7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring endosulfan, its metabolites, and other biomarkers of exposure and effect to endosulfan. 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

Endosulfan, in its pure form, is a crystalline substance consisting of α- and β-isomers in the ratio of approximately 7:3. It is an organochlorine pesticide, and analysis of biological and environmental samples for endosulfan commonly results in the detection of other organochlorine pesticides and polychlorinated biphenyls. These can interfere with the determination of endosulfan unless adequate cleaning and separation techniques are used. Detection of low levels of endosulfan typically involves extraction of samples with organic solvents, a clean-up step to remove lipids and other materials that may interfere with analysis, high-resolution gas chromatography (HRGC) to separate endosulfan from other compounds in the extract, and confirmation of endosulfan by electron capture detector (ECD) or mass spectroscopy (MS). Method blanks and control samples should be used to verify method performance and ensure that the reagents and glassware are not introducing contaminants that might interfere with the determination of endosulfan isomers or endosulfan sulfate.

The method of choice for the determination of α- and β-endosulfan in blood, urine, brain, and adipose tissue is gas chromatography (GC) equipped with an electron capture detector (ECD) (Cerrillo et al. 2005; Fernandez et al. 2007; Guardino et al. 1996). This is because GC/ECD is relatively inexpensive, is simple to operate, and offers a high sensitivity for halogens (Griffith and Blanke 1974). Fernandez et al. (2007) used a GC/MS isotope dilution method for detection of a variety organochlorine pesticides in human milk samples. Detection limits ranged from 0.1 to 3 ng/mL.

A rapid headspace solid-phase microextraction (SPME) based method for the detection of organic pollutants in human serum using GC coupled with electron impact ionization mass spectrometry was described (Flores-Ramirez et al. 2014). SPME is a simple, solvent-free method of extraction for sample preparation. Detection limits for α- and β-endosulfan and endosulfan sulfate were in the ppb range.

Vidal et al. (1998) discuss a GC-tandem mass spectrometry (GC-MS-MS) method using solid-phase extraction (SPE) for the analysis of α- and β-endosulfan in urine.

Cappiello et al. (2014) describe a method using GC coupled to a quadrupole mass spectrometric (qMS) detector using liquid-solid extraction followed by SPE for the analysis of α- and β-endosulfan and endosulfan sulfate in human fetal and newborn tissues. Detection limits ranged from 1.2 to 2.0 ng/g.

Mariani et al. (1995) have used GC in conjunction with negative ion chemical ionization mass spectrometry to determine α- and β-endosulfan in plasma and brain samples with limits of detection reported to be 5 ppb in each matrix. Details of commonly used analytical methods for several types of biological media are presented in Table 7-1.

Lozowicka (2013) describe a method based on matrix solid phase dispersion (MSPD) extraction followed by GC/nitrogen-phosphorus detection (NPD)-ECD for multipesticide residues in honeybees. The use of MSPD and cleanup was reported to be an efficient extraction technique with detection limits for α- and β-endosulfan and endosulfan sulfate of 0.005, 0.006, and 0.005 µg/kg, respectively.

7.2 ENVIRONMENTAL SAMPLES

Reliable analysis of endosulfan residue concentrations in environmental samples usually involves detection of the α- and β-isomers plus endosulfan sulfate (a degradation product of endosulfan). GC/ECD has been the most widely used analytical technique for determining low-ppb to parts-per-trillion (ppt) levels of α- and β-endosulfan and endosulfan sulfate in air, water, waste water, sediment, soil, fish, and various foods (EPA 1992, 1994, 1996a, 1996b, 1996c, 1997d, 1997e, 1997f, 2007; FDA 1994, 1999a, 1999b; Gale et al. 2009; Halsall et al. 1997; Hung et al. 2002; Wania et al. 2003). Both GC and high performance liquid chromatography (HPLC) have been used to separate endosulfan and its major metabolites endosulfan ether, endosulfan sulfate, endosulfan lactone, and endosulfan diol (Kaur et al. 1997).

Table 7-1. Analytical Methods for Determining Endosulfan in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Adipose tissue, placenta	Extraction with hexane and fractionated with HPLC; gravimetric determination of lipid content	GC/ECD/MS	Results reported in ng/g fat, placenta	93.99% (β-endosulfan); 100.03% (endosulfan sulfate)	Cerrillo et al. 2005
Umbilical cord blood	Serum extraction with organic solvents, clean-up using acid treatment with sulfuric acid, elution with HPLC	GC/ECD	Results reported in ng/mL serum	93.99% (β-endosulfan); 100.03% (endosulfan sulfate)	Cerrillo et al. 2005
Human milk	Extraction by shaking with methanol and sodium oxalate; further extraction procedures used organic solvents and clean-up with sulfuric acid treatment, followed by elution with HPLC	GC/ECD	Results reported in ng/mL milk	93.99% (β-endosulfan); 100.03% (endosulfan sulfate)	Cerrillo et al. 2005
Human milk	Heating, shaking for 30 minutes at 37 °C to homogenize, re-freezing, then extraction and clean-up with 2:1 acetone/ hexane	GC/MS (isotope dilution method)	Results reported in ng/g lipid	No data	Damgaard et al. 2006
Placenta	Placenta homogenate dissolved in hexane, elution in glass column then HPLC, drying, dissolution in hexane	GC/ECD	0.1–3 ng/mL (for all organochlorine pesticides)	84–102% (for all organochlorine pesticides)	Fernandez et al. 2007
Blood	Homogenization of sample followed by extraction with methanol and centrifugation; isolation of pesticides using SPE	GC/ECD	Approximately 0.2 µg/L (ppb)	No data	Guardino et al. 1996
Serum	Headspace SPME	GC/electron impact ionization MS	1.95 ng/mL (α-endosulfan); 0.33 ng/mL (β-endosulfan); 1.09 ng/mL (endosulfan sulfate)	At 15 ng/mL: 75.4% (α-endosulfan); 83.5% (β-endosulfan); 77.3% (endosulfan sulfate)	Flores-Ramirez et al. 2014

Table 7-1. Analytical Methods for Determining Endosulfan in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Plasma, brain (alpha and beta)	Brain: homogenization with ethanol, centrifugation, phase separation and evaporation of ethanol and addition of internal standard Plasma: extraction with hexane and then as for brain samples	GC/NICI MS	5 ng/mL for plasma (ppb); 5 ng/g (ppb) for brain; 8–31% RSD	85–93%	Mariani et al. 1995
Liver and brain	Liquid-solid extraction with acetone and n-hexane; SPE cleanup	GC/qMS	Liver and brain: 2.0 ng/g (α-endosulfan); 2.0 ng/g (β-endosulfan); 1.2 ng/g (endosulfan sulfate)	Liver (8.0 ng/g): 99% (4% RSD) (α-endosulfan); 95% (7%RSD) (β-endosulfan); 97% (4% RSD) (endosulfan sulfate) Brain (8.0 ng/g): 83% (2% RSD) (α-endosulfan); 91% (3%RSD) (β-endosulfan); 96% (6% RSD) (endosulfan sulfate)	Cappiello et al. 2014
Honeybees	MSPD extraction and cleanup using Florisil, sodium sulfate, and acetonitrile; evaporation; dissolution with hexane/acetone (9:1 v/v)	GC/NPD-ECD	0.005 μg/kg (α-endosulfan); 0.006 μg/kg (β-endosulfan); 0.005 μg/kg (endosulfan sulfate)	89.4–90.3% (3.15–3.59% RSD) (α-endosulfan); 92.5–107.8% (0.72–12.04% RSD) (β-endosulfan); 96.5–106.8% (0.58–2.13% RSD) (endosulfan sulfate)	Lozowicka 2013

ECD = electron capture detector; GC = gas chromatography; HPLC = high-performance liquid chromatography; MC = microcoulometric detector; MS = mass spectrometry; MSPD = matrix solid phase dispersion; NICI = negative ion chemical ionization; NPD = nitrogen-phosphorus detector; qMS = quadrupole mass spectrometry; RSD = relative standard deviation; SPE = solid phase extraction

The most common methods of sampling and measuring endosulfan in the atmosphere involve high-volume air samplers, where air is forced through a collection device. The collection medium is either glass fiber filters (GFFs) or polyurethane foam plugs (PUFs). The samples are then analyzed with GC/MS. This technique can measure endosulfan levels in air at the picogram level (Halsall et al. 1997; Su et al. 2007). The use of passive air samplers with XAD-2 resin filters is also common for measuring endosulfan concentrations in air. These samples are extracted with dichloromethane and methanol and analyzed with GC/ECD. Wania et al. (2003) reported detection limits of 0.15 pg/μL for α-endosulfan and 0.08 pg/μL for β-endosulfan. Gale et al. (2009) used semipermeable membrane devices (SPMDs) with low-density polyethylene tubing filled with triolein to detect endosulfans in indoor air. The samples were analyzed with GC/MS and GC/ECD and reported mean endosulfan concentrations as ng per SMPD.

GC/ECD or a halogen-specific detector (HSD) (Method 8080) is the technique recommended by EPA's Office of Solid Waste and Emergency Response for determining α- and β-endosulfan and endosulfan sulfate in water and waste water at low-ppb levels (EPA 1994). At these low concentrations, identification of endosulfan residues can be hampered by the presence of a variety of other pesticides. Consequently, sample clean-up on a Florisil® column is usually required prior to analysis (EPA 1994).

Methods 508, 508.1, and 525.2 (EPA 1997d, 1997e, 1997f) are applicable to drinking water and groundwater and can determine α- and β-endosulfan and endosulfan sulphate at concentrations as low as 7 ppt using liquid solid extraction (LSE) and GC/ECD.

GC/ECD and GC/MS (EPA Method 608) are the methods recommended for determining α-endosulfan, β-endosulfan, and endosulfan sulfate in municipal and industrial discharges (EPA 1996c). Sample clean-up on Florisil® column and an elemental sulfur removal procedure are used to reduce or eliminate interferences. Sensitivity is in the sub-ppb range. Recoveries and precision are good.

Chary et al. (2012) describe a method for detection of α- and β-endosulfan and endosulfan sulfate in river water and waste water at parts-per-trillion levels using stir-bar-sorptive extraction followed by liquid desorption and GC coupled with triple-quadrupole mass spectrometry (GC/QqQ-MS-MS).

Multiresidue methods for fatty and non-fatty foods (fruits, vegetables, seeds, dairy, eggs, meats) published by FDA (FDA 1994, 1999a, 1999b). Alamgir Zaman Chowdhury et al. (2013) and Andrascikova et al. (2013) also described a multiresidue method for vegetables and oranges, respectively, using GC/MS. Limits of detection are generally in the sub-ppm to ppb range.

Dreher and Podratzki (1988) developed an enzyme immunoassay technique for detecting endosulfan and its degradation products (i.e., endosulfan diol, endosulfan sulfate, endosulfan ether, and endosulfan lactone) in aqueous media. The enzyme immunoassay technique is based on detecting antibodies raised against the diol of endosulfan by immunizing rabbits with an endosulfan-hemocyanin conjugate. Minor problems were encountered with coupling of the detecting enzyme (peroxidase) to the conjugate and with cross-reactivity with the pesticide endrin. Although the enzyme immunoassay technique does not require sample extraction, and it is rapid and inexpensive, it is not yet in common use in environmental residue analysis. A detection limit of 3 µg/endosulfan/L of sample was achieved (Dreher and Podratzki 1988; Frevert et al. 1988). Immunoassays have also been reported for endosulfan (both isomers), endosulfan sulfate, and endosulfan diol in water and soil (Lee et al. 1997a, 1997b) with limits of detection reported to be 0.2 µg/L for water and 20 µg/kg in soil. Details of commonly used analytical methods for various environmental media are presented in Table 7-2.

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 endosulfan is available. Where adequate information is not available, ATSDR, in conjunction with 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 endosulfan.

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.

Table 7-2. Analytical Methods for Determining Endosulfan in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (vapor and particulate)	High volume air sampler using GFF and PUFs	GC/MS	No data	No data	Halsall et al. 1997; Hung et al. 2002
Air	Passive air sampler with XAD-2 resin filter and extraction with dichloromethane and methanol.	GC/ECD	0.15 pg/µL (α-endosulfan); 0.08 pg/µL (β-endosulfan)	No data	Wania et al. 2003
Indoor air	Semipermeable membrane device (SMPD) using low-density polyethylene tubing filled with triolein	GC/MS; GC/ECD	No data	No data	Gale et al. 2009
Drinking water, ground-water	Extraction of water with methylene chloride, removal of water from extract, volume reduction to 5 mL after solvent exchange to methyl-*t*-butyl ether	GC/ECD	0.015 µg/L (ppb) (α-endo-sulfan); 0.024 µg/L (β-endosulfan); 0.015 µg/L (endosulfan sulfate)	87% (10% RSD) (α-endosulfan); 92% (11% RSD) sulfate: 102% (15% RSD) (endosulfan sulfate)	EPA 1997d (Method 508)
Drinking water, ground-water	Extraction of water using C_{18} extraction disks (LSE); elution using ethyl acetate and methylene chloride; volume reduction	GC/ECD	<0.007 µg/L (α- and β-endosulfan and endosulfan sulfate)	92.6% (17.8% RSD at 0.03 µg/L) (α-endosulfan); 87.9% (18.6% RSD at 0.03 µg/L) (β-endosulfan) 106% (11.5% RSD at 0.03 µg/L) (endosulfan sulfate)	EPA 1997e (Method 508.1)
Drinking water	Extraction of sample using LSE; solvent elution using ethyl acetate and methylene chloride; volume reduction	GC/MS	α-endosulfan: 0.11 µg/L (α-endosulfan); 0.074 µg/L (β-endosulfan); 0.093 µg/L (endosulfan sulfate)	121% (6.1% RSD) (α-endosulfan); 128% (3.9% RSD) (β-endosulfan) 116% (5.4% RSD) (endosulfan sulfate)	EPA 1997f (Method 525.2)
Waste water	Extraction of sample with methylene chloride; water removal/ volume reduction	GC/MS	5.6 µg/L (endosulfan sulfate)		EPA 2012d (Method 625)

Table 7-2. Analytical Methods for Determining Endosulfan in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water; waste water	Extraction of sample (Method 3510, 3520) with direct injection	GC/ECD	0.49 µg/L (α-endosulfan); 6.1 µg/L (β-endosulfan); 2.7 µg/L (endosulfan sulfate)	45-153% (α-endosulfan); 202% (β-endosulfan); 26–144% (endosulfan sulfate)	EPA 1994 (Method 8080A)
River water; waste water	SBSE followed by liquid desorption with 1:1 acetonitrile and methanol	GC/QqQ-MS-MS	River water: 2 ng/L (ppt) (α-endosulfan); 2 ng/L (β-endosulfan); 2 ng/L (endosulfan sulfate) Waste water: 5 ng/L (ppt) (α-endosulfan); 5 ng/L (β-endosulfan); 10 ng/L (endosulfan sulfate)	76% (10% RSD) (α-endosulfan); β: 81% (12% RSD) (β-endosulfan); 83% (12% RSD) (endosulfan sulfate)	Chary et al. 2012
Liquid or solid	Extraction of sample with methylene chloride, methylene chloride and acetone or hexane and acetone (depending on solid content); clean-up	GC/ECD	1.3 µg/L (α-endosulfan, groundwater); 0.9 µg/L (β-endosulfan, groundwater); 0.51 µg/L (α-endosulfan, waste water); 0.54 µg/L (β-endosulfan, waste water	52% (α- and β-endosulfan; from sewage sludge); 47% (α-endosulfan, from stillbottoms); 49% (β-endosulfan from stillbottoms)	EPA 1996a (Method 8081A)
Various solid and liquid matrices	Extraction of sample with methylene chloride, hexane-acetone or methylene chloride-acetone (depending on solid content); clean-up	GC/ECD	No data	52–70% (α- and β-endosulfan; from sewage sludge); 41–47% (α-endosulfan, from stillbottoms); 46–49% (β-endosulfan from stillbottoms)	EPA 2007 (Method 8081B)

Table 7-2. Analytical Methods for Determining Endosulfan in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Various solid and liquid matrices	Extraction of sample with methylene chloride, hexane-acetone or methylene chloride-acetone (depending on solid content)	GC/AED	No data	100% (α-endosulfan); 110% (β-endosulfan II); 114 (endosulfan sulfate)	EPA 2007 (Method 8085)
Water, soil, sediment, waste	Extraction of sample using solvent or SPE	GC/MS	No data	Detection-107% (endosulfan sulfate); 96.3% (α-endosulfan in clay soil); 104% (β-endosulfan in clay soil); 101% (α-endosulfan in topsoil); 105% (β-endosulfan in topsoil)	EPA 1996b (Method 8270)
Municipal and industrial discharge	Extraction of sample with methylene chloride; water removal; exchange to hexane; volume reduction; clean-up on Florisil column and removal of elemental sulfur	GC/ECD	0.014 µg/L (α-endosulfan); 0.004 µg/L (β-endosulfan); 0.066 µg/L (endosulfan sulfate)	97% (α-endosulfan); 93% (β-endosulfan); 89%(endosulfan sulfate)	EPA 1996c (Method 608)
Municipal and industrial waste water; sludge	Extraction with methylene chloride and acetone, or acetonitrile and methylene chloride (depending on solids content); volume reduction and clean-up using GPC, column chromatography, or SPE; sulfur removal if needed	GC/ECD	α: 11 ng/L β: 8 ng/L sulfate: 7 ng/L	α: 18–158% β: 62–158% sulfate: 31–149%	EPA 1992 (Method 1656)
Non-fatty foods (<2% fat, <75% water)	Extraction with acetonitrile, partition into petroleum ether; cleanup using Florisil	GC/ECD	No data	>85% (α- and β-endosulfan and endosulfan sulfate)	FDA 1999b (PAM Method 303)
Fatty foods (>2% fat)	Extraction of fat using sodium sulfate, petroleum ether, by filtering, or by solvents; cleanup using solvent partitioning, Florisil	GC/ECD	No data	>85% (α- and β-endosulfan and endosulfan sulfate)	FDA 1994 (PAM Method 304)

Table 7-2. Analytical Methods for Determining Endosulfan in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Vegetables	Extraction with ethyl acetate, hexane, and acetone (3:1:1); concentration by vacuum rotary evaporator; cleanup using Florisil	GC/MS	0.0013–0.0041 mg/kg	79–106% (3.08–15.40 RSD)	Alamgir Zaman Chowdhury et al. 2013
Fruit (oranges)	DLLME with acetonitrile and carbon tetrachloride; centrifugation	GC/MS SIM	0.23 ng/g (ppb) (α-endosulfan); 0.19 ng/g (β-endosulfan); 0.09 ng/g (endosulfan sulfate)	99–121% (5–12% RSD) (α-endo-sulfan); 86–127% (4–25% RSD) (β-endo-sulfan); 73–120% (6–16% RSD) (endosulfan sulfate)	Andrascikova et al. 2013
Fish and shellfish	Extraction of fat using sodium sulfate; PLE with hexane and dichloromethane; cleanup by GPC	GC/NCI MS	0.03–2.48 ng/g (w/w)	No data	Helaleh and Al-Rashdan 2013
Milk	Extraction of milk with ethanol-ethyl acetate (9:95, v/v) with sodium sulfate; centrifugation and volume reduction	GC/ELCD	0.9 µg/kg (ppb) (α-endosulfan); 0.9 µg/kg (β-endosulfan); 1.8 µg/kg (endosulfan sulfate)	90% (5% RSD) (α-endosulfan); 91% (11% RSD) (β-endosulfan); 88% (11% RSD) (endosulfan sulfate)	Bennett et al. 1997

AED = atomic emission detector; DDLME = dispersive liquid-liquid microextraction; ECD = electron capture detector; EIA = enzyme-immunoassay; ELCD = electrolytic conductivity detector; GC = gas chromatography; GFF = glass fiber filter; GPC = gel permeation chromatography; HPLC = high-performance liquid chromatography; ITMS = ion trap mass spectrometer; LSE = liquid solid extraction; MS = mass spectrometry; NCI = negative chemical ionization; PLE = pressurized liquid extraction; PUF = polyurethane foam; QqQ = triple quadrupole analyzer; RSD = relative standard deviation; SBSE = stir-bar-sorptive extraction; SIM = selected ion monitoring; SPE = solid phase extraction; SPME = solid phase micro-extraction

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect

Exposure. GC/ECD and GC/MS are analytical techniques used for measuring endosulfan in cord blood, urine, placenta, and various biological tissues and excreta at low- and sub-ppb levels (Cerrillo et al. 2005; Fernandez et al. 2007; Guardino et al. 1996). These techniques are sensitive for measuring background levels of endosulfan in the population and levels of endosulfan at which health effects might begin to occur. Although accurate and reliable methods are available for analysis of endosulfan in biological tissues and fluids, insufficient data have been collected using these techniques to correlate the concentrations of endosulfan in biological materials with environmental exposure and health effects (see Chapter 3).

Effect. As mentioned in Section 3.8.2, Biomarkers Used to Characterize Effects Caused by Endosulfan, there are no specific biomarkers of effect for endosulfan. The main effect of acute-exposure to high amounts of endosulfan, as occurs in cases of intentional or accidental ingestion or dermal contact with endosulfan, is tremors and seizures. Other systemic effects may be secondary to the seizures. The effects of prolonged exposure to lower levels, as could be the case for exposure of the general population, are not known. If effects under that exposure scenario are eventually defined, then analytical methods with appropriate sensitivity should be available to determine the levels of endosulfan in blood and body tissues that are associated with those health effects.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. GC/ECD is the most prevalent analytical method for measuring low levels of α- and β-endosulfan and endosulfan sulfate in water, waste water, soil, sediment, and foods (EPA 1992, 1994, 1996a, 1996b, 1996c, 1997d, 1997e, 1997f, 2007; FDA 1994, 1999a, 1999b; Gale et al. 2009; Halsall et al. 1997; Hung et al. 2002; Wania et al. 2003). This technique is sensitive for measuring background levels of endosulfan in foods and water (media of most concern for potential human exposure to endosulfan) and levels of endosulfan at which health effects might begin to occur. The intermediate-duration oral MRL is 0.005 mg/kg/day, which translates to a required limit of detection of 0.175 mg/L, and these methods easily meet that need. GC/ECD or HSD is the method (Method 8080) recommended by EPA (1994) for detecting α- and β-endosulfan and endosulfan sulfate in water and waste water at low-ppb levels. GC/ECD has also been used to detect low-ppb levels of α- and β-endosulfan and endosulfan sulfate in foodstuffs, soil, and sediment.

7.3.2 Ongoing Studies

No ongoing studies regarding analytical methods sponsored by NIH or EPA were identified for endosulfan.