7. ANALYTICAL METHODS

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

The primary method for detecting methyl parathion and metabolites in biological tissues is gas chromatography (GC) coupled with electron capture (ECD), flame photometric (FPD), or flame ionization detection (FID). Sample preparation for methyl parathion analysis routinely involves extraction with an organic solvent (e.g., acetone or benzene), centrifugation, concentration, and resuspension in a suitable solvent prior to GC analysis. For low concentrations of methyl parathion, further cleanup procedures, such as column chromatography on silica gel or Florisil are required. Table 7-1 summarizes the analytical methods used to detect methyl parathion and its metabolites in biological tissues and fluids.

Methyl parathion was determined in dog and human serum using a benzene extraction procedure followed by GC/FID detection (Braeckman et al. 1980, 1983; DePotter et al. 1978). An alkali flame FID (nitrogen-phosphorus) detector increased the specificity of FID for the organophosphorus pesticides. The detection limit was in the low ppb (μg/L). In a comparison of rat blood and brain tissue samples analyzed by both GC/FPD and GC/FID, Gabica et al. (1971) found that GC/FPD provided better specificity. The minimum detectable level for both techniques was 3.0 ppb, but GC/FPD was more selective. The EPA-recommended method for analysis of low levels (<0.1 ppm) of methyl parathion in tissue, blood, and urine is GC/FPD for phosphorus (EPA 1980d). Methyl parathion is not thermally stable above 120 °C (Keith and Walters 1985).

Table 7-1. Analytical Methods for Determining Methyl Parathion and Metabolites in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Acidify and heat to hydroloyze; add NaOH to pH=11; extract with benzene-diethyl ether; reacidify and dry with sodium sulfate; derivatize with hexamethyl disilizane on GC column (PNP)	GC/ECD	50 µg/L (50 ppb)	95.4	Cranmer 1970; EPA 1980d
Urine	Acidify and heat to hydrolyze; add NaOH extract with anhydrous ethyl ether; derivatize with diazoethane; concentrate; add hexane; concentrate and cleanup on silica gel; elute with benzene-hexane (PNP)	GC/ECD	20 µg/L (20 ppb)	85–98	Shafik et al. 1973b
Urine, blood, tissues	Add acetone; centrifuge; extract on ion exchange column; derivatize with diazopentane; cleanup on silica gel if needed (metabolites)	GC/FPD	40–150 µg/L (40–150 ppb)	36–97	EPA 1980d; Lores and Bradway 1977
Blood, tissues	Homogenize, if tissue; mix sample with acetone; centrifuge; concentrate; saturate with sodium chloride; evaporate organic layer; cleanup on silica gel eluting with hexane-benzene; concentrate	GC/FPD	<100 ppb	No data	EPA 1980d
Serum	Extract with benzene; dry; resuspend in ethyl acetate	GC/FID	2 µg/L (2 ppb)	57–109	Braeckman et al. 1980; DePotter et al. 1978

EDC = electron capture detector; FID = flame ionization detector; FPD = flame photometric detector; GC = gas chromatography; NaOH = sodium hydroxide; PNP = paranitrophenol

Methyl parathion rapidly forms hydrolysis products after absorption by the body. 4-Nitrophenol and the alkyl phosphate, dimethyl phosphate, are major metabolites that are frequently found in biological fluids and tissues following exposure. Sample preparation steps are generally more extensive for the metabolites than for the parent compound. Usually, several extractions and a derivation are required prior to GC analysis. Total 4-nitrophenol has been measured in human and rat urine using GC/ECD of the diazoethane or hexamethyl disilizane derivatives of 4-nitrophenol (Cranmer 1970; Morgan et al. 1977; Shafik et al. 1973b). The minimum detectable level was 0.02 ppm. For the analysis of 4-nitrophenol in biological tissues and fluids, EPA recommends extraction with benzene-ether and derivatization with hexamethyl disilizane prior to analysis by GC/ECD (EPA 1980d). The diazoethane derivative of dimethyl phosphate was quantitatively measured in human urine by GC/FPD in the phosphorus mode (Morgan et al. 1977). EPA recommends GC/FPD for the detection of the diazopentane derivatives of dimethyl phosphate and other alkyl phosphates. Diazopentane derivatives are more easily resolved and separated from interfering compounds than diazoethane derivatives. The detection limit of dimethyl phosphate by the EPA method was 0.04–0.15 ppm (EPA 1980d; Lores and Bradway 1977; Shafik et al. 1973a). The problem with the use of the above metabolites for the analysis of methyl parathion exposure is that they are not specific. Other organophosphate insecticides may also form these degradates.

A recent method, still in development, for determining total 4-nitrophenol in the urine of persons exposed to methyl parathion is based on solid phase microextraction (SPME) and GC/MS; previously, the method has been used in the analysis of food and environmental samples (Guidotti et al. 1999). The method uses a solid phase microextraction fiber, is inserted into the urine sample that has been hydrolyzed with HCl at 50 EC prior to mixing with distilled water and NaCl and then stirred (1,000 rpm). The fiber is left in the liquid for 30 minutes until a partitioning equilibrium is achieved, and then placed into the GC injector port to desorb. The method shows promise for use in determining exposures at low doses, as it is very sensitive. There is a need for additional development of this method, as the measurement of acetyl-cholinesterase, the enzyme inhibited by exposure to organophosphates such as methyl parathion, is not an effective indicator of low-dose exposures.

Organophosphates, such as methyl parathion, are known to inhibit cholinesterase activity. A method has been developed to measure the extent of this inhibition and relate it to organophosphate exposure (EPA 1980d; Nabb and Whitfield 1967). In this EPA-recommended method, blood is separated into plasma and red blood cell fractions. The fractions are treated with saline solution, brought to pH 8 with sodium hydroxide, and dosed with acetylcholine perchlorate. The ensuing acetic acid releasing enzyme reaction

is automatically titrated using an automatic titrator. This method is sensitive, simple, and fast, but is not specific for methyl parathion.

In a study of the metabolism of methyl parathion in intact and subcellular fractions of isolated rat hepatocytes, a high performance liquid chromatography (HPLC) method has been developed that separates and quantitates methyl parathion and six of its hepatic biotransformation products (Anderson et al. 1992). The six biotransformation products identified are methyl paraoxon, desmethyl parathion, desmethyl paraoxon, 4-nitrophenol, *p*-nitrophenyl glucuronide, and *p*-nitrophenyl sulfate. This method is not an EPA or other standardized method, and thus it has not been included in Table 7-1.

7.2 ENVIRONMENTAL SAMPLES

The predominant method of analyzing environmental samples for methyl parathion is by GC. The detection methods most used are FID, FPD, ECD, and mass spectroscopy (MS). HPLC coupled with ultraviolet spectroscopy (UV) or MS has also been used successfully. Sample extraction and cleanup varies widely depending on the sample matrix and method of detection. Several analytical methods used to analyze environmental samples for methyl parathion are summarized in Table 7-2.

In air, methyl parathion has been determined to the sub-ppt (ng/m^3) level by GC equipped with FPD or a nitrogen-phosphorus detector (NPD). Sample preparation methods varied from simple extraction and concentration (EPA 1980d, 1987d; Jackson and Lewis 1978; Seiber et al. 1989) to inclusion of column cleanup and fractionation steps (Stanley et al. 1971; Tessari and Spencer 1971). The widest variation in methods centered around sample collection. Multilevel collectors (EPA 1980d; Jackson and Lewis 1978; Stanley et al. 1971), resins (Seiber et al. 1989), and nylon cloth (Tessari and Spencer 1971) have all been used successfully. Recoveries ranged from 53 to over 100%. The best recovery and sensitivity data was reported by Seiber et al. (1989) during studies of atmospheric methyl parathion concentrations in the Sacramento Valley area of California. Using a macroreticular resin sampler, extraction with ethyl acetate, and GC/NPD analysis, over 85% of injected methyl parathion was recovered with a sensitivity of 0.2 ng/m^3 (sub-ppt). However, the precision of the method was low. The EPA-recommended method is similar, employing a glass fiber filter/solid sorbent sampler, extraction with diethyl ether in hexane, and analysis by GC/FPD (EPA 1980d). Both of these methods detect methyl paraoxon, the oxidized metabolite of methyl parathion, as well. Methyl parathion has also been detected in hazardous waste incinerator effluents. Using GC/FID and GC/MS, detection limits of 4.8 and 2.0 ng and precisions of 6 and 10%, respectively, were achieved (James et al. 1985).

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collect on hexylene glycol-alumina adsorbent sampler; extract; cleanup with Florisil	GC/FPD	0.1 ng/m^3	53.4	Stanley et al. 1971
Air	Collect on XAD-4 macroreticular resin; extract with ethyl acetate	GC/NPD	0.2 ng/m^3	85–111	Seiber et al. 1989
Air	Collect on solid sorbent; extract with diethyl ether in hexane	GC/FPD	No data	72–105	EPA 1980d
Water (run-off)	Collect on XAD-2 macroreticular resin; extract with diethyl ether	HPLC/UV	2–3 µg/L	99.75	Paschal et al. 1977
Water	Extract with benzene plus anhydrous potassium carbonate; concentrate; cleanup on silica gel	GC/ECD	0.1 µg/L	79	Lee et al. 1984
Water	Extract with methylene chloride; concentrate; cleanup on silica gel	GC/FPD	No data	93	EPA 1980d
Water, plant tissue	Extract with acetonitrile; filter if necessary	HPLC/UV/EC	No data (water); 50 µg/kg (plants)	95–99	Clark et al. 1985
Sediments	Dry with sodium sulfate; extract with acetone/methylene chloride; concentrate	GC/FPD	No data	73–95	Belisle and Swineford 1988
Water, plant tissue, animal tissue	Extract with hexane; cleanup with hexane/acetonitrile	GC/ECD	0.1 µg/L (water); 0.01 mg/kg (tissue)	100	Kadoum 1968

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Plant tissue	Extract with ethyl acetate and sodium sulfate; filter through silanized glass wool	GC/TID	No data	No data	AOAC 1984
Food (butter fat)	Extract and cleanup on semipreparative HPLC column; elute with methylene chloride-hexane	GC/ECD	No data	No data	Gillespie and Walters 1986

EC = electrical conductivity detector; ECD = electron capture detector; FPD = flame photometric detector; GC = gas chromatography; HPLC = high performance liquid chromatography; NPD = nitrogen phosphorus detector; TID = thermionic detector; UV = ultraviolet spectroscopy

Analysis of water for methyl parathion in the ppb (ng/L) range has been done using GC/ECD, GC/FPD, and HPLC/UV. With water samples, the primary problems are concentration of the sample and selectivity of the method. Water samples generally contain only trace amounts of methyl parathion. Usually, other pesticides and interfering compounds are present. Several concentration, cleanup, and separation techniques have been tested in an attempt to improve the sensitivity and selectivity of analysis by GC/ECD (Agostiano et al. 1983; Kadoum 1968; Kawahara et al. 1967; Le Bel et al. 1979; Lee et al. 1984). EPA recommends fractionation on silica gel prior to detection by GC/FPD. The FPD detector is selective for organophosphates. Recoveries for these methods ranged from 74 to 94% with detection limits in the sub- and low-ppb range. HPLC/UV and HPLC/UV/electrochemical detectors have been used to simplify sample preparation and increase selectivity (Clark et al. 1985; Paschal et al. 1977). High recoveries (>99%) and precision, as well as detection limits in the low-ppb range, were reported.

Analysis of methyl parathion in sediments, soils, foods, and plant and animal tissues poses problems with extraction from the sample matrix, cleanup of samples, and selective detection. Sediments and soils have been analyzed primarily by GC/ECD or GC/FPD. Food, plant, and animal tissues have been analyzed primarily by GC/thermionic detector or GC/FPD, the recommended methods of the Association of Official Analytical Chemists (AOAC). Various extraction and cleanup methods (AOAC 1984; Belisle and Swineford 1988; Capriel et al. 1986; Kadoum 1968) and separation and detection techniques (Alak and Vo-Dinh 1987; Betowski and Jones 1988; Clark et al. 1985; Gillespie and Walters 1986; Koen and Huber 1970; Stan 1989; Stan and Mrowetz 1983; Udaya and Nanda 1981) have been used in an attempt to simplify sample preparation and improve sensitivity, reliability, and selectivity. A detection limit in the low-ppb range and recoveries of 100% were achieved in soil and plant and animal tissue by Kadoum (1968). GC/ECD analysis following extraction, cleanup, and partitioning with a hexane-acetonitrile system was used.

Using a simple, modified GC method with nitrogen-phosphorus detection (GC/NPD), Pappas et al. (1999) determined methyl parathion residues in apples with a recovery of 88–108% and a limit of detection of 2 ppb. Recent work by Sheridan and Meola (1999) suggests that analysis using GC coupled with tandem or ion trap MS (MS/MS) is a highly selective method capable of achieving clear compound identification and identity confirmation, with identification of compounds present in agricultural samples at the ppb level. The method is not as susceptible to interfering co-extractives as methods involving selective detectors; GC/MS/MS was able to detect methyl parathion in pears down to 2 ppb, while a selective detection method was limited to the level “<3 ppb” (Sheridan and Meola 1999).

Using established extraction and cleanup methods, followed by GC/FPD and GC/thermionic detection, Carey et al. (1979) obtained detection limits in the ppb range and recoveries of 80–110% in soil and 70–100% in plant tissue. Good sensitivity and recovery were maintained in a simplified extraction procedure of sediments followed by GC/FPD analysis (Belisle and Swineford 1988). Bound methyl parathion residues that were not extracted with the usual methods were extracted using supercritical methanol by Capriel et al. (1986). They were able to remove 38% of the methyl parathion residues bound to soil, but 34% remained unextractable, and 28% could not be accounted for.

HPLC has been recommended as a cleanup and fractionation procedure for food samples prior to analysis by GC/ECD (Gillespie and Walters 1986). The advantages over the AOAC-recommended Florisil column are that it is faster, requires less solvent, and gives better resolution. HPLC coupled with various detectors MS, MS/MS, UV/electrochemical detector, or UV/polarographic detection has been tested as a rapid, simplified separation and detection system to replace GC (Betowski and Jones 1988; Clark et al. 1985; Koen and Huber 1970). Recoveries, detection limits, and precisions were generally good, but further work is needed before the techniques are adopted for general use.

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 methyl parathion 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 methyl parathion.

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 or eliminate the uncertainties of human health assessment. 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. Sensitive, accurate methods exist for the measurement of erythrocyte and plasma cholinesterase levels (EPA 1980d; Nabb and Whitfield 1967). Organophosphates, including methyl parathion, inhibit cholinesterases. There are some problems with the reliability of this method because normal erythrocyte cholinesterase values vary widely (Midtling et al. 1985; Tafuri and Roberts 1987) and plasma cholinesterase can be suppressed by a variety of diseases (Henry 1984; Tafuri and Roberts 1987). Further studies to improve the reliability of cholinesterase levels might be useful in establishing this as a reliable measure of organophosphate exposure. Studies are needed regarding the measurement of methyl paraoxon in biological tissues, as this is the most toxic metabolite of methyl parathion.

Sensitive analytical methods exist to measure methyl parathion (Braeckman et al. 1980; DePotter et al. 1978; EPA 1980d) and some of its metabolic products (Anderson et al. 1992; Cranmer 1970; EPA 1980d; Lores and Bradway 1977; Morgan et al. 1977; Shafik et al. 1973b) at background levels and levels at which biological effects occur. The most sensitive and selective method for methyl parathion is currently GC/FPD (EPA 1980d; Gabica et al. 1971). The most sensitive and selective method for metabolites is derivitization followed by GC/FPD analysis (EPA 1980d; Lores and Bradway 1977); however, the metabolites found following methyl parathion exposure are not specific for methyl parathion.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Analytical methods exist to measure low levels of methyl parathion in air (EPA 1980d, 1987d; Jackson and Lewis 1978; Seiber et al. 1989; Stanley et al. 1971; Tessari and Spencer 1971), water (Agostiano et al. 1983; Clark et al. 1985; Kadoum 1968; Kawahara et al. 1967; Le Bel et al. 1979; Lee et al. 1984), soil, and other media (Alak and Vo-Dinh 1987; AOAC 1984; Belisle and Swineford 1988; Betowski and Jones 1988; Capriel et al. 1986; Carey et al. 1979; Clark et al. 1985; Gillespie and Walters 1986; Kadoum 1968; Koen and Huber 1970; Stan 1989; Stan and Mrowetz 1983; Vdaya and Nanda 1981). These methods can be used to identify potentially contaminated areas to determine if there is a risk to human health. The media of most concern for human exposure are air, water, and soil. Sensitive methods exist to measure both background levels and levels at which health effects occur. Gas chromatography continues to be the most frequently used technique for the separation and identification of methyl parathion. Paired with an ECD or FPD, the detection limit is generally in the low- to sub-ppb range for air (EPA 1980d), water (Agostiano et al. 1983; Clark et al. 1985; Kadoum 1968; Kawahara et al. 1967; Le Bel et al. 1979; Lee et al. 1984), soil, and plant and animal tissue (Belisle and Swineford

1988; Carey et al. 1979; Kadoum 1968). Some problems still exist with sample preparation and separation, which affect the precision, accuracy, and specificity of analyses. Further studies to improve sample preparation and selectivity of detection might be beneficial in improving the reliability of existing methods.

7.3.2 Ongoing Studies

No ongoing studies concerning the methods of analysis of methyl parathion in biological samples and environmental media were located.