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 mirex and chlordecone, their metabolites, and other biomarkers of exposure and effect to mirex and chlordecone. 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

The most commonly used methods for measuring mirex in blood, tissues (including adipose tissue), milk, and feces are gas chromatography (GC) or capillary GC combined with electron capture detection (ECD) or mass spectrometry (MS). Tables 6-l and 6-2 summarize the applicable analytical methods for determining mirex and chlordecone, respectively, in biological fluids and tissues. Sample preparation for biological matrices involves solvent extraction followed by clean-up steps. Biological samples are often contaminated with other compounds such as polychlorinated biphenyls (PCBs); therefore, additional clean-up steps and/or confirmation techniques are employed to assure reliable results.

Mirex can be extracted from blood using hexane, acetone-hexane, hexane-ethyl ether, or petroleum ether and acetone (Bristol et al. 1982; Caille et al. 1987; Korver et al. 1991; Stahr et al. 1980; Waliszewski and Szymczynski 1991). Blood samples are often contaminated with other compounds such as PCBs. The use of adsorption chromatography as a clean-up step is effective in achieving separation of PCBs from mirex in blood (Korver et al. 1991). Other clean-up methods for blood and tissue samples include concentrated sulfuric acid wash (Waliszewski and Szymczynski 1991), and Florisil column clean-up (Mes 1992). For measuring mirex in blood, sensitivity of GC/ECD is in the sub-parts per billion (ppb) range (Korver et al. 1991). Recovery of mirex from blood is generally

TABLE 6-1. Analytical Methods for Determining Mirex in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood serum	Denatured; solvent extraction; clean-up on silica gel	GC/ECD; confirmation by capillary GC/HRMS	0.2 ppb	70	Korver et al. 1991
Blood serum or whole blood	Acidification; solvent extraction; clean-up with concentrated sulfuric acid; optional silica gel column clean-up if PCBs are present	GC/ECD	No data	94.1 (serum); 93.3 (whole blood)	Waliszewski and Szymczynski 1991
Whole blood	Homogenization; centrifugation; filtered; redissolve dried residue in hexane; clean-up on Florisil column	Capillary GC/ECD; confirmation by capillary GC/MS	0.04 ng/g	80 (mean of all pesticides)	Mes 1992
Whole blood	Solvent extraction	GC/ECD; confirmation of metabolite by GC/MS	No data	92–99 (average)	Stahr et al. 1980
Plasma	Solvent extraction	GC/ECD	10 ng/mL	94.4	Caille et al. 1987
Tissue	Homogenization; solvent extraction; clean-up on Florisil column	GC/ECD	0.03 ppm (liver) 0.017 ppm (adipose)	>95 (average)	Stein and Pittman 1979
Tissue	Homogenized; solvent extraction	GC/ECD	0.001 µg/mg (tissues)	72.5 (liver); 81.3 (kidney)	Caille et al. 1987
Adipose tissue	Clean-up by Florisil column chromatography and GPC	GC/ECD; GC/MS	No data	96	Macleod et al. 1982
Adipose tissue	Dissolution in hexane; clean-up on Florisil column	GC/ECD	No data	89–92	EPA 1980e

TABLE 6-1. Analytical Methods for Determining Mirex in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Adipose tissue	Solvent extraction; clean-up by Florisil column chromatography	Capillary GC/ECD; confirmation by GC/MS	0.24 ng/g	86 (mean, all pesticides)	Mes 1992
Adipose tissue	Sample is dry macerated; solvent extraction; liquid-liquid partition; clean-up on Florisil column	GC/ECD; confirmation by GC/MS	0.05–0.1 ppm	No data	Kutz et al. 1985
Adipose tissue	Sample is dry macerated; solvent extraction; liquid-liquid partition; clean-up on Florisil column	GC/ECD	No data	No data	Holt et al. 1986
Adipose tissue	Dissolution in hexane; clean-up on Florisil column	GC/ECD	No data	89–92.3	Watts et al. 1980
Adipose tissue	Solvent extraction; GPC separation; clean-up on Florisil column	Capillary GC/ECD; confirmation by capillary GC/MS	1.8 ng/g (mirex); 1.9 ng/g (photomirex)	96.1–106 (mirex); 93.9–106 (photomirex)	LeBel and Williams 1986
Adipose tissue	Homogenization; Unitrex fractionation; clean-up by silica gel column fractionation	GC/ECD	~0.02 ppm	55	Head and Burse 1987
Milk	Soxhlet extraction; clean-up on deactivated Florisil column	GC/MS; capillary GC/ECD; confirmation by GC/MS	0.05 ng/g (GC/ECD); 1 ng/g (GC/MS)	66 (average)	Bush et al. 1983a, 1983b
Milk	Solvent extraction; addition of hexane; clean-up on Florisil-silicic acid column	Capillary GC/ECD; confirmation by capillary GC/MS-MID	~1 ppb	70–106 (all pesticides)	Mes et al. 1986

TABLE 6-1. Analytical Methods for Determining Mirex in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Milk	Solvent extraction; GPC separation; Florisil column clean-up	Dual column capillary GC/ECD	0.5 ng/g (estimated)	100	Rahman et al. 1993
Milk	Ultrasonic homogenization; solvent extraction; acid clean-up	Capillary GC/MS-SIM	10 µg/kg	75–85 (all pesticides)	Mussalo-Rauhamaa et al. 1993
Rat brain	Homogenization; clean-up on Florisil column fractionation	GC/ECD	10 ng/mL	No data	Bush and Barnard 1982
Feces	Homogenization; solvent extraction; clean-up on alumina/Florisil column	GC/ECD	No data	No data	Gibson et al. 1972

ECD = electron capture detection; EPA = Environmental Protection Agency; GC = gas chromatography; GPC = gel permeation chromatography; HRMS = high-resolution mass spectrometry; MID = multiple ion detection; MS = mass spectrometry; SIM = selected ion monitoring

TABLE 6-2. Analytical Methods for Determining Chlordecone in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood serum or whole blood	Acidification; solvent extraction; clean-up with concentrated sulfuric acid	GC/ECD	No data	84.7 (serum); 85.1 (whole blood)	Waliszewski and Szymczynski 1991
Blood	Solvent extraction from acidified blood; liquid-liquid partition	GC/ECD	≤10 µg/L in 1-mL serum specimen	24.7 (blood); 43.2 (serum)	Caplan et al. 1979
Plasma	Solvent extraction	GC/ECD	10 mg/mL	87.4	Caille et al. 1987
Blood, serum, plasma	Acidification; solvent extraction	GC/ECD	No data	>95	Blanke et al. 1977
Tissue (liver, kidney, adipose)	Liver, kidney: homogenization; solvent extraction Adipose: dissolution in solvent; centrifugation	GC/ECD	.10 µg/100 ng (1 ppm)	73.2 (liver); 58.5 (kidney)	Caille et al. 1987
Urine, saliva	Acidification; solvent extraction	GC/ECD	5 ppb	>95	Blanke et al. 1977
Stool, bile	Homogenization; acidification; solvent extraction; clean-up using liquid-liquid partition	GC/ECD	5 ppb	73.5 (bile)	Blanke et al. 1977
Bile	Dilution with water; treatment with buffer, enzyme, or acid; solvent extraction; clean-up with H_2SO_4	GC/ECD; GC/MS	No data	No data	Fariss et al. 1980

ECD = electron capture detection; GC = gas chromatography; H_2SO_4 = sulfuric acid; MS = mass spectrometry

good (≥70%) (Caille et al. 1987; Korver et al. 1991; Stahr et al. 1980; Waliszewski and Szymczynski 1991). Precision is generally very good for blood samples (≤10% relative standard of deviation [RSD]) (Korver et al. 1991; Stahr et al. 1980). The low RSDs indicate good repeatability of the procedures (Waliszewski and Szymczynski 1991). Sample storage may adversely affect recovery (Bristol et al. 1982) and precision (Bristol et al. 1982; Stahr et al. 1980). Confirmation of mirex in blood can be accomplished by using GC/MS (Korver et al. 1991; Mes 1992).

Mirex can be extracted from tissues using hexane, hexane-acetone, hexane-ethyl ether, or petroleum ether (Caille et al. 1987; EPA 1980e; Head and Burse 1987; Kutz et al. 1985; LeBel and Williams 1986). Clean-up methods include liquid-liquid partitioning (adipose tissue) (Kutz et al. 1985), gel permeation chromatography (GPC) (adipose tissue) (LeBel and Williams 1986; Macleod et al. 1982), and Florisil column clean-up (liver and adipose tissue) (EPA 1980e; Kutz et al. 1985; Mes 1992; Macleod et al. 1982; Stein and Pittman 1979). For measuring mirex in tissues, sensitivity of GC/ECD is in the sub-ppm to sub-ppb range (Kutz et al. 1985; LeBel and Williams 1986; Mes 1992; Stein and Pittman 1979). Recovery of mirex from tissues is generally good (≥70%) (Caille et al. 1987; EPA 1980d; LeBel and Williams 1986; Macleod et al. 1982), as is precision (<20% RSD) (EPA 1980d; Caille et al. 1987; LeBel and Williams 1986). Confirmation of mirex in adipose tissue can be accomplished using GC/MS (Kutz et al. 1985; LeBel and Williams 1986; Mes 1992). Photomirex has been measured in adipose tissue by GC/MS (LeBel and Williams 1986).

Capillary GC/ECD, dual column capillary GC/ECD, and capillary GC/MS have been used for quantitation of mirex in milk with sensitivity in the low to sub-ppb range (Bush et al. 1983b; Mes et al. 1986; Mussalo-Rauhamaa et al. 1993; Rahman et al. 1993). Recovery data for milk are generally very good (≥70%) (Mes et al. 1993; Mussalo-Rauhamaa et al. 1993; Rahman et al. 1993), but precision data were not reported.

Mirex can be extracted from feces with hexane-acetonitrile and the extract cleaned up on alumina/Florisil columns, then analyzed using GCYECD. Sensitivity, precision, and accuracy data for feces were not reported (Gibson et al. 1972).

The most commonly used method for measuring chlordecone in blood is GC combined with ECD (Blanke et al. 1977; Caille et al. 1987; Caplan et al. 1979; Waliszewski and Szymczynski 1991).

Sample preparation involves an extraction procedure. Chlordecone is unique among the chlorinated pesticides since it has a ketone functional group that readily forms a hydrate in the presence of water (Caplan et al. 1979). This hydrate formation permits selective extraction of chlordecone from all other chlorinated pesticides (Caplan et al. 1979). Although recoveries for the selective extraction procedure were low (<50%) because multiple extractions were not performed, sensitivity was maintained and precision was good (<7% RSD) (Caplan et al. 1979). Another preparation step that allowed better recovery (>80%) of chlordecone from blood involved extraction with petroleum ether and acetone followed by a sulfuric acid clean-up step (Waliszewski and Szymczynski 1991). Results of this method were reproducible, with precision being <7% RSD (Waliszewski and Szymczynski 1991). Sensitivity was not reported for this method (Waliszewski and Szymczynski 1991). Extraction of plasma and tissues with hexane-acetone gave low-to-adequate recoveries (58.5-87.4%), but again, reproducibility was good, with precision being <6% RSD (Caille et al. 1987). Method detection limits for measuring chlordecone in blood samples are in the low ppb range (Caille et al. 1987; Caplan et al. 1979). Confirmation techniques for chlordecone include GC/MS and GC with microcoulometric detection (Blanke et al. 1977), and for chlordecone and its breakdown products, GC/chemical ionization (CI) MS (Harless et al. 1978).

Chlordecone can be extracted from tissues with hexane-acetone, then analyzed by GC/ECD. Sensitivity is 1 ppm, and recoveries of 73.2% (liver) and 58.5% (kidney) were reported (Caille et al. 1987). No methods for measuring chlordecone in human milk were located.

GC/ECD is the most commonly used method to measure chlordecone in urine and saliva, and chlordecone and its metabolites (chlordecone alcohol and the glucuronide conjugates) in feces and bile (Blanke et al. 1977; Fariss et al. 1980). For the liquid samples, using acetone in hexane to extract chlordecone from acidified samples gave good recoveries (95%) and required no clean-up step (Blanke et al. 1977). Stool and bile samples required a clean-up procedure prior to analysis. Sensitivity was 5 ppb. For the bile samples, precision was adequate (<20% RSD) (Blanke et al. 1977). No other data were reported. Chlordecone and its metabolites (chlordecone alcohol and the glucuronide conjugates) were detected by GC/ECD in feces and bile (Blanke et al. 1978; Fariss et al. 1980). Chlordecone alcohol was isolated from feces (Wilson and Zehr 1979).

6.2 ENVIRONMENTAL SAMPLES

Methods exist for determining mirex and chlordecone in air (ambient and occupational), water, sediment and soil, biota and fish, and foods. Most involve separation by GC with detection by ECD or MS. Tables 6-3 and 6-4 summarize some of the applicable analytical methods used for determining mirex and chlordecone, respectively, in environmental samples.

The most commonly used methods for measuring mirex or its degradation products in air are packed column or capillary GC/ECD. Air samples are collected using polyurethane foam (PUF), then the PUF plugs are Soxhlet-extracted (Durrell and Sauer 1990; ASTM 1991; Lewis et al. 1977). For air samples, sensitivity of GC/ECD is in the sub-ppb range (Durrell and Sauer 1990). Recovery is excellent (>98%), although precision was not reported (Lewis et al. 1977). Confirmation of mirex may be accomplished using GC/MS (ASTM 1991) or dual capillary column GC/dual detector (Durrell and Sauer 1990).

Mirex has been measured in water samples using GC and capillary GC coupled with ECD or MS detection (Driscoll et al. 1991; Durrell and Sauer 1990; Hargesheimer 1984; Sandhu et al. 1978). Samples are extracted with dichloromethane (Hargesheimer 1984) or hexane (Driscoll et al. 1991; Sandhu et al. 1979). Clean-up methodologies which have been applied to water samples are chromic acid treatment (Driscoll et al. 1991) and Florisil column fractionation (Sandhu et al. 1978). For water samples, sensitivity is in the low ppb (Durrell and Sauer 1990) to low parts per trillion (ppt) range (Hargesheimer 1984; Sandhu et al. 1978). Precision is acceptable (<20% RSD) (Driscoll et al. 1991; Dun-e11 and Sauer 1990; Sandhu et al. 1978). The sensitivity of GC/MS analysis is in the sub-ppb range (Hargesheimer 1984); recovery and precision data were not reported (Hargesheimer 1984). A chromic acid digestion extraction technique was compared to conventional solvent extraction for recovery of mirex and photomirex from river water samples (Driscoll et al. 1991). The digestion technique was more efficient than conventional solvent extraction, with better recoveries and superior precision (Driscoll et al. 1991). The better precision obtained with sample digestion may be due to lack of emulsions, which allowed better phase separation and, therefore, more reproducible recoveries (Driscoll et al. 1991). Sensitivity data were not reported. Confirmation can be accomplished using dual capillary GC/dual detector system (ECD and electrolytic conductivity detector, ELCD) (Durrell and Sauer 1990).

TABLE 6-3. Analytical Methods for Determining Mirex in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collection on particulate filter and polyurethane foam; Soxhlet extraction; alumina column clean-up	GC/ECD	0.1 ng/m^3 [a]	>95	Lewis et al. 1977
Indoor air	Collection on filter and PUF plug; solvent extract; optional alumina column clean-up (ASTM D 4861)	GC/ECD or capillary GC/ECD; confirmation on second GC column	.01 µg/m^3 [a]	90–110	ASTM 1991
Water	Solvent extraction	GC/MS (CI-SIM)	0.005 ppb	No data	Hargesheimer 1984
River water	Hexane extraction coupled with chromic acid digestion	Capillary GC/ECD	No data	99.4 (mirex); 100.9 (photomirex)	Driscoll et al. 1991
Drinking water (groundwater)	Solvent extraction; clean-up on Florisil column	GC/ECD	10 ng/L	66.7	Sandhu et al. 1978
Seawater, rain	Solvent extraction	Dual capillary GC/dual detector (ECD, ELCD)	IDL: 8.4 pg/µL (ECD); 11.5 pg/µL (ELCD)	No data	Durell and Sauer 1990
Waste water	Solvent extraction; optional Florisil column clean-up (EPA Method 617)	GC/ECD; confirmation by GC/MS	.015 µg/L	89.1	EPA 1992b
Lake sediments	Ultrasonic solvent extraction; clean-up on Florisil column; separation of mirex and photomirex from PCBs using charcoal-polyurethane column	GC/ECD	No data <0.05 ppm	99.9–100 (mirex); 95.1–99.1 (photomirex)	Chau and Babjak 1979
Sediment	Solvent extraction; liquid-liquid partition; GPC separation; clean-up on Florisil; copper powder to remove sulfur; nitration/alumina column to remove PCBs	GC/ECD	≥10 ppb	93 (mirex) 92 (photomirex) (solvent standards)	Norstrom et al. 1980a

TABLE 6-3. Analytical Methods for Determining Mirex in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Sediment	Sonication extraction; liquid-liquid partition; Florisil column clean-up	GC/ECD and capillary GC/MS	No data	98.6 (solvent standards by GC/MS)	Onuska et al. 1980
Soil	Simultaneous steam-distillation-solvent extraction (SDE)	Capillary GC/ECD	1 ppb	78%	Seidel and Lindner 1993
Sediment	Solvent sonication extraction; GPC separation	Capillary GC/ECD; confirmation by GC/MS	.002 ppb	90–95%	Sergeant et al. 1993
Copepods and mixed micro-crustaceans	Homogenization; solvent extraction; column clean-up	Dual capillary GC/ dual detector (ECD, ELCD)	IDL: 8.4 pg/μL (ECD); 11.5 pg/μL (ELCD)	No data	Durell and Sauer 1990
Fish	Extraction using GPC; clean-up on Florisil column; mirex separated from PCBs and other aromatic compounds by nitration/alumina column technique	GC/ECD	≥10 ppb	93 (mirex); 92 (photomirex)	Norstrom et al. 1980a
Fish	Soxhlet extraction of blended sample; clean-up and fractionation on Florisil column	GC/ECD	0.055 ppb	95.8–102	Quintanilla-Lopez et al. 1992
Fish	Homogenization; solvent extraction; clean-up on Florisil column	Capillary GC/MS	low pg	98.6 (standard solutions)	Onuska et al. 1980
Fish	Solvent extraction; GPC separation; Florisil column clean-up	GC/MS	0.1-2 ng/g	No data	Hellou et al. 1993
Fish	Homogenization; Soxhlet extraction; GPC separation, Florisil column clean-up	Dual column GC/ECD	.5 ng/g (estimated)	100	Rahman et al. 1993

TABLE 6-3. Analytical Methods for Determining Mirex in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Fish eggs and aquatic macro-invertebrates	Homogenization using tissuemizer; clean-up on Florisil column	Capillary GC/ECD	1 ppm	93.5	Bush and Barnard 1982
Herring gull eggs	Solvent extraction; clean-up; separation from PCBs by nitration/alumina column technique	GC/ECD; confirmation by capillary GC/MS	No data	95 (mirex) 94–100 (degradation products, except 5,10-dihydromirex)	Norstrom et al. 1980b
Fruit and vegetables	Extraction and Florisil clean-up (AOAC Method)	GC/ECD	No data	95.5 (apples); 103 (cauliflower)	Krause 1973
Green pepper	Solvent extraction; GPC separation	GC/MSD	No data	No data	Stan 1989
Poultry fat	Liquification; GPC clean-up	GC/ECD	<0.5 ppm	90	Ault and Spurgeon 1984
Fish and butterfat	Fractionation on unactivated Florisil column; liquid-liquid partition; activated Florisil column clean-up	GC/ECD	No data	90.8 average (fish); 103.9 average (butterfat)	Bong 1977
Non-fatty foods	Homogenization	Capillary GC/MS	0.5 µg/g (estimated)	89	Liao et al. 1991
Milk	Mixed with water and methanol; SPE clean-up	Capillary GC/ECD; confirmation using second column	0.7 µg/L	70 (average)	Manes et al. 1993
Milk	Solvent extraction; Florisil column clean-up	Capillary GC/ECD	~1 ppb	99.3	de la Riva and Anadon 1991

TABLE 6-3. Analytical Methods for Determining Mirex in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Milk	Oxalate/solvent extraction; GPC separation; optional clean-up on alumina; Florisil column clean-up, if needed	GC/ECD; GC/ECD or capillary GC/ECD for confirmation	.0005 ppm	93–95	Trotter and Dickerson 1993
Fatty foods	Dissolution in solvent; SPE clean-up; H_2SO_4/SPE clean-up	GC/ECD	No data	84.5	Di Muccio et al. 1991

[a]Sample detection limit depends upon sampling rate and duration

AOAC = Association of Official Analytical Chemists; ASTM = American Society for Testing and Materials; CI = chemical ionization; ECD = electron capture detection; ELCD = electrolytic conductivity detector; EPA = Environmental Protection Agency; GC = gas chromatography; GPC = gel permeation chromatography; H_2SO_4 = sulfuric acid; IDL = instrumental detection limit; MS = mass spectrometry; MSD = mass selective detector, Na_2SO_4 = sodium sulfate; PCBs = poylchlorinated biphenyls; PUF = polyurethane foam; SIM = selective ion monitoring; SPE = solid phase extraction

TABLE 6-4. Analytical Methods for Determining Chlordecone in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Occupational air	Collection using filter and impinger; solvent extraction	GC/ECD	10 ng/sample	No data	NIOSH 1984
Air	Collection on glass fiber filters; solvent extraction	GC/ECD; confirmation by GC/ELCD; GC/MS	No data	95	Hodgson et al. 1978
Water	pH adjustment to 11; extraction with methylene chloride (EPA Method 625)	GC/MS	18 µg/L (secondary effluent)	7 (distilled water); 11 (secondary effluent)	Spingarn et al. 1982
River water	Prefiltration; addition of XAD-2 resin; vacuum filtration; solvent extraction; clean-up on Florisil column	GC/ECD	<0.3 ng/L	90.7	Harris et al. 1980
Water	pH adjustment; extraction with methylene chloride	capillary GC/MS	5 ppt	No data	Hargesheimer 1984
Water	Solvent extraction; Florisil column clean-up optional	GC/ECD	40 ppt	90–96 (distilled water); 90–92 (river water)	Moseman et al. 1977
Water	Solvent extraction; clean-up on Florisil column	GC/ECD	20 ng/L	100	Saleh and Lee 1978
Sediment	Air dried; homogenization; solvent extraction; clean-up on Florisil column	GC/ECD	10 µg/kg	103	Saleh and Lee 1978
Sediment and soil	Dried; Soxhlet extraction; Florisil column clean-up	GC/ECD; GC/MS	10–20 ppb	99 (sediment); 86 (soil)	Moseman et al. 1977
Fish and shrimp	Homogenization; solvent extraction; clean-up and fractionation on Florisil column	GC/ECD	<1 ppb	80–105	Mady et al. 1979

TABLE 6-4. Analytical Methods for Determining Chlordecone in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Finfish and shellfish	Homogenization; solvent extraction; clean-up with GPC; clean-up with micro Florisil column to remove PCBs	GC/ECD; GC/MS	10–20 ppb	80–94 (fish); 84 (oyster)	Moseman et al. 1977
Finfish	Homogenization; Soxhlet extraction; clean-up using micro-Florisil column	GC/ECD; confirmation using GC/ELCD; GC/MS	No data	No data	Hodgson et al. 1978
Finfish liver and entrails	Homogenization; solvent extraction; liquid-liquid partition; clean-up using micro-Florisil column	GC/ECD; confirmation using GC/ELCD; GC/MS	No data	80	Hodgson et al. 1978
Clams and oysters	Homogenization; solvent extraction; liquid-liquid partition; clean-up using micro-Florisil column	GC/ECD; confirmation using GC/ELCD; GC/MS	No data	82 (clam); 80 (oyster)	Hodgson et al. 1978
Lake trout, crab, oysters	Solvent extraction; liquid-liquid partition	GC/ECD	<0.005 ppm	79.9–86.4 (chlordecone); 79.4–85.2 (monohydrochlordecone) 74.2–81.3 (dihydrochlordecone)	Carver and Griffith 1979
Beef fat, pork fat, and poultry fat	Dissolved in solvent; clean-up using GPC	Capillary GC/ECD	~0.10 ppm	58–73 (beef fat); 58–81 (pork fat); 63–77 (poultry fat)	Goodspeed and Chestnut 1991
Milk	Solvent extraction; concentration; sulfuric acid clean-up	GC/ECD	4 mg/m^3	91–93.2	Posyniak and Stec 1980

ECD = electron capture detection; ELCD = electrolytic conductivity detector; EPA = Environmental Protection Agency; GC = gas chromatography; GPC = gel permeation chromatography; MS = mass spectrometry; PCBs = polychlorinated biphenyls

Mirex and photomirex have been measured in soil and sediment samples using GC and capillary GC/ECD. Soil and sediment samples are usually solvent extracted, then cleaned up using Florisil columns and GPC. Recovery of mirex and photomirex from sediment samples is generally excellent (>90%) (Chau and Babjak 1979; Norstrom et al. 1980a; Onuska et al. 1980; Sergeant et al. 1993) with very good precision (<20 %RSD) (Norstrom et al. 1980a; Onuska et al. 1980). Sensitivity is in the low ppb to low ppt range (Norstrom et al. 1980a; Sergeant et al. 1993).

Mirex and its degradation products have been measured in biota using GC/ECD, capillary GC/ECD and capillary GC/MS techniques (Bush and Barnard 1982; Hellou et al. 1993; Norstrom et al. 1980a; Onuska et al. 1980; Quintanilla-Lopez et al. 1992). Samples are homogenized and most commonly extracted with solvent shake-out (Hellou et al. 1993; Norstrom et al. 1980a) or Soxhlet extraction (Quintanilla-Lopez et al. 1992). The clean-up techniques that are most commonly used are Florisil columns (Bush and Barnard 1982: Hellou et al. 1993; Norstrom et al. 1980a) and GPC (Hellou et al. 1993; Norstrom et al. 1980a). An additional nitration step has been used to separate mirex and photomirex from PCBs (Norstrom et al. 1980a). Sensitivity of GC/ECD analysis is in the low to subppb range. Recoveries are excellent (>90%), and precision is good (<20% RSD). Mirex and its degradation products have been measured in gull eggs using GC/ECD with capillary GC/MS confirmation (Norstrom et al. 1980b).

Packed and capillary GC/ECD or GC/MS have been used to measure mirex in foods, including fruits, vegetables, and fatty foods (Bong 1977; de la Riva and Anadon 1991; Di Muccio et al. 1991; Krause 1973; Liao et al. 1991; Manes et al. 1993; Stan 1989; Trotter and Dickerson 1993). Food samples are most commonly homogenized and extracted with solvent, then cleaned up using GPC (Stan 1989; Trotter and Dickerson 1993), Florisil columns (de la Riva and Anadon 1991; Krause 1973), or SPE columns (Di Muccio et al. 1993; Manes et al. 1993). Sensitivity is in the low to sub-ppb range for both GC/ECD and GC/MS techniques (de la Riva and Anadon 1991; Liao et al. 1991; Manes et al. 1993; Trotter and Dickerson 1993). Good to excellent recovery (>85% to >90%) and good precision (<20% RSD) were obtained for most methods (Bong 1977; Di Muccio et al. 1991; Trotter and Dickerson 1993). Confirmation was accomplished using a different capillary column (Manes et al. 1993; Trotter and Dickerson 1993). GC/ECD has been used to measure mirex in fatty foods with excellent recovery and good precision; however, the method is not suitable when PCBs are present (Ault and Spurgeon 1984).

The major analytical problem in the measurement of mirex and photomirex in environmental samples is co-elution with interferents. Confirmation techniques have been developed to assure reliable results. A dual-column, dual-detector GC analysis has been used to prevent false-positive identifications due to interfering compounds and to avoid misidentification (Durrell and Sauer 1990). The two detectors used were ECD and ELCD. MS techniques have been used to assure correct identification (Hargesheimer 1984; Hellou et al. 1993; Liao et al. 1993; Onuska et al. 1980; Stan 1989) and also to confirm GC/ECD measurements (Sergeant et al. 1993). Chemical procedures have been used as well. Perchlorination (Hallett et al. 1978) and nitration (Norstrom et al. 1980a, 1980b) have been used to convert co-eluting PCBs to compounds easily separable from mirex.

The most commonly used methods for measuring chlordecone and its degradation products in air, water, soil, sediment, fish, shellfish, and animal fat are similar to those used for mirex (i.e., GC/ECD techniques and confirmation by GC/MS). Because of the polar nature of chlordecone, the removal of chlordecone from the different types of environmental samples was accomplished using extraction with polar solvents (Moseman et al. 1977). The clean-up steps generally used for the environmental samples include Florisil column chromatography and GPC.

Air samples are collected using filters, or filters and impingers, and extracted with benzene and methanol (Hodgson et al. 1978; NIOSH 1984). Sensitivity is in the low ppb range for GC/ECD. Recovery is very good ($\geq$85%); precision is acceptable ($\leq$25% RSD) (Hodgson et al. 1978; NIOSH 1984). Confirmation of the identity of chlordecone in air was accomplished using both GC/MS and GC/ELCD (Hodgson et al. 1978).

Water samples are usually solvent extracted and may be analyzed directly by GC/MS (Spingam et al. 1982). Sensitivity is in the low ppb range, but recovery is low (7-11%) and precision is poor (48% RSD). Extracts may be cleaned up on Florisil columns and analyzed by GC/ECD (Garman et al. 1987; Moseman et al. 1977; Saleh and Lee 1978). Recoveries were very good (>90%) with sensitivity of GC/ECD being in the low to sub-ppt range (Harris et al. 1980; Moseman et al. 1977; Saleh and Lee 1978); precision data were not reported. Detection limits were lowered to sub-ppt levels by passing large volumes of water through XAD-2 resin, then extracting the resin (Harris et al. 1980). Recovery was very good (91%) as was precision (4% RSD).

Sediment and soil samples are homogenized and extracted. Clean-up procedures are required prior to analysis by GC/ECD or GC/MS techniques (Lopez-Avila et al. 1992; Moseman et al. 1977; Saleh and Lee 1978; Tieman et al. 1990). For sediment, soil, and sludge, recoveries were good (>85%) with sensitivity in the low ppb range (Moseman et al. 1977; Saleh and Lee 1978). Precision is good (<6% RSD) (Saleh and Lee 1978). Analytical difficulties (unacceptable recovery; not detectable using second capillary GC column) were reported (Lopez-Avila et al. 1992; Tieman et al. 1990).

Fish samples are extracted and cleaned up using liquid-liquid partitioning or Florisil columns prior to analysis by GC/ECD (Carver and Griffith; Hodgson et al. 1978; Mady et al. 1979; Moseman et al. 1977). Recoveries are good for chlordecone ($\geq$ 80%) (Carver and Griffith 1979; Hodgson et al. 1978; Mady et al. 1979) and the monohydro and dihydro degradation products (Carver and Griffith 1979). Precision is good (Carver and Griffith 1979; Mady et al. 1979) and sensitivity is in the low ppb range (Carver and Griffith 1979; Mady et al. 1979).

Few methods for measuring chlordecone in foods are available. Lower recoveries (58-81%) were obtained with GC/ECD for beef, pork, and poultry fat samples using GPC clean-up before analysis (Goodspeed and Chestnut 1991). Precision varied greatly (7.1-47.7% RSD) because of the lower recoveries; sensitivity was not reported.

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 mirex and chlordecone 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 mirex and chlordecone.

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. There are reliable methods for detecting, quantifying, and identifying mirex and chlordecone in biological samples. These include packed column and capillary GC/ECD and packed column and capillary GC/MS. These methods are sensitive enough to measure background levels in the population and levels at which biological effects occur. These methods are accurate and reliable for measuring mirex in blood (Korver et al. 1991; Mes 1992) and chlordecone in blood (Caille et al. 1987). Sensitivity for these methods is in the low to sub-ppb range. Sensitive (low to sub-ppb range) and accurate methods are available to measure mirex in tissues (Caille et al. 1987; LeBel and Williams 1986; Mes 1992). Improved recovery data and greater sensitivity for measuring chlordecone in tissues are needed (Caille et al. 1987). For milk, fecal, bile, urine, and saliva samples, sensitivity, recovery, and precision data are needed to more fully evaluate the reliability of these methods as predictors of environmental exposure to both mirex and chlordecone (Blanke et al. 1977; Bush et al. 1983b; Gibson et al. 1972).

Biochemical indicators of renal dysfunction (increased urinary protein and/or histopathological changes of the kidneys) have been associated with exposure to both mirex (NTP 1990) and chlordecone (Larson et al. 1979b). Microsomal enzyme induction as shown by changes in urinary D-glucaric acid has also been associated with exposure to both mirex and chlordecone (Guzelian 1985; Morgan and Roan 1974). Although these changes are not specific for mirex or chlordecone, these parameters may provide information about renal damage and hepatic effects in exposed populations. Tremorgrams have been used to assess tremors associated with chlordecone exposure in humans (Taylor et al. 1978). An infrared reflection technique and oculography have been used to assess the oculomotor disturbances caused by chlordecone (Taylor et al. 1978). Standard tests for memory and intelligence can be used to determine the presence of encephalopathy, but in the absence of baseline pre-exposure levels for individuals, subtle changes may be difficult to detect. Decreased sperm count has been observed following exposure to mirex or chlordecone (Chu et al. 1981a; Yarborough et al. 1981). The existing analytical methods that are discussed for exposure can reliably measure mirex or chlordecone in blood, urine, and tissues at the levels at which these effects occur.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Reliable methods for detecting mirex and chlordecone in environmental media include GC/ECD, capillary GC/ECD and capillary GC/MS. In general, the methods are sensitive and accurate enough to measure background levels of mirex and chlordecone in the environment and levels at which health effects occur. Methods of adequate sensitivity (low ppb to sub-ppb), accuracy, and specificity are available for determining levels of mirex in air (Dun-e11 and Sauer 1990; Hoff et al. 1992; Lewis et al. 1977), water (Durrell and Sauer 1990; Hargesheimer 1984; Sandhu et al. 1978), and soils and sediment (Norstrom et al. 1980a; Sergeant et al. 1993; Seidel and Lindner 1993). Sensitive, accurate methods are also available for measuring chlordecone in air (NIOSH 1984), water (Garman et al. 1987; Harris et al. 1980; Saleh and Lee 1978: Spingarn et al. 1982), and soil and sediment (Moseman et al. 1977; Saleh and Lee 1978). Methods for measuring mirex and chlordecone in aquatic species and food are reliable and accurate and provide detection limits in the low ppm to ppb range. These include methods for determining mirex in fish and other aquatic species (Bush and Barnard 1982; Hellou et al. 1993; Norstrom et al. 1980a; Quintanilla-Lopez et al. 1992; Rahman et al. 1993) and food (Ault and Spurgeon 1984; Liao et al. 1991; Manes et al. 1993; Trotter and Dickerson 1993). Similarly, there are acceptable methods for determining chlordecone in fish and other aquatic species (Carver and Griffith 1979; Mady et al. 1979) and food (Goodspeed and Chestnut 1991; Posyniak and Stec 1980). More information on the precision of these methods for measuring mirex and chlordecone in water and improved sensitivity, recovery, and precision data in foodstuffs are needed to better assess the risk of exposure for these media. Research investigating the relationship between levels of mirex and chlordecone measured in air, water, soil, and food and observed health effects could increase our confidence in existing methods and/or indicate where improvements are needed. No data were located regarding measurement of mirex in soil samples.

6.3.2 Ongoing Studies

Research is being conducted at the State University of New York at Albany, sponsored by the National Institute of Environmental Health Sciences, to improve chemical analysis of environmental media for PCBs and selected pesticides, including mirex. No other studies involving rnirex or chlordecone were located in the FEDRIP database.