7. ANALYTICAL METHODS

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

This chapter summarizes the methods available for the analysis of toxaphene in biological and environmental media. In designing a study and choosing a method, it is very important that adequate attention be paid to the extent of validation and field applicability. Some of the EPA methods have been validated, while some of the literature methods have not. It is the analyst's responsibility to determine the data quality needed before initiating the application of a particular method.

The analytical methods used to quantify toxaphene in biological and environmental samples are summarized below.

7.1 BIOLOGICAL MATERIALS

Table 7-1 lists the applicable analytical methods for determining toxaphene in biological samples. The analysis and chemical characterization of toxaphene is difficult because of the extreme complexity of the compound. Commercial toxaphene is a complex mixture of chlorinated camphene derivatives containing more than 670 components (Jansson and Wideqvist 1983). Furthermore, widespread contamination from ubiquitous PCBs, 1,1-dichloro-2-2-bis (chlorphenyl)-ethane (DDE), and other organochlorine pesticides, which are also complex multi-isomeric chemicals, often interferes with toxaphene's analysis. Hence, identification of toxaphene in biological and environmental samples almost invariably involves rigorous sample preparation and clean-up procedures prior to chromatographic analysis (de Geus et al. 1999; Gooch and Matsumura 1985; Matsumura et al. 1975; Nelson and Matsumura 1975).

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human tissues (toxaphene and some metabolites)	Maceration of tissue into a fine slurry; addition of anhydrous Na_2SO_4 and acetone; filtration of solution and addition of water and saturated Na_2SO_4 solution to extract; extraction with chloroform; addition of 5% KOH to chloroform extract; extraction with water; water removal (Na_2SO_4); evaporation and dissolution of residue in acetone	TLC	1 µg/sample	94	Tewari and Sharma 1977
Tissues	Grinding of sample (20 g, wet weight) containing internal standards anhydrous sodium sulfate followed by extraction with 1:1 dichloromethane:hexane, volume reduction; cleanup using GPC and Florisil	GC/NCIMS	~10 ppb	77–107 at 40–50 ppb	Fowler et al. 1993
Human breast milk	Centrifugation of milk sample; freeze-drying of fat concentrate; dissolution in acetone and cooling to -60°C; re-dissolution of residue in hexane and shaking with concentrated H_2SO_4; cleanup using silica gel column	GC/ECD and GC/NCIMS	100 ng/g	No data	Vaz and Blomkvist 1985
Human breast fat	Homogenization and extraction with petroleum ether; removal of water from extract with anhydrous Na_2SO_4; volume reduction	GC/ECD	No data	No data	Head and Burse 1987
Stomach washings and urine (toxaphene and some metabolites)	Filtration of sample and wash of residue with water; addition of saturated solution of Na_2SO_4 and extraction with hexane; filtration of extract through anhydrous Na_2SO_4 and evaporation to dryness; dissolution of residue in acetone	TLC	1 µg/sample	94	Tewari and Sharma 1977
Human blood	Addition of 60% H_2SO_4 to blood sample; extraction with hexane:acetone (9:1); centrifugation and evaporation to dryness; dissolution of residue in hexane	GC/ECDGC/MC	No data 10–40 ppb	100 100	Griffith and Blanke 1974

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human blood	Addition of sample to a solution of dilute H_2SO_4 and 10% sodium tungstate; filtration of solution and wash of residue with water; water removal with (Na_2SO_4) and extraction with hexane; filtration of extract through anhydrous Na_2SO_4 and evaporation to dryness; dissolution of residue in acetone	TLC	1 µg/sample	94	Tewari and Sharma 1977

ECD = electron capture detection; GC = gas chromatography; GPC = gel permeation chromatography; MC = microcoulometry; NCIMS = negative chemical ionization mass spectrometry; TLC = thin-layer chromatography

Cautions regarding potential transformations of toxaphene components during sample clean-up operations are described below in Section 7.2. The determination of trace amounts of toxaphene in human tissues and fluids has been restricted to a limited number of analytical techniques. These include gas chromatography equipped with either an electron capture detector (GC/ECD), or a microcoulometric detector (GC/MC), or negative ion chemical ionization mass spectrometry (GC/NCIMS), and thin-layer chromatography (TLC).

The most prevalent analytical technique employed to determine trace amounts of toxaphene in biological and environmental samples is GC/NCIMS because it has shown the greatest sensitivity to these types of chlorinated compounds (Lau et al. 1996; Xia et al. 2009). Vaz and Blomkvist (1985) developed a GC/NCIMS method to quantitatively and selectively detect components of toxaphene at ppb (ng/g) levels in human breast milk. These authors demonstrated that several mass (M) fragments containing mainly (M-35)-ions can be identified, thereby giving relatively simple mass spectra. More important, however, fragmented ions from contamination by other organochlorine compounds were not detected because they gave weak NCIMS spectra. One disadvantage of GC/NCIMS is the potential for obtaining false negative results for certain congeners (Lau et al. 1996; Santos et al. 1997; Xia et al. 2009).

An alternative method is gas chromatography/electron impact/mass spectrometry (GC/EI/MS) (Lau et al. 1996). This method is less sensitive than GC/NCIMS; however, it is better at overcoming interferences (Lau et al. 1996; Xia et al. 2009). In efforts to improve sensitivity, methods using high resolution GC/EI coupled with tandem MS/MS have been developed (Chan et al. 1998; Gouteux et al. 2002; Skopp et al. 2002a; Xia et al. 2009).

GC/ECD has also been widely used as a low-cost and sensitive method for toxaphene analysis. Griffith and Blanke (1974) and Head and Burse (1987) employed GC/ECD for analysis of toxaphene in human blood and breast fat, respectively. MS detection techniques have been favored over ECD since ECD has lower selectivity and higher risk for the coelution of congeners (Bordajandi et al. 2006; de Geus et al. 1999; Fowler 2000; Lau et al. 1996). A number of studies have explored multidimensional gas chromatography (MDGC) or similar techniques coupled with ECD as a way to increase selectivity (Bordajandi et al. 2006; De Boer et al. 1997; Korytar et al. 2003; Shoeib et al. 2000). Enantiomeric determination of chiral toxaphene congeners has been achieved using MDGC/ECD (Bordajandi et al. 2006).

Detection of the individual toxaphene congener enantiomers, referred to as enantioselective determination, has been demonstrated using both GC/NCIMS and MDGC/ECD (Bordajandi et al. 2006; de Geus et al. 1999; Vetter and Luckas 1995, 2000). Enantiomers tend to show differences in biological behavior due to chiral-specific interactions despite their identical physical properties (Vetter and Luckas 2000). The enantiomers of a single congener may be biodegraded or metabolized at different rates and they may show differences in toxicity. Therefore, analysis of the enantiomeric ratios of the congeners found in biological and environmental samples may provide further insight into the environmental fate and toxicity of toxaphene.

Identification of low ppb levels of toxaphene in human blood was achieved by GC/MC (Griffith and Blanke 1974). The advantages of GC/MC are that the system is linear and more specific, and a lower temperature is generally required to vaporize the compound in the GC column.

A radioreceptor assay has been described for the determination of toxaphene in whole blood (Saleh and Blancato 1993). The method is based on the ability of toxaphene to displace 35S tertiary butylbicyclo-phosphorothioate from the chloride channel of isolated gamma-aminobutyric acid receptor ionophore complexes. Unlike chromatographic methods, this approach requires no sample clean-up, needs only 0.1 mL of blood, and is sensitive to toxaphene concentrations in blood of 2 ppb. An advantage of this method is that it assays those toxaphene isomers that are toxic to the nervous system by exploiting the known receptor-based mechanism of that toxicity.

In addition to direct measurement of toxaphene in biological media, it is also possible to determine the level of metabolites in biological tissues and fluids. Tewari and Sharma (1977) developed a TLC method for determination of toxaphene and its metabolites (dechlorinated and dehydrochlorinated toxaphene) in urine, stomach washings, and blood. A detection limit of $1x10^{-6}$ g of toxaphene per sample was achieved. The authors employed a series of solvent systems and chromogenic reagents on silica gel plates impregnated with silver reagents and copper sulfate for separation of the pesticides. The TLC technique is, however, laborious and time consuming.

Despite the availability of advanced instrumental methods, the accurate quantitative determination of the level of toxaphene is difficult because of inherent differences between the GC fingerprint pattern of the technical toxaphene standard and the pattern found in human fluid extracts containing toxaphene. These differences reflect changes caused by metabolism and degradation of the original compound (Lamb et al. 2008).

7.2 ENVIRONMENTAL SAMPLES

Table 7-2 lists the methods used for determining toxaphene in environmental samples. Residues of toxaphene are detectable in the environment because of its use as a piscicide and its use as a pesticide on field crops, fruits, vegetables, and uncultivated lands. The identification and quantification of toxaphene in environmental samples is complicated by changes in the numbers and relative sizes of constituent peaks (components) due to the difference in their rates of degradation, sorption, and volatilization in the environment. In addition, quantitative analysis can be further hindered by the lack of purified, individual congeners, although improvements in this area are being made (Foreid et al. 2000; Gill et al. 1996; Muir and de Boer 1993; Vetter et al. 2000). This is important because of the differing detector response factors of the different congeners, a problem of particular relevance to mass spectrometric detection methods (Xu et al. 1994). Most recently, the focus of analytical toxaphene research has been to develop methods capable of sensitive, selective, and accurate determination of the many different individual toxaphene congeners present in samples (Bordajandi et al. 2006; EPA 2010a; Gill et al. 1996; MacEachen and Cocks 2002; Vander Pol et al. 2010; Vetter et al. 2005; Xia et al. 2009).

Since the formerly used commercial form of toxaphene, called technical toxaphene, undergoes "weathering" through environmental transformation and degradation processes, methods that are strictly based on technical toxaphene analysis may not give the most accurate picture regarding the form that humans may be exposed to in the environment. Therefore, recent efforts have also been made to differentiate between the congener profiles for technical toxaphene and weathered toxaphene (EPA 2010a).

A number of potential problems in the procedures used to isolate toxaphene components (chlorobornanes) have been noted and compiled after a workshop on the analytical chemistry of toxaphene (Muir and de Boer 1993). Extraction/clean-up procedures that include treatments with sulfuric or nitric acid modify the toxaphene peak profile. Gel permeation chromatography (GPC) or column chromatography on alumina were judged suitable for the isolation of lipids from toxaphene and related organochlorines. The use of base hydrolysis for the removal of lipids would degrade chlorobornanes and is not recommended. It has also been reported that oxygen in the chemical ionization (CI) source during mass spectrometric detection can produce fragment ions from PCBs that appear to be derived from chlorobornanes and this can lead to errors in quantitation (Andrews et al. 1993; Muir and de Boer 1993). Other researchers claim that the

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Trapping on chromasorb 102; extraction with hexane	GC/ECD	0.234–0.926 ng/m^3	100	Thomas and Nishioka 1985
Air	Collection of air sample in an air sampling train equipped with prefilter and ethylene glycol; dilution of ethylene glycol with water and extraction with hexane; extraction of prefilter with hexane; pooling of extracts before drying and concentration	GC/ECD	1–10 ng/m^3	No data	Kutz et al. 1976
Air	Adsorption onto PUF using a high volume sampling pump; extraction with hexane and volume reduction	GC/ECD; GC/MS	0.10 pg/m^3 (10,000 m^3 sample)	No data	Barrie et al. 1993
Ambient air	High volume sampler consisting of glass fiber filter with PUF backup adsorbent and flow rate approximately 200–280 L/minute for 24 hours; extraction of filter and PUF in soxhlet with 5% ether in hexane; cleanup using alumina column chromatography and concentration using K-D (EPA Method TO4)	GC/ECD (EPA Method 608)	Generally >1 ng/m^3	No data	EPA 1984a
Drinking water	Extraction of sample with 15% dichloromethane in hexane; water removal using anhydrous Na_2SO_4; extract volume reduction	GC/ECD or GC/MC or GC/electrolytic conductivity and GC/MS	0.001–0.01 µg/L (single component pesticide sample) 0.050–1.0 µg/L (multiple component pesticide sample)	No data	EPA 1987a
Drinking water	Extraction of sample with dichloromethane, water removal and solvent exchange to methyl-t-butyl ether (EPA Method 508)	GC/ECD (capillary column)	No data	No data	EPA 1989

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Drinking water, groundwater, soil, sludges, wastes	Extraction of sample with organic solvent and cleanup using Florisil column	GC/ECD	0.24 µg/L (drinking water) to 24 mg/L (non-water miscible waste)	No data	EPA 1986b
Drinking water	Extraction of sample with acetone on a water sampling apparatus equipped with porous polyurethane plugs; elution of extract through activated Florisil column with diethyl ether in petroleum ether	GC/ECD and GC/MS	0.01 ng/L	100	EPA 1976b
Drinking water	Extraction and concentration from water using SPE followed by elution with dichloro-methane	GC/MS (SIM)	0.32 µg/L	95.4 (3.7% RSD) at 10 µg/L	EPA 2012
Tap water, groundwater, river water	Isolation of compounds from water using C_{18} SPE followed by recovery of adsorbed analytes with supercritical carbon dioxide containing acetone	GC/ion trap MS	7.4 µg/L (ppb, w:v)	105 (18% RSD) at 25 µg/L	Ho et al. 1995
Waste water	Extraction with dichloromethane	Tandem MS	5 µg/sample		Hunt et al. 1985
Waste water	Extraction with dichloromethane, solvent exchange to hexane; Florisil cleanup	GC/ECD (packed column)	0.24 µg/L	96	EPA 1984c
Waste water	Extraction with 15% dichloro-methane in hexane followed by water removal with sodium sulfate and concentration with K-D; additional cleanup, if needed, by partition with acetonitrile to remove fats and oils or fractionation using a Florisil column	GC/ECD	No data	96	EPA 1992a
Municipal and industrial discharge water	Adjustment to pH=11 and extraction with dichloromethane; concentration using K-D after drying	GC/MS	No data	No data	APHA 1998a

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Municipal and industrial discharges	Extraction with dichloromethane (no pH adjustment) and solvent exchange to hexane during concentration; magnesia-silica gel cleanup and concentration	GC/ECD	0.24 µg/L	80	APHA 1998b
Municipal and industrial waste water, sludges	(1) If solids <1%, extraction with dichloromethane. (2) For nonsludges with solids 1–30%, dilution to 1% and extraction with methylene chloride. If solids >30%, sonication with methylene chloride/acetone. (3) For sludges: if solids <30%, treatment as in #2 above. If solids >30%, sonication with acetonitrile then methylene chloride. Back extraction with 2% sodium sulfate. Water removal with sodium sulfate, concentration using K-D, purification using GPC, Florisil, and/or SPE	GC with ECD, MC, or electrolytic conductivity	910 ng/L (lower if many interferences)	76–122 at 5,000 ng/L is accept-able	EPA 1992b
Primary sludge	Extraction of sample with hexane: dichloromethane: acetone (83:15:2); extract concentration and cleanup on Florisil column and elution with 20% acetone in hexane	GC/ECD and GC/MS	No data	85–93	EPA 1982b
Soil, water	Extraction of sample with organic solvent or mixture of organic solvents, depending on the sample matrix, followed by open-column, chromatographic cleanup	GC/ECD or GC/ELCD (EPA Method 8081B)	No data	No data	EPA 2007a
Soil, water	Extraction of sample with organic solvent or mixture of organic solvents, depending on the sample matrix, followed by open-column, chromato-graphic cleanup	GC/NIMS (EPA Method 8276)	No data	No data	EPA 2010a

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil	Addition of water and extract with methanol:toluene (1:1); loading of extract onto chromaflex column containing Florisil; concentration of sample; addition of 43% methanolic KOH solution and refluxing followed by extraction with hexane and Florisil column cleanup	GC/MS and HPLC	0.05 µg/g	76–91	Crist et al. 1980
Soil	Soxhlet extraction using methylene chloride or sonication with methylene chloride:acetone (1:1, v/v); GPC or SPE cleanup	GC/EC-NIMS	100 µg/kg	No data	Brumley et al. 1993
Soil	Extraction of sample (1 g) with dichloromethane:acetone (1:1) using sonication; removal of water with a sodium sulfate column; solvent exchange to isooctane; Florisil cleanup	GC/NCIMS	50 µg/kg (ppb, w:w)	90–109 (10% RSD)	Onuska et al. 1994
Soil	Extraction of soil; introduction of extract with enzyme-toxaphene conjugate into tube containing immobilized toxaphene antibody	Colorimetric immunoassay	0.5 µg/g (0.5 ppm)	118% >0.25–5.0 µg/g	EPA 1996
Sediment, and mussel tissue	Extraction of sample with hexane; elution from alumina column and concentration of eluent	HPLC followed by GC/FID or GC/ECD	<1 ng/g	95–100	Petrick et al. 1988
Pesticide formulation	Extraction of sample using 50% methanolic KOH; elution with ether from Florisil	GC/ECD	1 ng/sample	No data	Gomes 1977
Pesticide formulation	Removal of solvent (xylene) from pesticide sample by reduced pressure; extraction with hexane	Open tubular GC column and GC/TLC	No data	No data	Saleh and Casida 1977
Pesticide formulation	Extraction of sample with hexane	TLC	1 µg/sample	No data	Ismail and Bonner 1974
Pesticide formulation	Dissolution of sample in hexane and loading onto alumina column; elution with hexane, then 20% methylene chloride in benzene and finally 100% methanol	GC/ECD or GC/FID	No data	No data	Seiber et al. 1975

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Cotton leaves	Extraction of sample with water and petroleum ether; addition of methanolic KOH and heat treatment; concentration of extract	TLC followed by GC/ECD	0.16–0.45 µg/cm^2	No data	Bigley et al. 1981
Non-fatty foods	Extraction of homogenized sample with solvent (acetone, acetonitrile, or acetonitrile/water, depending on moisture and sugan content) followed by water removal and Florisil cleanup	GC/ECD (PAM1 methods 302, 303)	<0.2 ppm	>80	FDA 1994a
Various produce	50 g homogenized sample extracted with acetonitrile, filtered, and salt added to affect phase separation; evaporation to near dryness and reconstitution in benzene	GC/ECD	2 ppm	No data	Hsu et al. 1991
Fruits and vegetables	Extraction with acetone in blender; filtration and extraction with petroleum ether/di-chloromethane; solvent evaporation and dissolution of residue in minimum amount of acetone	GC/ECD	No data	No data	WHO 1984
Cucumber	Blending of sample with acetone followed by extraction with petroleum ether and dichloromethane (1:1); water removal (Na_2SO_4) and concentration followed by Florisil column cleanup	GC/ECD or FID	4.34 ppm	113	Luke et al. 1975
Fortified extracts (various foods)	Preparation of sample solution with acetone or hexane; addition of diphenylamine and zinc chloride solution and evaporation to dryness; heating of residue (250°C) for a few minutes and dissolution of residue complex in acetone	Spectro-photometer (absorbance at 640 nm)	<1 ppm	69–100	Graupner and Dunn 1960
Molasses	Dilution of sample with water; extraction with hexane: isopropanol	GC/ECD	0.03 mg/kg	No data	WHO 1984

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Fatty foods	Extraction of fats and residues from homogenized sample by dissolution in an organic solvent followed by isolation of the residues from the fat using Florisil	GC/ECD	<0.2 ppm	>80	FDA 1994b
Meat	Blending with ethyl acetate followed by drying (Na_2SO_4) and filtration; treatment of extract with KOH and heat; extraction with hexane; Florisil column cleanup	GC/ECD	No data	76–79	Boshoff and Pretorius 1979
Bovine defibrinated whole blood	Dilution of blood with water and extraction with hexane	GC/ECD	0.58 µg/mL	73.4	Maiorino et al. 1980
Bovine defibrinated whole blood	Addition of sample to 88% formic acid and shaking on a vortex mixer; extraction with hexane and extraction of hexane with 5% potassium carbonate; extract volume reduction	GC/ECD	0.465 µg/mL	71.7	Maiorino et al. 1980
Bovine defibrinated whole blood	Addition of sample to 88% formic acid followed by mixing and loading onto Florisil column; elution with 6% diethyl ether in petroleum ether; volume reduction and washing with hexane	GC/ECD	0.026 µg/mL	103.4	Maiorino et al. 1980
Lard	Extraction with petroleum ether; centrifugation; removal of water from extract with anhydrous Na_2SO_4; volume reduction	GC/ECD	1.37 µg/g	46.5–107.3	Head and Burse 1987
Poultry fat	Rendering of fat followed by direct analysis	GC/ECD	0.475–0.908 ppm	92.6–96.9	Ault and Spurgeon 1984
Milk fat	Centrifugation and fractionation using Florisil column	GC/ECD and GC/MS	<10 ppb (ECD) 7 ppb (MS)	No data	Cairns et al. 1981
Milk and butter	Addition of sample to KOH followed by heat treatment and extraction with hexane; centrifugation and cleanup using Florisil	GC/ECD	No data	78–88	Boshoff and Pretorius 1979

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human breast milk	Centrifugation of milk sample; freeze-drying of fat concentrate; dissolution in acetone and cooling to -60°C; re-dissolution of residue in hexane and shaking with concentrated H_2SO_4; cleanup using silica gel column	GC/ECD and GC/NCIMS	100 ng/g	No data	Vaz and Blomkvist 1985
Fish (whole)	Blending of frozen sample with dry ice and anhydrous Na_2SO_4; extraction in a column with hexane: acetone (1:1), followed by methanol	GC/NCIMS	75 pg/sample	98	Swack-hamer et al. 1987
Fish tissues	Extraction of tissues with a mixture of hexane and acetone followed by a second extraction with hexane and diethyl ether; evaporation and dissolution of lipid extract in hexane; shaking of extract with H_2SO_4 to remove lipid	GC/NCIMS	No data	No data	Jansson and Wideqvist 1983
Fish tissue	Homogenization of 10 g sample with hexane:acetone (1:2.5) under acid condition, extraction twice more with 10% diethyl ether in hexane. Treatment with 98% H_2SO_4 and cleanup using GPC and silica gel chromatography	GC/NCIMS	No data	94 (RSD= 11%) at 19 ng/g	Jansson et al. 1991
Fish	Homogenization of 20 g sample followed by extraction with hexane/acetone, addition of internal standards (^{13}C-PCBs), and cleanup using GPC and Florisil	GC/HRMS (SIM)	10 ppb (wet weight)	No data	Andrews et al. 1993

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Fish	Homogenization of 10 g sample blended with 80 g sodium sulfate; extraction with 50% acetone in hexane. Lipid extraction with 60% cyclohexane in dichloro-methane; cleanup on 1% water deactivated silica	ECNI GC/MS	No data	105% at 10 μg	Glassmeyer et al. 1999
Fish tissue	Pulverization of tissue with anhydrous sodium sulfate and extraction with acetone; solvent exchange to hexane and volume reduction; cleanup using dry-packed Florisil, wet-packed Florisil and silica gel	GC/MS (SIM)	0.1 ng/g	90 (RSD= 7%) at 100 ng	Jarnuzi and Wakimoto 1991

ECD = electron capture detector; ECNI = electron capture, negative ionization detector; ELCD = electrolytic conductivity detector; FID = flame ionization detector; FTIR = Fourier transform infrared spectroscopy; GC = gas chromatograph; GPC = gel permeation chromatography; HPLC = high performance liquid chromatography; HRMS = high resolution mass spectrometry; K-D = Kuderna-Danish concentration; MC = microcoulometry; MS = mass spectrometry; NCIMS = negative ion chemical ionization mass spectrometry; PCBs = polychlorinated biphenyls; PUF = polyurethane foam; SIM = selected ion monitoring; RSD = relative standard deviation; SIM = selected ion monitoring; SPE = solid phase extraction; TLC = thin-layer chromatography; v/v = volume/volume; wt/wt = weight/weight

problem of residual oxygen in the ion source does not present a major problem (Fowler et al. 1993). In order to minimize problems with interferences during analysis, it is recommended that toxaphene components be isolated as completely as possible from PCBs and that the presence of oxygen in the ion source be minimized.

GC/ECD, sometimes in combination with GC/MS, is the most frequently used analytical method for characterization and quantification of toxaphene in air, drinking water, fish, and other environmental samples (Boshoff and Pretorius 1979; Cairns et al. 1981; EPA 1976c, 1985, 2007a; Kutz et al. 1976; Luke et al. 1975; Thomas and Nishioka 1985; WHO 1984; Wideqvist et al. 1984). Analysis of the sample includes extraction in organic solvent; a Florisil silica, gel permeation, or TLC clean-up step; and detection by GC (Atuma et al. 1986; Ault and Spurgeon 1984; EPA 1976b; Head and Burse 1987; Ismail and Bonner 1974; Maiorino et al. 1980; Saleh and Casida 1977; Seiber et al. 1975). A typical gas chromatogram contains a series of hills and valleys with three main peaks (EPA 1982b; Gomes 1977). Detection limits of toxaphene residues in fish and drinking water were 50 ng of toxaphene per g of sample and 1 ng of toxaphene per g of sample, respectively (EPA 1976c, 1987a). GC/ECD is the standardized method used by EPA (method 8081B) for determining toxaphene in water and soil samples (EPA 2007a). EPA method 8270c (GC/MS, electron impact ionization) is not recommended for toxaphene because of limitations in sensitivity arising from the multicomponent nature of toxaphene (EPA 2007b). More recently, EPA Method 8276 has been developed to detect congeners typically found in weathered toxaphene such as p-26, p-40, p-41, p-44, p-50, p-62, Hx-Sed, and Hp-Sed (EPA 2010a). This method uses fused-silica, open tubular capillary columns with negative ion mass spectrometry (NIMS) and is considered an appropriate alternative to EPA Method 8081.

Archer and Crosby (1966) developed a confirmatory method for toxaphene analysis in environmental samples that involved dehydrohalogenating (in 50% methanolic potassium hydroxide) the residue extract prior to GC analysis. The gas chromatogram indicated one main peak and several minor peaks. Also, the detector response was doubled, thereby increasing the sensitivity of this procedure. While this method was also rapid, its main application was in samples where toxaphene was the major residue. In samples with multiple organochlorine pesticide residues, it would be difficult to measure accurately all of the residues and quantify the amount of toxaphene (Archer and Crosby 1966; Bigley et al. 1981; Crist et al. 1980; Gomes 1977). Recoveries from various samples are generally good with detection limits at levels of <1 ppm.

The tandem MS method has been used as an alternative to GC/MS. This method employs the technique of collision-activated dissociation on a triple quadruple mass spectrometer. This facilitates direct and rapid qualitative and semiquantitative analysis of toxaphene samples in both liquid and solid environmental matrices at the 10–100 ppb level (Hunt et al. 1985). Additional features of tandem MS include the elimination of most wet chemical and chromatographic separation steps, detection of both known and unknown compounds by molecular weight and functional group, and a total analysis time per sample of <30 minutes. A disadvantage is that tandem MS is somewhat less specific than GC/MS in the identification of some isomeric compounds.

Techniques developed by Jansson and Wideqvist (1983) and modified by Swackhamer et al. (1987) indicated that toxaphene can be detected at 75 pg per sample (approximately 1.2 ng/g) in fish using methane NCIMS. The authors noted that the NCIMS technique is more specific and 100 times more sensitive than EI or chemical ionization (CI) mass spectrometry and GC/ECD. In combination with a selected ion monitoring program, specific fragment ions can be monitored without any preseparation column chromatography to eliminate other organochlorine pesticides that coelute with toxaphene (Swackhamer et al. 1987). Furthermore, NCIMS spectra are less complex than EI or CIMS spectra and contain higher mass ions due to successive losses of chloride and hydrochloride from the molecular ion. Jansson et al. (1991) reported a GC/NCIMS method for toxaphene in fish that allowed detection of levels below 19 ng/g. Methods based on GC/NCIMS generally give lower limits of detection than GC/ECD methods and thus, are recommended for the best sensitivity (Muir and de Boer 1993).

Shafer et al. (1981) reported that the combined data of a gas chromatograph coupled to a Fourier-transform infrared spectrometer (GC/FT-IR) and GC/MS provide complementary information that leads to a better understanding and identification of the EPA's priority pollutants (including toxaphene) in air. Both GC/FT-IR and GC/MS separations were performed quickly and efficiently on wall-coated open tubular capillary columns.

A semi-specific spectrophotometric method for toxaphene analysis in fortified extracts of various foods was developed by Graupner and Dunn (1960). It was based on measuring the absorbance at 640 nm of a greenish-blue diphenylamine-toxaphene complex that was formed by reacting a sample extract with diphenylamine in the presence of zinc chloride. Several other organochlorine pesticides also reacted under these conditions, but only a few formed complexes that absorbed appreciably at 640 nm, thereby causing some interference with toxaphene analysis. A detection limit of <1 ppm of toxaphene was reported (Graupner and Dunn 1960).

Petrick et al. (1988) employed high-performance liquid chromatography (HPLC) as a clean-up technique prior to GC analysis. Petrick and co-workers efficiently separated toxaphene residues from other organochlorinated compounds in fat-rich samples with quantitative recovery. A detection limit of less than 1 ng of toxaphene per gram of sample was achieved by GC/ECD. The authors noted that the HPLC technique is highly efficient and reproducible and has a low consumption of solvents and high sample loading capacity.

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 toxaphene 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 toxaphene.

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 are available for detecting and quantifying levels of toxaphene in the blood and milk fat of humans. The precision, accuracy, reliability, and specificity of these methods have been reported. These methods are sufficiently sensitive to determine background levels of toxaphene in the general population and levels at which adverse health effects would begin to occur. Pharmacokinetic data indicate that toxaphene rapidly redistributes to fat; therefore, blood levels would be useful for identifying very recent exposures to toxaphene. Levels in milk fat are retained somewhat longer, but these levels decrease within weeks of cessation of exposure.

A highly sensitive and specific NCIMS technique has been employed to detect components of toxaphene at ppb levels in breast milk without the interference of other organochlorine pesticides (Vaz and Blomkvist 1985). GC/ECD and GC/MS can also detect trace amounts of toxaphene in human tissues and fluids following an efficient sample preparation and rigorous clean-up procedures. TLC has been used for analysis of toxaphene metabolites (Tewari and Sharma 1977). There is a growing need for research and development of highly sensitive and quantitative methods for determination of toxaphene metabolites. These methods would be useful, since they would allow investigators to assess the risks and health effects of long-term, low-level exposure to toxaphene.

Currently, no methods are available to quantitatively correlate monitored levels of toxaphene in tissues or fluids with exposure levels or toxic effects in humans. If methods were available, they would provide valuable information on systemic effects following exposure to trace levels of toxaphene.

Effect. No specific biomarkers of effect have been clearly associated with toxaphene poisoning. Some biological parameters have been tentatively linked with toxaphene exposure, but insufficient data exist to adequately assess the analytical methods associated with measurement of these potential biomarkers.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Human exposure to toxaphene occurs primarily by inhalation of ambient air, ingestion of contaminated foodstuffs, and contact with contaminated soil and surface water. Reliable analytical methods are available to detect background levels of toxaphene in a wide range of environmental matrices. Toxaphene levels of 75 pg/sample (approximately 1.2 ng/g) can be detected in fish using the NCIMS technique (Swackhamer et al. 1987). However, there is a need to implement more refined software to process efficiently the data generated by the NCIMS technique. GC/ECD is the standardized analytical method used by EPA (2007a) to determine toxaphene in soil and water samples at ppb levels. A newer EPA method (8276) has been developed as an alternative to method 8081, which uses GC/NIMS (EPA 2010a). GC/ECD, GC/MS, and tandem MS can detect and quantify toxaphene in air, soil, plant material, fish, water, milk, fat, and meat at ppb levels. The MRL for intermediate oral exposure to toxaphene is 0.002 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 70 ppb (70 μg/L or 70 μg/kg) in either medium. The methods reported for drinking water have limits of detection far below this value (EPA 1976b, 1987a, 1989, 1986b; Ho et al. 1995). The needed sensitivities can be achieved for produce (Hsu et al. 1991; Luke et al. 1975), molasses (WHO 1984), and fish (Andrews et al. 1993; Jarnuzi and Wakimoto 1991; Swackhamer et al. 1987). Limits of detection in FDA methods are reported

as “<0.2 ppm” and are thus inadequate for these MRLs. Additional analytical methods for detecting low levels of toxaphene are needed for foods other than produce.

Little is known about the toxic properties of toxaphene congener metabolites in the environment (Bidleman et al. 1993). Additional analytical methods specifically targeted at toxaphene metabolites and degradation products are needed to support such investigations.

7.3.2 Ongoing Studies

No ongoing studies concerning techniques for measuring and determining toxaphene in biological and environmental samples were reported.