as developed by Di Muccio and co-workers (1990) has been applied to vegetable oils (Di Muccio et al. 1991) for analysis of 18 organochlorine pesticides, including endrin. The relatively recent application

Table 6-1. Analytical Methods for Determining Endrin and Metabolites in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Lipid material	Extraction with pet. ether, partition with acetonitrile, addition of satd. aq NaCl to back-extract pesticides to pet. ether, additional processing	GC/ECD or GC/MS	No data	No data	Walters 1986
Human viscera (endrin)	Extraction of homogenized sample with diethyl ether, volume reduction and clean-up using column chromatography on charcoal, alumina, and sodium sulphate; elution using ethyl ether; solvent removal and redissolution in acetone	TLC	No data	No data	Ganguly and Bhattacharyya 1973
Human serum (endrin)	Combination of serum with methanol followed by extraction with hexane/ethyl ether and clean-up using Florisil	GC/ECD	1 ppb	112.6–121.6 (16.6% RSD)	Burse et al. 1990
Human milk (endrin)	Mixing of milk with Florisil and elution with petroleum ether/dichloromethane (80:20, v/v); solvent removal and redissolution in hexane	GC/ECD, GC/MS	0.003 ppm (3 ppb)	No data	Alawi et al. 1992
Breast adipose (endrin)	Placement of adipose into extraction cell between layers of alumina followed by SFE with CO_2 and CO_2 modified with 5% dichloromethane; analyte recovery into cyclohexane; clean-up using neutral alumina	GC/ECD	10 ppb	73	Djordjevic et al. 1994

Table 6-1. Analytical Methods for Determining Endrin and Metabolites in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (anti-12-hydroxy-endrin glucuronide)	Addition of sodium metaperiodate to urine followed by heating to 70 °C for 45 minutes, addition of carbonate buffer, and extraction with hexane; confirmation of analyte by conversion to 12-ketoendrine with chromium trioxide in pyridine	GC/ECD	No data	92 at 10.5 ppm	Baldwin and Hutson 1980

GC = gas chromatography; GPC = gel permeation chromatography; ECD = electron capture detector; MS = mass spectrometry; MSD = mass selective detector; SFE = supercritical fluid extraction; TLC = thin layer chromatography

of supercritical fluid extraction (SFE) (Hopper and King 1991; Lopez-Avila et al. 1990; Snyder et al. 1992) to a large number of both organochlorine and organophosphorus pesticides (including endrin and its aldehyde and ketone) showed the efficacy of this method, although it was determined to be more expensive than traditional Soxhlet methodology. SFE involves the use of carbon dioxide at high pressure for extraction. The technique is potentially advantageous because of the mildness of the extraction, and because solvent removal is easy (reduced pressure simply allows CO_2 to convert from a supercritical state to a gas). As the emphasis shifts towards reduced use of organic solvents in general, and chlorinated solvents in particular, because of environmental concerns and costs associated with disposal, SFE can provide a quick payback and continued savings.

As noted above for biological sample analysis, detection of endrin and its derivatives may be accomplished using either ECD or mass spectrometry (MS). The same concerns about chemical transformation of endrin, endrin aldehyde, and endrin ketone during sample preparation as discussed for biological samples are valid for environmental samples. Bentabol and Jodral (1995) showed that the use of sulfuric acid completely destroyed endrin during its isolation from cheese. Many of the EPA methods, such as Method 508 for drinking water (EPA 1988e), include steps to measure the degradation of endrin into endrin aldehyde and endrin ketone that results from the procedure. Active sites within the GC injector or column can also impact conversions (Grob and Wagner 1993). An immunoassay method has been reported as a screening procedure for endrin in selected vegetables (Wigfield and Grant 1992). Table 6-2 gives the analytical methods for the determination of endrin and the aldehyde and ketone in environmental samples.

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 endrin 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 endrin.

Table 6-2. Analytical Methods for Determining Endrin and Transformation Products in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air[a]	Low volume sampler with glass fiber filter and ethylene glycol trap	GC/ECD	No data	No data	Harkov 1986
Air[a]	Adsorption/solvent extraction with polyurethane foam plug	HRGC/MS	No data	No data	Ligocki and Pankow 1985
Water	Extracted with dichloromethane/hexane, dry with sodium sulfate, concentrated.	GC/ECD	0.05 ppb	No data	ASTM 1988
Water	Extracted with dichloromethane, exchanged to hexane, concentrated.	GC/ECD	0.006 ppb	95±2.1[b]	EPA 1982a
Water	Extracted with dichloromethane at pH 11 and 2, concentrated.	GC/MS	No data	No data	EPA 1982b
Water	Solid phase extraction with Empore disk. Eluted with ethyl acetate. Dried with sodium sulfate, concentrated.	GC/ECD	4 ppt	86	Tomkins et al.1992
Water	Solid phase extraction. Eluted with pentane, concentrated.	GC/ECD	0.1 ppb	100	Russo et al. 1993
Water	Solid phase extraction followed by supercritical fluid extraction.	GC/MS	No data	136	Tang et al. 1993
Water	Acidified water. Extracted through Empore Disk with ethyl acetate followed by dichloromethane. Dried with sodium sulfate, concentrated.	GC/MS	No data	126–128	Kraut-Vass and Thoma 1991
Well water	Extracted/concentrated using reverse phase SPE columns. Eluted analytes withmethanol.	GC (detection not specified)	No data	58–67	Hogmire et al. 1990
Runoff water	Samples solvent extracted with methylene chloride, concentrated and cleaned up with Florisil.	GC/ECD	No data	No data	Marsh 1993

Table 6-2. Analytical Methods for Determining Endrin and Transformation Products in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Sea water	Samples filtered then extracted with pentane. Solvent dried then partitioned with sodium hydroxide. Organic fraction cleaned up with alumina and silica.	GC/ECD	<0.1 ppt	97	Cruz et al. 1993
Soil and solid	Extracted from sample, waste clean-up	GC/MS	No data	No data	EPA 1986b
Soil and solid	Extracted from sample, waste clean-up	HRGC/MS	No data	No data	EPA 1986c
Soils	1) Supercritical fluid extraction with CO_2 premixed with 3% methanol.	GC/MS	No data	97	Snyder et al. 1992
	2) Soil mixed with sodium sulfate. Soxhlet extracted with 1:1 hexane/acetone. Dried extracts with sodium sulfate, concentrated. Solvent exchanged to MTBE.	GC/ECD		97	
	3) Sonication extraction with 1:1 dichloromethane/acetone. Dried extracts with sodium sulfate, concentrated. Solvent exchanged to MTBE.				
Soils	Liquid extraction by concentric rotation. Extract dried with anhydrous sodium sulfate.	GC/ECD	2–30 ppb	90–110	Carey et al. 1976
Milk	Extraction with ethyl acetate-methanol-acetone (2:4:4). Clean-up with solid-phase extraction.	GC/ECD	≈2 ppb	108–116	Prapamontol and Stevenson 1991

Table 6-2. Analytical Methods for Determining Endrin and Transformation Products in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Milk	Solid phase extraction of milk mixed with 0.5% toluene. Eluted with hexane.	GC/ECD	No data	75.55	Barcarolo et al. 1988
Human milk	Homogenized sample with Florisil. Eluted with 80:20 pet, ether/ dichloromethane. Evaporated eluate. Dissolved residue in hexane.	GC/ECD	No data	No data	Alawi et al. 1992
Human milk (?)	Sample previously cleaned up by Florisil added to sulfuric acid-impregnated Kieselguhr SPE. Eluted with pet. ether. Transferred to isooctane.	GC/ECD	No data	Endrin converted to ketone	Di Muccio et al. 1990
Food	Food was chopped, then homogenized with dichloromethane. Dried with sodium sulfate. Solvent exchanged to cyclohexane. Sample cleaned up by GPC. Concentrated to 1.0 mL isooctane.	GC/ECD	10 ppt	No data	Davies 1988
Food	Blended food mixed with pelletized diatomaceous earth. Added mixture to extraction column. Extracted with SC-CO_2. Eluted nonfat samples from Florisil trap with acetone.	GC/ECD	No data	82–99	Hopper and King 1991
Olive oil	Oil dissolved in hexane, then transferred to SPE column. Eluted with acetonitrile. Residue after drying cleaned up using Florisil. Hexane/benzene/ethyl acetate used to elute fraction for analysis.	GC/ECD	No data	101	Di Muccio et al. 1991

Table 6-2. Analytical Methods for Determining Endrin and Transformation Products in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Apple	Samples blended/filtered and brought up in acetone. Partitioned into methylene chloride, dried and concentrated.	ELISA	10–30 ppm	23–82 (spiked samples)	Wigfield and Grant 1992

[a] Method applicable to chlorinated pesticides similar to endrin, such as aldrin and dieldrin
[b] Relative recovery, percent ± standard deviation, percent

ECD = electron capture detector; ELISA = enzyme-linked immunosorbent assay; GC = gas chromatography; HRGC = high resolution gas chromatography; MS = mass spectrometry; MTBE = methyl-*tert*-butyl ether; ppb = parts per billion; ppm = parts per million; ppt = parts per trillion; SPE = solid phase extraction

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. Endrin has been measured in various human samples including tissues, blood, and breast milk (Alawi et al. 1992; Burse et al. 1990; Djordjevic et al. 1994; Ganguly and Bhattacharyya 1973). Methods have been published for use with animal tissues and could serve as the basis for work with human samples (see Table 6-l). The determination of the glucuronide of anti-12-hydroxyendrin in urine (Baldwin and Hutson 1980) provides for a biomarker of exposure that might be specific to endrin; anti-12-hydroxyendrin can undergo dehydrogenation to form 12-ketoendrin. If a concurrent exposure to keto-endrin occurred, 12-hydroxyendrin could be formed by an *in vivo* reduction of 12-ketoendrin. The extent to which this could occur is not known. Baldwin and Hutson (1980) claimed 92% recovery for the method and did not detect any interferences in its application to urine from workers in an endrin synthesis plant. Based on this information, the method appears to be reliable. Work has been conducted to show that, as a result of endrin-induced oxidative stress, certain lipid metabolites are excreted in the urine of rats. Thus the levels of formaldehyde, acetaldehyde, malondialdehyde, and acetone were excreted in significantly increased quantities following exposure to endrin. These data probably reflect the response of the organism to a general xenobiotic-induced oxidative stress, and are not endrin-specific. In addition, various neurological effects can result from exposure to endrin (see Section 2.6.2), but these are not specific to endrin, endrin aldehyde, or endrin ketone.

Cyclodiene pesticides, of which endrin and its oxidized analogs are representative, can also be estimated by receptor-assay technique. Cyclodiene pesticides exert their mode of action by altering central nervous system membrane ion transport. In work reported by Saleh et al. (1993), a labeled amino acid, GABA, that binds to the chloride channel receptor is displaced by endrin (and other similar molecules), and thus serves as an assay for these pesticides. The GABA receptor was shown to be a potentially useful biomarker for organochlorine pesticides such as lindane, toxaphene, endrin, chlordane, and others. The assay involves small quantities of blood (0.1 mL), and requires only that

the plasma be separated from the blood for direct analysis. Sensitivity for several cyclodiene pesticides was in the low-to-mid-picomole range.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. The MRL for chronic oral exposure to endrin is 0.0003 mg/kg/day. Assuming a 70-kg individual and oral intakes of either 2 L/day of water or 2 kg/day of food, analytical methods would need to have sensitivities below 10.5 ppb (10.5 (μg /L or 10.5 (μg /kg) in either medium. The methods reported for drinking water have limits of detection (LODs) far below this value and are adequate (ASTM 1988; EPA 1982a, 1988e; Russo et aI. 1993; Tomkins et al. 1992). The needed sensitivities can be achieved for some foods (Alawi et al. 1992; Davies 1988; Nakamura et al. 1994; Prapamontol and Stevenson 1991; Schmitt et al. 1985). The LODs of FDA methods (1994a, 1994b) are within a factor of 2-3 of those needed to measure chronic exposure but can be used to monitor intermediate acute exposure (MRL = 0.002 mg/kg/day) where LODs of 70 ppb are needed. Additional methods for foods are needed to measure concentrations relevant to the chronic oral MRL. Many of the reported methods have not been validated for the oxygenated derivatives of endrin (endrin aldehyde and endrin ketone). Although many of the methods should work for these derivatives, this needs to be shown. No MRLs have been established for inhalation exposures.

6.3.2 Ongoing Studies

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, is developing methods for the analysis of endrin and other volatile organic compounds in blood. These methods use purge and trap methodology, high resolution CG and magnetic sector MS which gives detection limits in the low parts per trillion (ppt) range.

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, is developing methods for the analysis of endrin and phenolic compounds in urine. These methods use high resolution GC and magnetic sector MS which gives detection limits in the low parts per trillion (ppt) range.

No ongoing studies concerning techniques for measuring and determining endrin, endrin aldehyde, or endrin ketone in biological and environmental samples were reported.