7. ANALYTICAL METHODS

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

Gas chromatography (GC) with an electron-capture detector (ECD) has been used to measure methoxychlor concentrations in human milk, serum, semen, and adipose tissue (LeBel and Williams 1986; Mes 1981; Steinberg et al. 1989; Szymczynski and Waliszewski 1982). In GC, samples dissolved in a volatile solvent are injected into a heated column with a stationary phase consisting of silica coated with a liquid phase. An inert gas carries the sample through the column, and the partitioning of methoxychlor between the mobile and stationary phases gives it a characteristic retention time that is used to identify it. ECDs use a radioactive source such as ^{63}Ni to generate electrons that are captured by the chlorine atoms of methoxychlor. Reduction in electron flow by this capture produces a signal for methoxychlor.

Most methoxychlor in biological samples is dissolved in fat, and thus, samples are prepared for GC/ECD by extraction of the fat with organic solvents. The fat extract is generally "cleaned up" by gel permeation chromatography (GPC), which separates methoxychlor from higher molecular weight lipids, and/or by passage through a Florisil® column which retains lipids and other contaminants (LeBel and Williams 1986; Mes 1981; Szymczynski and Waliszewski 1982). These methods provide 71–104% recovery of methoxychlor (LeBel and Williams 1986; Szymczynski and Waliszewski 1982). Methoxychlor has been determined in human serum in women living in agricultural areas of Spain (Frenich et al. 2000). Samples of serum were purified using a hexane extraction procedure followed by cleanup using high performance liquid chromatography (HPLC) and detection using tandem mass spectrometry

(GC/MS/MS). Using this method, the analytical limit of detection (LOD) was found to be 2.0 µg/L and the and the percent recovery was 113%. The advantage of using GC/MS/MS is a much higher sensitivity than GC/ECD. However, the GC/ECD gives greater precision, accuracy, and limit of detection values. In serum, the detection limit for methoxychlor was reported to be 0.24–4.07 mg/L (Steinberg et al. 1989). No data were located on the specificity or precision of these methods for biological samples.

As discussed previously, because methoxychlor is metabolized fairly quickly, measurement of metabolites may prove to be more useful for assessing exposure than measurement of methoxychlor itself. Pure samples of methoxychlor impurities (such as, 1,1,2,2-tetrachloro-2-(4-methoxyphenyl)ethane, 1,1-dichloro-2,2-(4-methoxyphenyl)ethene, and 1,1,1-trichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane), that may also be metabolic products of methoxychlor, may be analyzed by GC with a flame ionization detector (FID), HPLC with an ultraviolet (UV) detector, or by GC with detection by mass spectrometry (MS) (Lamoureux and Feil 1980; West et al. 1982); however, no information was located concerning the application of these methods to detecting metabolites in biological samples.

Table 7-1 summarizes the methods used for sample preparation and analysis of methoxychlor in biological samples.

7.2 ENVIRONMENTAL SAMPLES

Methoxychlor in environmental samples is also usually measured using GC/ECD (APHA 1992; Ault and Spurgeon 1984; EPA 1989b, 1990a, 1990c; Gillespie and Walters 1986; Hopper and King 1991; Hsu et al. 1991; Ivey et al. 1983). A halogen electrolytic conductivity detector (HECD) may be used instead of, or in conjunction with, an ECD, and GC/MS may be used to confirm the identity of methoxychlor (EPA 1990b; Hopper and King 1991; NIOSH 1978). Use of MS as the primary detection system can be achieved by establishing a library of reference pesticide spectra and inclusion of appropriate standards (Liao et al. 1991). Recent work has concentrated on improving methods for extraction of methoxychlor from environmental samples. Since methoxychlor in air is usually associated with particulate matter, standard methods involve collection of air samples on glass fiber or polyurethane foam and extraction with organic solvents prior to GC analysis (EPA 1990a; NIOSH 1978). Methoxychlor has detected in dust samples at levels of 0.6–3.5 µg/g using GC/MS selective ion monitoring (Rudel et al. 2001). Reports in the development and application of semipermeable membrane devices (SPMDs) describe new

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human serum	Extract with hexane/ diethyl ether, concentrate, acidify (H_2SO_4), centrifuge, extract aqueous layer with hexane, cleanup via HPLC	GC/MS/MS	500 ng/L	113	Frenich et al. 2000
Human serum	No data	GC/ECD and GC/MS	0.24–4.07 mg/L	No data	Steinberg et al. 1989
Adipose tissue	Extract with acetone/hexane, cleanup with GPC and FAC	GC/ECD and GC/MS	No data	71–98	LeBel and Williams 1986
Human milk	Extract with ethanol/hexane/ethyl ether, cleanup via GPC	GC/ECD	10 mg/kg	No data	Hooper et al. 1997
Human milk	Extract with hexane or benzene, cleanup with FAC	GC/ECD	No data	No data	Mes 1981
Human semen	Extract with petroleum ether, cleanup with FAC	GC/ECD	No data	104	Szymczynski and Waliszewski 1982

ECD = electron capture detector; FAC = Florisil® adsorption chromatography; GC = gas chromatography; GPC = gel permeation chromatography; HPLC = high performance liquid chromatography; MS = mass spectrometry.

approaches to determining bioavailable contaminants in a variety of aquatic systems (Petty et al. 1998). The SPMD membrane mimics the diffusion transfer of organic contaminants through the respiratory membranes of aquatic organisms. Methoxychlor in water, soil, solid waste, and food is also generally extracted with organic solvents, and the extracts are cleaned up by chromatographic methods using GPC, Florisil® adsorption, Attagel®, magnesia-silica gel, and/or semi-preparative HPLC (APHA 1992; Ault and Spurgeon 1984; EPA 1990c; Gillespie and Walters 1986; Hopper and King 1991; Hsu et al. 1991; Ivey et al. 1983). Modifications to extraction and cleanup methods have been proposed, which reduce the amount of solvent required or increase the speed of analyses (Kraut-Vass and Thoma 1991; Patterson 1991). An analytical procedure for the identification of methoxychlor in a drinking water sample matrix has been developed that involves the use of a two-step pre-concentration followed by a separation step (Fung and Mak 2001). The pre-concentration steps employ a solid-phase extraction (C18 SPE) and cleanup followed by stacking with reverse polarity prior to separation using micellar electrokinetic capillary chromatography (MEKC). The recovery of methoxychlor using the SPE method is 89%. The MEKC procedure was found to give analytical results that are comparable with GC-MS and LC-MS and is more sensitive than LC. The detection limit of methoxychlor in drinking water was determined to be 0.041 µg/L. Supercritical fluid extraction, a process using carbon dioxide liquified above 31 EC at high pressure, provides efficient extraction of large samples (Hopper and King 1991). These extraction methods typically provide recoveries of 84–100%. Gas chromatographic measurements can detect about 0.2 µg methoxychlor per L of hexane extract, with actual detection limits for soil and waste dependent upon matrix interferences, extraction efficiency, and cleanup procedures (EPA 1990b, 1990c). Although detection limits were not reported for analysis of methoxychlor by GC/ECD (Hopper and King 1991; Hsu et al. 1991) a detection limit of 0.05 µg/g food was reported for analysis by GC/MS (Liao et al. 1991). Pure samples of metabolic products of methoxychlor may be analyzed by GC with a FID, HPLC with an UV detector, or by GC with detection by MS (Lamoureux and Feil 1980; West et al. 1982).

Table 7-2 summarizes representative methods used for sample preparation and analysis of methoxychlor in environmental samples.

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 methoxychlor is available. Where adequate information is

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
House dust	Extract with ether/hexane, chromatograph on florisil with acetone/hexane	GC/MS-SIM	No data	No data	Rudel et al. 2001
House dust	Extract with diethyl ether/hexane, cleanup with FAC	GC/MS	0.02–0.1 µg/g	No data	Lewis et al. 1999
Air	Collect on glass fiber filter, extract with isooctane	GC/HECD	7.7 mg/m^3	103	NIOSH 1978
Indoor air	Collect on polyurethane foam, extract with diethyl ether/hexane	GC/ECD	0.01 µg/m^3	65	EPA 1990a
Drinking Water	Adjust pH to 3.0 (HCl), soild phase extraction (eluted with methanol)	MEKC equipped with a variable UV detector	0.041 µg/m^3	89	Fung and Mak 2001
Water	SPMD, extract with hexane, and fractionate	Capillary column GC/ECD	0.01 µg/-SPMD	No data	Petty et al. 1998
Water	Collect analyte on SPE cartridge, elute with hexane/propanol	Capillary column GC/ECD	0.01–0.018 µg/L	No data	Kolpin et al. 1998
Water	Extract with diethyl ether/hexane or methylene chloride/hexane, cleanup with magnesia-silica gel	GC/ECD	No data	No data	APHA 1992
Water	Extract with hexane	GC/ECD	0.96 µg/L	98–100	EPA 1989b
Soil/solid waste	Sonicate, extract with hexane, cleanup with GPC	GC/ECD or GC/HECD	0.12–18 mg/kg wet weight	No data	EPA 1990b

Table 7-2. Analytical Methods for Determining Methoxychlor in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil/solid waste	Extract with hexane/acetone or methylene chloride/ acetone, cleanup with silica gel, GPC, and/or FAC	Capillary column GC/ECD	5.7–860 µg/kg wet weight	53–80	EPA 1990c
Food	Extract with SC-CO_2	GC/ECD	No data	90–105	Lehotay et al. 1995
Food	Extract with SC-CO_2 and cleanup with GPC and FAC	GC/ECD and GC/HECD	No data	87–112	Hopper and King 1991
Food	Extract with acetonitrile	GC/MS	0.05 µg/g	94	Liao et al. 1991
Food	Extract with acetonitrile, cleanup with Attagel® (in benzene), Florisil® (in hexane), or C_{18} (in acetonitrile)	GC/ECD	No data	89–125	Hsu et al. 1991
Edible fat	Dissolve in hexane, filter, HPLC on semi-preparative silica column	GC/ECD	No data	89–95	Gillespie and Walters 1986
Edible fat	Dissolve in methylene chloride/cyclohexane, cleanup with GPC	GC/ECD	No data	84–86	Ault and Spurgeon 1984
Human milk	Extract with ethanol/hexane/ethyl ether, cleanup via GPC	GC/ECD	10 mg/kg	No data	Hooper et al. 1997
Milk	Cleanup with Florisil®, elute with hexane	GC/ECD	0.005 ppm	84–96	Ivey et al. 1983

C_{18} = Carbon 18; ECD = electron capture detector; FAC = Florisil® absorption chromatography; GC = gas chromatography; GPC = gel permeation chromatography; HECD = halogen electrolytic conductivity detector; HPLC = high performance liquid chromatography; MEKC = Micellar electrokinetic capillary chromatography; MS = mass spectrometry; SC-CO_2 = superficial fluid-carbon dioxide; SIM = selective ion monitoring; SPE = solid phase extraction; SPMD = semipermeable membrane device

not available, ATSDR, in conjunction with the National Toxicology Program (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 methoxychlor.

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. Methoxychlor can be measured in a variety of human tissues and fluids, including serum (LeBel and Williams 1986), milk (Mes 1981), semen (Steinberg et al. 1989), and adipose tissue (Szymczynski and Waliszewski 1982). Detection limits are not well characterized, but are probably about 0.2 µg/L in extracts of biological samples (EPA 1990b; Steinberg et al. 1989) and 0.05 µg/g in food (Liao et al. 1991). Based on the low frequency of methoxychlor detection in biological samples, it would be helpful if suitable sample extraction and analysis procedures could be developed to measure background levels of methoxychlor in the general population as well as levels at which biological effects occur. These data would be valuable in monitoring environmentally exposed populations. Adaptation of methods for measuring pure samples of methoxychlor metabolic products (Lamoureux and Feil 1980; West et al. 1982) to biological samples could provide additional data on exposure to methoxychlor. Since methoxychlor is rapidly metabolized to phenolic derivatives, some of which are estrogenic, additional studies that investigate the applicability or adaptability of methods for methoxychlor to metabolites of methoxychlor in biological media would be useful in developing other biomarkers of exposure.

No data were located concerning methods to measure biological markers of methoxychlor effects. Methoxychlor produces effects on the reproductive system of exposed animals primarily by the estrogenic action of its metabolites (see Chapter 3). In exposed female rodents, methoxychlor exposure may affect the estrus cycle (Gray et al. 1988; Harris et al. 1974; Martinez and Swartz 1992; Okazaki et al. 2001; Tegeris et al. 1966), serum hormone levels (Cummings and Gray 1987, 1989; Cummings and Laskey 1993; Gray et al. 1988), and the uterus and ovaries (Bal 1984; Chapin et al. 1997; Cummings and Gray 1987, 1989; Cummings and Laskey 1993; Cummings and Perreault 1990; Dikshith et al. 1990; Gray et al.

1988, 1989; Harris et al. 1974; Martinez and Swartz 1992; Mitsumori et al. 2000; Okazaki et al. 2001; Swartz 1994; Tegeris et al. 1966; Tullner 1961), and may reduce fertility (Bal 1984; Chapin et al. 1997; Gray et al. 1989; Harris et al. 1974; Haskell Laboratories 1966). In the female offspring of exposed female rodents, reproductive development, as indicated by age at vaginal opening and first estrus, may be accelerated (Chapin et al. 1997; Gray et al 1989; Harris et al. 1974). In male rodents, methoxychlor exposure may affect sexual maturity (age of preputial separation) (Gray et al. 1989, 1999), serum hormone levels (Cummings and Gray 1989; Goldman et al. 1986; Gray et al. 1989; Stoker et al. 1999), testes (Bal 1984; Chapin et al. 1997; Dikshith et al. 1990; Gray et al. 1989, 1999; Hodge et al. 1950; Tullner and Edgcomb 1962; Wenda-Rozewicka 1983), prostate (Okazaki et al. 2001; Shain et al. 1977; Stoker et al. 1999; Welshons et al. 1999), fertility (Chapin et al. 1997; Gray et al. 1989, 1999; Harris et al. 1974; Wenda-Rozewicka 1983), and behavior (Parmigiani et al. 1998; vom Saal et al. 1995). However, these effects are not specific to exposure to methoxychlor, but could occur following exposure to other chemicals with estrogenic activity as well. Since several *in vitro* studies have indicated the presence in human hepatic microsomes of enzymatic activities similar to those in rats that are responsible for the metabolism of methoxychlor to its estrogenic metabolites (Dehal and Kupfer 1994; Stresser and Kupfer 1998), it is likely that similar effects may occur in exposed humans. Research into biomarkers for the estrogenic effects of methoxychlor would be most useful if performed in conjunction with development of sensitive, specific, and reliable methods for measuring these biomarkers.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for detection of methoxychlor in air (EPA 1989b), water (EPA 1990a), soil (EPA 1990b), solid waste (EPA 1990c), and food (Liao et al. 1991) are all based on GC. The media most likely to be of concern for human exposure to methoxychlor are soil and food. Developments in GC analysis for methoxychlor include development of capillary columns, which provide better resolution than packed columns (EPA 1990c; Lopez-Avila et al. 1990; Tessari and Winn 1991), and the use of MS as the primary means of detection, which is much more specific than retention time monitored by ECD or halogen electrolytic conductivity detector, HECD (Liao et al. 1991; Stan 1989). Immunoassays, which have been developed for other pesticides (Stanker et al. 1989), may provide a rapid, inexpensive, and sensitive, method for detecting methoxychlor in environmental samples. Another area of interest is the complete and selective extraction of methoxychlor from complex environmental samples, such as soil and food; supercritical-fluid extraction shows promise in this area (Hopper and King 1991; Lopez-Avila et al. 1990; Walters 1990). Supercritical-fluid extraction and GC/MS, if validated in additional studies, appears to have the sensitivity, specificity, and reliability to measure background levels of methoxychlor in the environment and levels at which health effects occur.

Methods have been developed to separate methoxychlor-degradation products (West et al. 1982). These methods are based on complimentary chromatographic techniques using normal phase HPLC and GC. High molecular weight impurities found in technical grade and formulated methoxychlor that are difficult to separate by GC are cleanly separated by HPLC. HPLC separation products are then readily analyzed by GC/MS. However, it is not known if this technique can be used reliably for the analysis of environmental samples. Further studies using HPLC GC/MS are needed to determine if the technique could be used in the analysis of air, water, or soil samples contaminated with methoxychlor degradation products.

7.3.2 Ongoing Studies

The application of microwave assisted solvent extraction, coupled with solid phase extraction cleanup to the recovery of methoxychlor from crops (beets, cucumbers, lettuce, peppers, and tomatoes have been studied thus far) shows promise in screening foods for pesticide residues, and pesticide recoveries compare favorably with standard extraction methods (Pylypiw et al. 1997). Ongoing studies to improve methods for analysis of methoxychlor include further research on applications of supercritical-fluid extraction procedures to recover methoxychlor and other compounds from solid matrices (Ashraf-Khorassani et al. 1992; Lopez-Avila et al. 1992). In addition, research to optimize sensitivity, specificity, reproducibility, and analysis time using fused silica capillary columns in a dual-column, dual-detector arrangement, is providing promising results for analysis of food and solid matrices (Hopper 1991; Lopez-Avila et al. 1992).

No information was located concerning on-going studies for improving methods of analysis of methoxychlor metabolites, or other biomarkers of exposure and effect for methoxychlor in biological materials.