7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and monitoring DDT, DDE, and DDD, their metabolites, and other biomarkers of exposure and effect to DDT, DDE, and DDD. 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

Table 7-1 lists the analytical methods used for determining DDT in biological fluids and tissues. DDT, DDE, and DDD residues have been measured in biological samples such as adipose tissue, skin lipids, blood serum, urine, milk, and other samples primarily by gas chromatographic (GC) methods. GC methodology provides high resolution and a reproducibility of retention time, which is ideal for distinguishing between the *p,p'*- and *o,p'*-isomers of the compounds, especially when using GC capillary columns (Mukherjee and Gopal 1996). The GC separation method has been historically coupled with electron capture detection (ECD) quantitative techniques (Fishbein 1974). For example, the GC/ECD methodology proposed by Cranmer et al. (1972b) has detected DDT, DDE, and DDD in human urine at levels as low as 50 pg/sample. In human serum, detection limits of between 2 and 7 pg/g serum for DDT, DDE, and DDD have been reported for the GC/ECD quantitative method (Atume and Aune 1999). With the wider availability of gas chromatography-mass spectrometry (GC/MS) instrumentation in analytical laboratories, GC/MS detection methods have been used to quantify DDT and its metabolites (Akiyama et al. 2000; Gill et al. 1996). Both the GC/ECD and GC/MS analytical methods are suitable for the analysis of DDT, DDE, and DDD. However, the GC/ECD method typically provides greater detection sensitivity, whereas the GC/MS method has the advantage of providing qualitative information to determine the specificity of the analysis. Various authors cited in Table 7-1 used GC methods to monitor the residues of these compounds in blood, serum, semen, liver, human milk, and adipose tissue, which were detectable at the ppm and ppb level. Since DDT partitions in fat, analyses are often performed on

Table 7-1. Analytical Methods for Determining DDT, DDE, and DDD in Biological Samples

Sample matrix	Sample preparation	Analytical method	Sample detection limit	Percent recovery	Reference
Blood/plasma/serum	Extract with hexane	GC/ECD	2 ppb (DDT); 1ppb (DDE); 2 ppb (DDD)	>90% (DDT); 100–110% (DDE); No data (DDD)	EPA 1980b; Nachman et al. 1972
Blood/plasma/serum	Extract with methanol and hexane-ethyl ethers; cleanup with Florisil	GC/ECD	0.8 ppb (DDE); No data (DDT, DDD)	90–100% (DDE); No data (DDT, DDD)	McKinney et al. 1984
Blood	Extract with hexane; concentrate to 5 mL	GC/ECD HERL_004	No data	No data	EMMI 1997
Serum	Extract with hexane; cleanup with Florisil	GC/ECD	91 pg/g serum (DDE)	83.1–85.5% (PCB)	Greizerstein et al. 1997
Semen	Extract with acetone; cleanup with Florisil	GC/ECD	No data	96–97% (DDT); 91.4% (DDE); 91.4% (DDD)	Waliszewski and Syzmczneki 1983
Urine	Extract with acetic acid in hexane followed by methylation	HPLC/NAA	0.01 mg/mL (DDT); No data (DDE, DDD)	No data	Opelanio et al. 1983
Urine	Extract with hexane	GC/ECD	2 pg (DDE); No data (DDT, DDD)	93.2–106.2% (DDE); No data (DDT, DDD)	Muhlebach et al. 1985
Liver, kidney, human milk	Macerate sample with acetonitrile; cleanup with Florisil	GC/ECD	No data	81% (DDD); No data (DDT, DDD)	Ando 1979; EPA 1980b

Table 7-1. Analytical Methods for Determining DDT, DDE, and DDD in Biological Samples *(continued)*

Sample matrix	Sample preparation	Analytical method	Sample detection limit	Percent recovery	Reference
Muscle	Homogenized and extracted with hexane	GC/ECD	2 pg (DDE); No data (DDT, DDD)	93.2–106.2% (DDE); No data (DDT, DDD)	Muhlebach et al. 1985
Human milk	Triple solvent extraction with ethanol, hexane, and hexane-ethyl ether; Florisil cleanup	GC/ECD	2 ppb (DDE); No data (DDT, DDD)	81–108% (DDE); No data (DDT, DDD)	McKinney et al. 1984
Human milk	Extract with hexane; cleanup with Florisil	GC/MS	2 ppb (DDT); 1.5 ppb (DDD); No data (DDE)	80–100% (DDD); No data (DDT, DDE)	Krauthacker et al. 1980
Human milk	Head-space solid-phase extraction; desorption from solid phase in GC injector	GC/ECD	0.08 µg/L (*p,p*'-DDT) 2.79 µg/L (*o,p*'-DDT) 1.92 µg/L (*p,p*'-DDE) 1.36 µg/L (*o,p*'-DDE) 1.62 µg/L (*p,p*'-DDD) 1.85 µg/L (*o,p*'-DDD)	No data	Röhrig and Meisch 2000
Milk	Remove proteins with ethanol; extract with hexane; cleanup with Florisil	GC/ECD	6 pg/g serum (DDE)	95.1% (PCB)	Greizerstein et al. 1997
Milk/adipose tissue	Extract with hexane and petroleum ether; cleanup with GPC	GC/ECD HERL_026	No data	No data	EMMI 1997
Adipose tissue	Digest with perchloric-acetic acid; extract with n-hexane	GC/ECD	2 pg (DDE); No data (DDT, DDD)	93.2–106.2% (DDE); No data (DDT, DDD)	Muhlebach et al. 1985

Table 7-1. Analytical Methods for Determining DDT, DDE, and DDD in Biological Samples *(continued)*

Sample matrix	Sample preparation	Analytical method	Sample detection limit	Percent recovery	Reference
Adipose tissue	Extract with petroleum ether, cleanup with Florisil	GC/ECD	No data	85–100% (DDT); No data (DDD, DDE)	EPA 1980b
Feces	Hexane extraction; evaporate and reconstitute with isooctane	GC/ECD	20 ppb (DDT, DDE); No data (DDD)	92–111% (DDT); 96–109% (DDE); No data (DDD)	Saady et al. 1992
Lymph	Co-extract with ether; final extraction with cyclopentanone	HPLC	No data	96.4% (DDT); No data (DDE, DDD)	Noguchi et al. 1985
Skin lipids	Purify with GPC, wash with sulfuric acid	GC/ECD	No data	96–109% (DDE); No data (DDT, DDD)	Sasaki et al. 1991b
Milk ($MeSO_2$-DDE[a])	Liquid-gel partitioning followed by adsorption and GPC cleanup	capillary GC/MS	No data	80%, mean ($MeSO_2$-DDE)	Noren et al. 1996
Lung, blubber, liver ($MeSO_2$-DDE)	Extraction with GPC cleanup	GC/ECD GC/AED	No data	No data	Janak et al. 1998

[a]DDE methyl sulfone

AED = atomic emission detection; ECD = electron capture device; GC = gas chromatography; GPC = gel permeation chromatography; HERL= Health and Environmental Research Laboratory of the Environmental Protection Agency; HPLC = high performance liquid chromatography; MS = mass spectrometry; NAA = neutron activation analysis; PCB = polychlorinated biphenyls

adipose tissue, milk fat, or a lipid extract of serum or other material. In the latter case, the results may be reported on a lipid or fat basis (i.e., ng DDT/g lipids). By reporting monitoring studies of DDT on a lipid basis, variability in results due to variability in fat content is reduced (McKinney et al. 1984; Phillips et al. 1989).

A methyl sulfonyl metabolite of DDE that has been found in many tissue samples, 3-methyl sulfonyl 2,2-bis(4-chlorophenyl)-1,1-dichloroethene, can be analyzed by GC/MS or GC/ECD (Janak et al. 1998; Norén et al. 1996), although gas chromatography-tandem mass spectrometry is the preferred instrumental technique for the analysis of 3-methyl sulfonyl 2,2-bis(4-chlorophenyl)-1,1-dichloroethene in complex biological matrices (Letcher and Norstom 1995). However, the use of atomic emission detection significantly improves its determination (Janak et al. 1998). While methods exist for measuring DDT, DDE, and DDD in liver, breast milk, and adipose tissue, the most frequently used sampling techniques utilize samples of blood, urine, and semen because of ease of sample collection. DDT, DDE, and DDD can also be measured in skin lipids collected by wiping the face with cotton (Sasaki et al. 1991b). Although these methods can detect and quantify levels of DDT, there is no information available to quantitatively correlate levels in these fluids with environmental levels or toxic effects.

7.2 ENVIRONMENTAL SAMPLES

DDT residues are found in the environment because of its slow transformation. DDT was used as an insecticide from the late 1940s until the early 1970s. Well-established analytical test procedures to analyze environmental samples use GC and MS (see Table 7-2). EPA methods 608 and 8081B are recommended to detect DDT, DDE, and DDD in surface water and municipal and industrial discharges (EPA 1982, 1998j). These are required procedures under the Clean Water Act. Behzadi and Lalancette (1991) described a modified isotope dilution (MID) GC/MS method to analyze DDT, DDE, and DDD in water and soil samples. Sample preparation for MID GC/MS does not require extensive extraction and cleanup compared to GC/MS. The detection limits are in the 0.001 µg/L (ppt) range, and recoveries range from 73 to 110% for soil and from 90 to 116% for water. EPA methods 8081B and 8270D are GC/MS methods used to determine DDT and its metabolites in soils with detection limits of 0.3–0.4 µg/kg (EPA 1998j, 1998k). GC/ECD and nitrogen-phosphorus detection (NPD) is used for the analysis of DDT in foods with a detection limit of 0.5 µg/kg (ppb) (Rodriguez et al. 1991). GC/ECD is also used for the analysis of DDT and its metabolites in fish, oysters, and waterfowl. Detection limits were reported in the ppb range and recoveries ranged from 66 to 97% (Blus et al. 1987; Ford and Hill

Table 7-2. Analytical Methods for Determining DDT, DDE, and DDD in Environmental Samples

Sample matrix	Sample preparation	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Separate with silicic acid column	GC/ECD	0.20 ng/m^3 (DDE); 0.16 ng/m^3 (DDT)	104–106% (DDT); 100% (DDE); No data (DDD)	Bidleman et al. 1978
Air	Filter collection and iso-octane extraction	GC	0.49–2.60 mg/m^3 (DDT); No data (DDD, DDE)	No data	NIOSH 1977
Air	Sample collection on glass fiber filter; Soxhlet extraction; cleanup with alumina	GC/ECD AREAL Method TO-4	No data	No data	EMMI 1997
Water	Extract using hexane followed by acetonitrile	GC	No data	85% (DDT); No data (DDD, DDE)	Kurtz 1977
Water	Extract using methylene chloride cleanup with Florisil	GC/ECD EPA Method 608	0.012 µg/L (DDT); 0.004 µg/L (DDE); 0.011 µg/L (DDD)	92% (DDT, DDD); 89% (DDE)	EPA 1982
Water	Extract at neutral pH with methylene chloride	GC/ECD or GC/ELCD EPA Method 8081B	0.081 µg/L (DDT); 0.058 µg/L (DDE); 0.050 µg/L (DDD).	121.1% (4.4'-DDT); 98.0% (4,4'-DDE); 86.8% (4,4'-DDD)	EMMI 1997; EPA 1998j
Water	Digest with chromic acid; extract with hexane	GC	No data	100% (DDT, DDE); No data (DDD)	Driscoll et al. 1991
Water	Extract with methylene chloride	MID GC/MS	0.012 µg/L (DDT); 0.007 µg/L (DDE); 0.008 µg/L (DDD)	93–110% (DDT); 73–110% (DDE); 76–110% (DDD)	Behzadi and Lalancette 1991
Water	Immersion solid-phase extraction, desorption from solid-phase in GC injector	GC/ECD	0.30 ng/L (DDT) 0.20 ng/L (DDE) No data (DDD)	32.3% (DDT); 103.8% (DDE); 113.6% (DDD)	Aguilar et al. 1999

Table 7-2. Analytical Methods for Determining DDT, DDE, and DDD in Environmental Samples *(continued)*

Sample matrix	Sample preparation	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Solid-phase extraction, eluted with methylene chloride/methanol (80:20)	GC/Ion trap MS	0.16 µg/L (DDT) 0.07 µg/L (DDE) No data (DDD)	24–77% (DDT); 27–51% (DDE); No data (DDD)	Eitzer and Chevalier 1999
Finished drinking water and groundwater	Extract with methylene chloride; solvent exchange to methyl *tert*-butyl ether	GC/ECD EPA Method 508	0.060 µg/L (DDT); 0.010 µg/L (DDE); 0.003 µg/L (DDD)	No data	EMMI 1997
Landfill leachate	Head-space solid-phase microextraction, desorption from solid-phase in GC injector	GC/ECD	0.1 µg/L	78.5% (DDT,DDE, and DDD)	Brás et al. 2000
Soil	Extract with hexane/acetone; cleanup with Florisil	GC/ECD AOAC 970.52	No data	No data	Helrich 1990
Soil	Extract with hexane/acetone; cleanup with Florisil	GC/ECD	No data	No data	Williams 1984
Soil	Extract with hexane-acetone or methylene chloride-acetone, cleanup by appropriate method.	GC/ECD or GC/ELCD EPA Method 8081B	0.0036 µg/kg (DDT); 0.0025 µg/kg (DDD); 0.0042 µg/kg (DDE)	121.1% (DDT); 98.0% (DDE); 86.8% (DDD)	EMMI 1997; EPA 1998j
Soil	Extraction with methylene chloride	GC/MS EPA Method 8270D	No Data	111–134% (DDT)	EPA 1998k
Soil	Extraction with methylene chloride	MID GC/MS	0.4 µg/kg (DDT); 0.3 µg/kg (DDD); 0.3 µg/kg (DDE)	91–109% (DDT); 90–116% (DDD); 93–104% (DDE)	Behzadi and Lalancette 1991

Table 7-2. Analytical Methods for Determining DDT, DDE, and DDD in Environmental Samples *(continued)*

Sample matrix	Sample preparation	Analytical method	Sample detection limit	Percent recovery	Reference
Food	Extract with acetonitrile into petroleum ether; cleanup with Florisil	GC/ECD	No data	>80%	McMahon and Burke 1978; Williams 1984
Food	Soxhlet extraction using redistilled hexane	on-line SEC-GC	10–50 µg/kg (DDE); No data (DDT, DDD)	No data	Grob and Kalin 1991
Food	Extract with n-hexane; cleanup with Florisil	GC-ECD/NPDMS	0.50–10 µg/kg (DDT, DDE); No data (DDD)	68–95% (DDT, DDE); No data (DDD)	Rodriguez et al. 1991
Food	Mixed ether extraction; cleanup with Florisil	DC-GC/ECD	0.05–1.5 ng (DDT, DDE); No data (DDD)	No data	Hopper 1991
Food	Mix sample with dried potassium bromide powder	IR/UV-SP	No data	No data	Gore et al. 1971
Milk	Solid-phase extraction, eluted with hexane, cleanup on neutral alumina, eluted with hexane	GC/ECD	0.12 µg/L (*p,p*'-DDT) 0.12 µg/L (*o,p*'-DDT) 0.07 µg/L (*p,p*'-DDE) 0.05 µg/L (*o,p*'-DDE) 0.07 µg/L (*p,p*'-DDD) 0.12 µg/L (*o,p*'-DDD)	100% (*p,p*'-DDT); 104% (*o,p*'-DDT); 93% (*p,p*'-DDE); 97% (*o,p*'-DDE); 106% (*p,p*'-DDD); 83% (*o,p*'-DDD); (spiked at 1 µg/L)	Yagüe et al. 2001
Animal fat	Extract with methylene chloride/cyclohexane; separation by GPC	GC/ECD AOAC 984.21	No data	No data	Helrich 1990
Plants	Extract with hexane/methanol/acetone	SP	No data	92–99% (DDT); No data (DDD, DDE)	Verma and Pillai 1991a
Plants	Extract with methylene chloride; organic phase concentrated	GC/HECD AOAC 985.22	No data	No data	Helrich 1990

Table 7-2. Analytical Methods for Determining DDT, DDE, and DDD in Environmental Samples *(continued)*

Sample matrix	Sample preparation	Analytical method	Sample detection limit	Percent recovery	Reference
Fish	Soxhlet extraction with hexane; cleanup with Florisil	GC/ECD	10 µg/kg	No data	Ford and Hill 1991
Fish	Extract with petroleum ether; cleanup with Florisil	GC/ECD AOAC 983.21	No data	No data	Helrich 1990

AOAC = Association of Official Analytical Chemists; AREAL = Atmospheric Research and Exposure Laboratory of the Environmental Protection Agency; DC = dual capacity; ECD = electron capture device; ELCD = electrolytic conductivity detector; EPA = Environmental Protection Agency; GC = gas chromatography; GPC = gel permeation chromatography; HECD = halogen-specific electron capture device; IR/UV infrared/ultraviolet; MID = modified isotope dilution; MS = mass spectrometry; NPD = nitrogenphosphorous detection; SEC = size exclusion chromatography; SP = spectro-photometry

1991; Long et al. 1991b; Lott and Barker 1993). The use of silica acid column chromatography purification methods in conjunction with GC/ECD quantitative techniques has provided a detection limit for DDT in air of 0.16 ng/m^3 (Bidleman et al. 1978). Spectrometry with an automatic quench correction facility was used for the analysis of DDT in rice, maize, and grain plants. Recoveries ranged from 92 to 99%, and detection limits were not reported (Verma and Pillai 1991a). Even though analytical methods exist for detection of DDT in almost all samples, many references did not state detection limits or accuracy of the method.

Alternative approaches are being developed to improve sample recoveries, speed analysis time, or lower detection sensitivities in the analysis of DDT, DDE, and DDD. Sample extraction techniques such as supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), and microwave extraction techniques have been applied to the analysis of DDT, DDE, and DDD to improve extraction of these compounds from soil (de Andrea et al. 2001; Fitzpatrick et al. 2000; Glazkov et al. 1999). SFE has been shown to improve recoveries of organochlorines from water samples over standard liquid/liquid extraction techniques (Glazkov et al. 1999). Solid-phase microextraction techniques can aid in improving sample clean-up and detection sensitivities (Aguilar et al. 1999; Brás et al. 2000; de Jager and Andrews 2000; Röhrig and Meisch 2000). In this technique, fiber-based solid-phase extractants can either directly extract DDT, DDE, and DDD through immersion in water or other aqueous samples (e.g., milk), or extract DDT, DDE, and DDD from the head-space over a sample as it is heated (Aguilar et al. 1999). Once the compounds have been adsorbed into the solid-phase fibers, the compounds are directly desorbed from the fibers in a GC injector and then detected using either electron-capture or mass spectrometric detection techniques. In addition to the instrumental analytical techniques, immunoassays offer good detection sensitivities (<0.1 µg/L) of DDT compounds in complex matrices, although the assay is not specific to one specific DDT compound (Abad et al. 1997; Beasley et al. 1998). These newer analytical methods show much promise towards improving the analysis DDT, DDE, and DDD in complex environmental and biological samples (Röhrig and Meisch 2000).

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 DDT, DDE, and DDD 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 DDT, DDE, and DDD.

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 for the analysis of DDT, DDE, and DDD in blood/plasma, semen, urine, liver, kidney, adipose tissue, skin lipids, human milk, and lymph are described in the literature. These methods are helpful in estimating the potential health risk of exposed populations. In certain cases, spike recoveries were performed in a variety of biological samples to determine the recovery efficiency and analytical sensitivity of the method. In some cases, information was unavailable on the detection limit and accuracy of a method. Obtaining detection limits and information on the accuracy of a method is important to effectively and precisely quantify the parent compound and metabolites in a biological system. Once tissue levels of DDT, DDE, and DDD are obtained, there is no acceptable methodology for extrapolating backwards from those tissue levels to the amount of exposure. Even in those studies in which volunteers were fed measured doses of DDT, such a relationship could not be determined because of bioaccumulation of DDT and its metabolites in adipose tissues and because of individual variability. Further research would help in understanding the relationship between exposure and DDT levels measured in body compartments.

Effect. No specific biomarkers of effect have been determined. Until these biomarkers are determined, methodology needed to identify them cannot be established.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Human exposure is most likely to occur from ingesting food contaminated with small amounts of DDT, DDE, or DDD. Analytical methods are available for measuring DDT, DDE, and DDD in air, water, soil, fish, waterfowl, plants, and food. Of the techniques available, MID GC/ECD appears to be the most sensitive for measuring background levels of DDT, DDE, and DDD in all environmental media.

No information was available on background levels of DDT, DDE, or DDD at which health effects occur. Although analytical techniques are available for measuring DDT, DDE, and DDD in environmental media, further information on the accuracy and precision of these techniques is needed.

7.3.2 Ongoing Studies

No ongoing studies were located on the analytical methods of DDT, DDE, or DDD.