6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring diisopropyl methylphosphonate, its metabolites, and other biomarkers of exposure and effect to diisopropyl methylphosphonate. 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 (NOSH). 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 may be included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision

6.1 BIOLOGICAL SAMPLES

Methods exist for measuring diisopropyl methylpbosphonate and its metabolites in biological materials. Metabolites of diisopropyl methylphosphonate bave been analyzed in plasma, urine, and fecal samples of mink and rats (Weiss et al. 1994). Cold acetonitrile is added to each sample, with fecal samples first being homogenized with distilled water. Each sample is then centrifuged, and the supernatant is separated and fractionated by reverse phase, high performance liquid chromatography and thin layer chromatography. Gas chromatography (GC), combined with detection by mass spectrometry (MS), and proton nuclear magnetic resonance are then used to identify the fractions (Weiss et al. 1994). Attempts to develop verifiable analytical techniques for the detection of IMPA, the major metabolite of diisopropyl methylphosphonate, were unsuccessful in one study (ATSDR 1996).

GC, utilizing flame ionization detection FID), has been used to measure diisopropyl methylphosphonate in meat, grain, or milk (Caton et al. 1994). Sample preparation steps include homogenization, filtration, dialysis, and extraction on a solid sorbent. Two common solid phase extractants, Tenax GC and octadecylsilane bonded silica gel (C18 Silica), were compared by Caton et al. (1994). They reported 70% recovery when using Tenax GC and 85% recovery wlren using C18 Silica. Sensitivity was not reported. Equilibrium experiments indicate that 8-10 mg of Tenax GC are required to achieve maximum recovery of each µg of diisopropyl methylphosphonate (Caton et al. 1994). By extrapolating these results to a complex

mixture sample in which several chemical species would be competing for sorption sites, the study authors estimated that 0.1-0.5 mg of Tenax GC should be used for each mg of plant or animal tissue. An alternative approach to measure diisopropyl methylphosphonate in meat or milk is based upon thermal desorption into an ion trap mass spectrometer (TDITMS) (Buchanan et al. 1995). Preparation of beef tissue samples requires combination of centrifugation, filtration, and absorption on solid sorbents. Milk samples did not require centrifugation and filtration. Overall recovery for diisopropyl methylphosphonate from the sorbent Tenax was found to be in the range of 20-50% (Buchanan et al. 1995). Small initial sample sizes are required to reach detection limits of 50-100 ppb, suggesting that it may be possible to extract small tissue samples from livestock by needle biopsy. This would eliminate the requirement of animal sacrifice for analysis Further, the time needed for sample preparation and analysis using TDITMS is shorter than conventional EPA methods, allowing for a greater number of samples to be included in the analysis. A summary of the analytical methods for various biological media is presented in Table 6-l.

6.2 ENVIRONMENTAL SAMPLES

Methods exist for determining levels of diisopropyl methylphosphonate in air, soil, and water. These methods include separation by GC coupled with FID and flame photometric detection (FPD), determination by infrared and Raman spectroscopy, separation by ionization mass spectrometry, determination utilizing piezoelectric crystals, and determination by gas-sensitive microsensors. Table 6-2 summarizes the methods that have been used to analyze environmental samples for diisopropyl methylphosphonate.

Methods used for analyzing diisopropyl methylphospbonate in air include real-time analyses utilizing interdigitated gate electrode-field effect transistors (IGEFET), coated and uncoated piezoelectric crystal detectors, gas chromatographs, and mass spectrometers. The IGEFET method utilizes electron-beam evaporated thin films of copper phthalocyanine (CuPc) to obtain a microelectronic integrated circuit sensor that can selectively detect parts-per-billion (ppb) concentration levels of diisopropyl methylphosphonate (Kolesar and Wiseman 1989; Kolesar et al. 1992). At specific frequencies, the interaction between adsorbed diisopropyl methylphosphonate and the thin film of CuPc produces a distinct change in the film's electrical resistance and reactance, allowing the IGEFET to selectively identify diisopropyl methylphosphonate.

A number of studies (Kristoff and Guilbault 1983; Milanko et al. 1992) have investigated the use of coated and uncoated piezoelectric crystals in the detection and analysis of diisopropyl methylphosphonate in air samples. Piezoelectric crystals have a natural resonant frequency of oscillation that can be utilized to detect

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Plasma	Add cold acetonitrile, centrifuge, separate supernatant, fractionate by reverse-phase HPLC and TLC	GC/MS and PNMR	No data	No data	Weiss et al. 1994
Urine	Add cold acetonitrile, centrifuge, separate supernatant, fractionate by reverse-phase HPLC and TLC	GC/MS and PNMR	No data	No data	Weiss et al. 1994
Fecal matter	Homogenize with distilled water, add cold acetonitrile, centrifuge, separate supernatant, fractionate by reverse-phase HPLC and TLC	GC/MS and PNMR	No data	No data	Weiss et al. 1994
Beef muscle	Homogenize, filter, remove macromolecular constituents by dialysis, isolate remaining constituents on solid sorbent	GC/FID or ITMS	No data	70–85%	Caton et al. 1994

TABLE 6-1 (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Beef muscle	Homogenize, dilute with distilled water, centrifuge and filter to remove water-insoluble particulates; extract onto solid-phase sorbents	ITMS	50–100 ppb	20–50%	Buchanan et al. 1995
Milk	Homogenize, dilute with distilled water, extract onto solid-phase sorbents	ITMS	50–100 ppb	20–50%	Buchanan et al. 1995
Milk	Homogenize, filter, remove macromolecular constituents by dialysis, isolate remaining constituents on solid sorbent	GC/FID or ITMS	No data	70–85%	Caton et al. 1994
Plant	Homogenize, filter, remove macromolecular constituents by dialysis, isolate remaining constituents on solid sorbent	GC/FID or ITMS	No data	70–85%	Caton et al. 1994

FID = flame ionization detection; GC = gas chromatography; HPLC = high performance liquid chromatography; ITMS = ion trap mass spectrometry; MS = mass spectrometry; PNMR = proton nuclear magnetic resonance; TLC = thin-layer chromatography

TABLE 6-2. Analytical Methods for Determining Diisopropyl Methylphosphonate in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	None (real time)	IGEFET	No data	No data	Kolesar et al. 1992
Air	None (real time)	Uncoated piezoelectric crystal detector	0.5 ng	No data	Kristoff and Guilbault 1983
Air	None (real time)	Coated piezoelectric crystal detector	No data	No data	Milanko et al. 1992
Air	None (real time)	GC/FPD	No data	No data	Sass and Parker 1980
Air	None (real time)	MIMS	300 ppt	No data	Syage et al. 1988
Soil	Extract with distilled water, filter, pass through C_{18} sorbent, elute with chloroform, add internal standard	GC/FID	No data	66±7%	Tornes et al. 1991
Soil	Extract using ultrasonic vibrations	GC/FID/FPD/MS	No data	No data	Sawyer et al. 1992
Soil	Extract with ethyl acetate, filter or separate, dry, concentrate	GC/AFID	No data	No data	Spanggord et al. 1979
Water	Extract with distilled water, filter, pass through C_{18} sorbent, elute with chloroform, add internal standard	GC/FID	No data	87±10%	Tornes et al. 1991
Water	Extract with methylene	GC/MS	No data	No data	Calgon 1977

TABLE 6-2 (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
	chloride, filter through anhydrous sodium sulfate, evaporate, concentrate				
Water	Extract with methylene chloride, separate, dry, concentrate	GC/FPD	9.05 µg/L	No data	Fasano et al. 1982
Water	Extract with ethyl acetate, filter or separate, dry, concentrate	GC/AFID	No data	No data	Spanggord et al. 1979

AFID = alkali-flame ionization detection; FID = flame ionization detection; FPD = flame photometric detection; GC = gas chromatography; IGEFET = interdigitated gate electrode field-effect transistor; ITMS = ion trap mass spectrometry; MIMS = multiphoton ionization mass spectrometry; MS = mass spectrometry

chemical contaminants such as diisopropyl methylphosphonate. A thin coating of an appropriate substance is applied to a crystal. The film selectively adsorbs the diisopropyl methylphosphonate, which increases the mass of the piezoelectric crystal, thereby decreasing the oscillation frequency (Brothers 1990). Although piezoelectric crystals are very sensitive, there is a major problem with this sensitivity. The chemically sensitive coating must be extremely selective in its adsorption of diisopropyl methylphosphonate. Otherwise, other contaminants may also adsorb to the crystal, altering its mass and causing the piezoelectric sensor to be unable to measure the diisopropyl methylphosphonate (Brothers 1990). Piezoelectric crystal coatings include Triton X- 100 (polyethylene glycol *p*-(1,1,3,3-tetramethylbutyl)phenyl ether), Aerosol-OT (sodium dioctylsulphosuccinate), dibutyl phthalate, and collodion (Milanko et a1.1992). Kristoff and Guilbault (1983) investigated the diisopropyl methylphosphonate detection ability of uncoated piezoelectric crystals with various metal electrodes (gold, silver, and nickel). They found that sensitivity increased and selectivity decreased in the following order: gold, silver, nickel. Sensitivity was found to decrease with increasing temperature (20-50° C) for the gold-plated crystal. Water vapor at 50% relative humidity was not a serious interference. The smallest weight of diisopropyl methylphosphonate detected at 10 μg L^{-1} was approximately 0.5 ng (Kristoff and Guilbault 1983).

Gas chromatography with a flame photometric detector (Sass and Parker 1980) and multiphoton ionization mass spectrometry (MI/MS) (Syage et al. 1988) have also been used to analyze diisopropyl methylphosphonate in air samples.

Soil samples of diisopropyl methylphosphonate are detected and quantified mainly by GC, FID, FPD, and MS (Sawyer et al. 1992; Spanggord et al. 1979; Tomes et al. 1991). A number of different preparation methods exist. In a study designed to determine chemical warfare agents in samples from a battlefield environment, a solid-phase extraction method was described that extracts soil samples by shaking them in distilled water (Tomes et al. 1991). The extract is then filtered to remove particulate matter and immediately passed through a cartridge containing 200 mg C_{18} sorbent wetted with methanol and water. The diisopropyl methylphosphonate is then eluted from the sample witb chloroform, an internal standard is added, and the sample is analyzed by GC using a 30m x 0.242 mm I.D. capillary column and FID (Tomes et-al. 1991). Percent recovery was 66 ± 7% from soil samples contaminated with 1 mg of diisopropyl methylphosphonate; no sample detection limit was given. Sawyer et al. (1992) described a method in which extraction is accomplished using ultrasonic vibrations prior to analysis by GC using a 15-m column and FID, FPD, and MS. No sample detection limits or percent recovery data were reported. In a study on the environmental fate of diisopropyl methylphosphonate at the RMA, Spanggord et al. (1979) described a method in which soil

samples are extracted with three volumes of ethyl acetate, filtered or separated, dried over anhydrous NA_2SO_4, and concentrated using rotary evaporation Triethyl phosphate (TEP) is added as an internal standard, and the sample is analyzed by GC using a 50-m SP2100 glass capillary column and alkali-flame ionization detection (AFID). Retention time for diisopropyl methylphosphonate was 9.82 minutes at a flow rate of 0.5 mL/minute N_2 at 110° C isothermal Altbough a detection limit was not specified, a detection level of 0.05 ppm was reported for soil extracts (Spanggord et al. 1979).

The most common method for analyzing water samples for diisopropyl methylphosphonate is GC with FID or FPD. One method described by Rosenblatt et al. (1975b) involves extraction of diisopropyl methylphosphonate from water with chloroform prior to GC analysis using a 5-foot-long, 0.25inch-diameter glass chromatographic column filled with OV-17/Reoplexon 400 CRG. A 1-ppm solution of diisopropyl methylphosphonate in chloroform was used as a standard (Rosenblatt et al. 1975b). Methods described by Tornes et al. (1991) and Spanggord et al. (1979) for analysis of diisopropyl methylphosphonate in soils are also applicable to diisopropyl methylphosphonate analysis in water samples. Tomes et al. (1991) reported an 87 ± 10% recovery rate from 30-mL water samples contaminated with 1 mg of diisopropyl methylphosphonate. Fasano et al. (1982) described a method for the quantification of diisopropyl methylphosphonate in water in the presence of dimethyl methylphosphonate (DMMP) and trimethyl phosphate (TMP). Samples are extracted with methylene chloride, dried, concentrated, and analyzed using a 10-foot-long, 2-mm-diameter I.D. glass GC column packed with 10% Carbowax 20M on 100/120 mesh Supelcoport. A sample detection limit of 9.05 μg/L was reported for diisopropyl methylphosphonate in water in the presence of 10 mg/L of DMMP (Fasano et al. 1982).

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 diisopropyl methylphosphonate 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 diisopropyl methylphosphonate.

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

Exposure. No biomarkers of exposure were identified that were specific to diisopropyl methylphosphonate. Although standard procedures exist for identifying diisopropyl methylphosphonate's primary metabolite (IMPA) in plasma, urine, and feces (Weiss et al 1994), the detection of IMPA is not unique to diisopropyl methylphosphonate exposure. IMPA is also a major metabolite of GB (Sarin) (Little et al. 1986). In addition, IMPA is cleared from the body rapidly, making it a useful indicator for recent exposure only.

Effect. No biomarkers of effect were identified that were specific to diisopropyl methylphosphonate. No specific target organs of diisopropyl methylphosphonate are known, and no toxic effects in humans can be positively linked to diisopropyl methylphosphonate exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods exist to detect diisopropyl methylphosphonate and its degradation products in air (Kolesar et al. 1992; Kristoff and Guilbault 1983; Milanko et al. 1992; Sass and Parker 1980; Syage et al. 1988), soil (Sawyer et al. 1992; Spanggord et al. 1979; Tomes et al. 1991), and water (Calgon 1977; Fasano et al. 1982; Spanggord et al. 1979; Tomes et al. 1991). These methods are relatively sensitive and reliable and have varying degrees of selectivity.

6.3.2 On-going Studies

No on-going analytical methods studies were located.