6. ANALYTICAL METHODS

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

Methods for the determination of chlorpyrifos and its metabolites are shown in Table 6- 1. Chlorpyrifos has been measured in human whole blood, plasma, and urine at concentrations as low as 10 ppb (Drevenkar et al. 1994; Jitsunari et al. 1989; Nolan et al. 1984). The chlorpyrifos oxygen analog (oxon) has been reported to be recoverable from serum and urine by hexane extraction, but no limit of detection (LOD) or recovery was reported (Drevenkar et al. 1993). The chlorpyrifos metabolite TCP has been measured at concentrations as low as 0.5 ng/mL weight per volume (0.5 ppb, w/v) in human blood and urine (Bartels and Kastl 1992; Jitsunari et al. 1.989; Nolan et al. 1984). The hydrolysis product diethyl phosphate (DEP) has been measured in urine and plasma (Drevenkar et al.1994; Takamiya 1994) and the hydrolysis product diethylthiophosphate (DETP) has been measured in plasma (Drevenkar et al. 1994) with LODs of approximately 50 ppb. Chlorpyrifos and its oxon can be extracted directly into organic solvent while TCP, DEP, and DETP can be isolated after acid hydrolysis of the conjugated forms. Chlorpyrifos and its oxon can be determined directly using gas chromatography (GC) and selective detection methods (see below). The metabolites TCP, DEP, and DETP are typically derivatized to improve the chromatography and, hence, detectability. No methods were found for chlorpyrifos and its metabolites in human tissue, but methods have been reported for animal tissue (see Table 6-2) (Brown et al. 1987; Clabom et al. 1968; Dishburger et al. 1977; Ivey and Clabom 1968; Lino and Noronha da Silveira 1994) and could most likely be applied to human tissues.

Table 6-1. Analytical Methods for Determining Chlorpyrifos and Metabolites in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood, urine (chlorpyrifos and TCP)	Blood: Chlorpyrifos extraction with acetone and solvent exchanged to hexane. Water was removed from the extract followed by clean-up using silica gel. TCP was recovered via SPE from separate aliquot of acidified blood. TCP elution from SPE with methanol then extraction into benzene and derivatization with N,O-bis(trimethylsilyl)acetamide. Urine: Chlorpyrifos extraction with hexane. Hydrolysis of conjugates of TCP with H_2SO_4 at 90 °C for 1 hour. TCP isolation via SPE, extraction into benzene, and derivatization as for blood.	Chlorpyrifos: GC/FPD; TCP: GC/ECD	Chlorpyrifos: No data; TCP: 10 ng/mL (10 ppb, w/v)	Chlorpyrifos: No data; TCP: 91.5% (4% RSD) at 0.1 µg/mL (0.1 ppm, w/v)	Jitsunari et al. 1989; Nolan et al. 1984
Urine (TCP)	TCP isolation from urine by acid hydrolysis of urine aliquots followed by extraction with diethyl ether. Residues dissolved in *o*-xylene followed by derivatization with N-(*tert*-butyldimethylsilyl)-N-methyl-trifluoroacetamide.	GC/NCIMS	0.5 ng/mL (0.5 ppb, w/v)	Relative recoveries 80.6 to 89.9% over concentration range of 4.1 to 411 ng/mL of urine	Bartels and Kastl 1992
Urine (DEP)	Inorganic phosphate removal by addition of $Ca(OH)_2$. DEP isolation using ion exchange and derivatization to pentafluorobenzyl derivative.	GC/FPD	No data	149% (9% RSD) at 0.50 ppm	Takamiya 1994

Table 6-1. Analytical Methods for Determining Chlorpyrifos and Metabolites in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Plasma (chlorpyrifos, DEP, DETP)	Chlorpyrifos extraction into hexane. DEP and DETP were recovered from hexane-extracted plasma as follows: plasma saturation with NaCl, acidification with 6N HCl, and extraction with diethyl ether. DEP and DETP methylation using diazomethane.	Chlorpyrifos: GC/ECD; DEP, DETP: GC/AFID	50 ng/mL (50 ppb, w/v)	DEP: 97% (3% RSD) at concentrations ≥ 2 µg/mL; DETP: 97% (11% RSD) at concentrations ranging from 0.1 to 2.8 µg/mL	Drevenkar et al. 1994
Serum and urine (chlorpyrifos, chlorpyrifos oxon, DEP, DETP)	Chlorpyrifos and its oxon recovered via extraction with hexane. Extracted sample was acidified and saturated with NaCl followed by extraction with diethyl ether. DEP and DETP derivatization with diazomethane.	GC/MS	No data	No data	Drevenkar et al. 1993

AFID = alkali flame ionization detector; DEP = diethyl phosphate; DETP = diethyl thiophosphate; GC = gas chromatography; ECD = electron capture detector; FPD = flame photometric detector; MS = mass spectrometry; NCIMS = negative ion chemical ionization mass spectrometry; TCP = 3,5,6-trichloro-2-pyridinol

Table 6-2. Analytical Methods for Determining Chlorpyrifos and Transformation Products in Environmental Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Known volumes of air drawn through XAD-2 adsorbent. Desorption with toluene.	GC/FPD (OSHA Method 62)	0.23 ppb (mole/mole); 0.003 mg/m^3	96.6 (5.3% standard error at 0.014 ppm)	OSHA 1986
Air	Known volumes of air drawn through polyurethane foam (PUF). Desorption via Soxhlet extraction using 5% diethyl ether in hexane. Extract volume reduction and further clean-up using Florisil if needed.	GC/ECD (EPA Method TO-10) May also use GC/FPD, GC/NPD, GC/MS	Approximately 0.01 µg/m^3 (0.7 ppt, mole/mole) This limit depends on the sampling volume	87 (20% RSD) for 10–1,000 ng/m^3 concentration and 24 h sampling	EPA 1988b
Air, surfaces	Air: Known volume of air pulled through ORBO-44 tubes (Supelpak 20) and elution with toluene. Surfaces: wiping with surgical gauze moistened with distilled water. Gauze extraction with toluene.	GC/ECD	Air: 83 ng/m^3 (5.8 ppt, mole/mole) Surface wipes: 0.6 ng/cm^2	Air: 85 (SD=6); Wipes: 84 (SD=10)	Fenske et al. 1990
Drinking Water (chlorpyrifos and TCP)	Chlorpyrifos: Water extraction with hexane. Water removal from extract followed by volume reduction. TCP: Water acidification, NaCl addition, and extraction with benzene. Water removal from extract followed by volume reduction.	TLC	100 ng/L or 100 ppb (w/v) for chlorpyrifos; 25 ng/L or 25 ppb (w/v) for TCP	Chlorpyrifos: 87 (8% RSD) at 5 ppb; TCP: 84 (5% RSD) at 5 ppb	Sherma and Slobodien 1984
Well water (drinking water)	Direct injection of 20 µL onto GC retention gap.	GC/ECD	<0.9 ppb (w/v)	95 (16% RSD) at 0.9 ppb	Gerhart and Cortes 1990

Table 6-2. Analytical Methods for Determining Chlorpyrifos and Transformation Products in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
River water, fish	Water: Water passage through C_{18} SPE cartridge and analyte elution with ethyl acetate. Solvent removal and redissolution in ethyl acetate. Fish: Sample lyophilization and Soxhlet extraction with ethyl acetate. Extract clean-up using SPE and GPC.	GC/NPD, GC/MS, GC/NCIMS	Water: 0.1 µg/L (0.1 ppb, w/v) NPD; 0.02 µg/L (0.02 ppb, w/v) NCIMS. Fish: 2 ng/g (2 ppt, w/w) NCIMS	Water: 94 (4% RSD) at 10–15 µg/L. Fish: 92–136 at 0.1 µg/g	Lacorte et al. 1993
Surface water	Water passage through XAD-2 and XAD-7 resins and analyte elution with methylene chloride. Internal standard addition, water removal and extract concentration.	GC/Ion Trap MS	0.005 ppb (w/v) or 5 ng/L	86.7 (17% RSD)	Mattern et al. 1991
Surface water	Water passage through C_8 SPE cartridge and elution of analytes with methanol.	HPLC/UV	5.0 ppb (w/v) or 5 µg/L	93 (14% RSD)	Bogus et al. 1990
Waste water	Extraction using methylene chloride. Water removal, solvent exchange to hexane and extract volume reduction.	GC/NPD or GC/FPD (P mode) (EPA Method 622)	0.3 µg/L (0.3 ppb, w/v)	98 (5.5% RSD over concentration range 1–50 µg/L)	EPA 1992b
Groundwater, soil, sludges, wastes	Aqueous samples: Extraction using methylene chloride; water removal and extract volume reduction. Soils, sludges, wastes: Extraction (sonication or Soxhlet) using methylene chloride after mixing sample with sodium sulfate. Additional clean-up using Florisil if needed.	GC/NPD or GC/FPD (EPA Method 8140); GC/MS (EPA Method 8270)	3 µg/L (3 ppb, w/v) for groundwater; 3 mg/kg (3 ppm, w/w) for high level soil and sludges	98 (5.5% RSD)	EPA 1986a (Method 8140); EPA 1986b (Method 8270).

Table 6-2. Analytical Methods for Determining Chlorpyrifos and Transformation Products in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Groundwater, soil, wastes	Extraction of aqueous samples at neutral pH using methylene chloride, water removal, and volume reduction. Extraction of solid samples with methylene chloride/acetone. Additional clean-up using Florisil if needed.	GC/NPD or GC/FPD (EPA Method 8141A)	0.7 µg/L (0.7 ppm, w/v) for groundwater; 5 mg/kg (5 ppm, w/w) for water-immiscible wastes	89±6% from water at 1.56 µg/L; 79±7% from soil at 52 µg/kg	EPA 1992a
Pesticide formulations (chlorpyrifos, TCP)	Liquids: Weighing sufficient sample to contain ca. 80 mg into vial and addition of 25 mL of acetonitrile containing 1,4-dibromonaphthalene (internal standard). Solids: As for liquids with added filtration step before analysis.	HPLC/UV	No data	No data	Helrich 1990a
Turkey and chicken (muscle, skin, heart, gizzard, brain, liver, fat)	Extraction of 250 mg of ground sample ground with petroleum ether. Water removal using sodium sulfate followed by centrifugation.	GC/ECD	0.05 ppm (w/w)	79–99 (at 0.05 and 0.10 ppm, w/w)	Hunt et al. 1969
Fatty and non-fatty foods (eggs, pasta)	Homogenization of sample with acetone (water addition needed for certain foods) and extraction with methylene chloride/acetone after NaCl addition. Water removal using sodium sulfate and extraction twice with methylene chloride. Water removal from extract and solvent evaporation. Further extract clean-up using carbon/Celite, Extrelut-3, or C_{18}-SPE. Solvent evaporation and redissolution in benzene.	GC/FPD	5.2 ppb (w/w)	80 at 0.03 ppm spike	Leoni et al. 1992

Table 6-2. Analytical Methods for Determining Chlorpyrifos and Transformation Products in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Beef fat	Tissue extraction sweep co-distillation to isolate analytes.	GC/ECD	No data	83.5% (5.3% RSD) at 0.16 mg/kg (0.16 ppm, w/w)	Luke and Richards 1984
Rumen content, liver	Homogenization of 5 g sample with methanol:methylene chloride (1:9, v/v). Water removal from extract followed by volume reduction prior to clean-up using GPC and silica SPE.	GC/FPD	0.01 to 0.05 µg/g (ppm, w/w)	Rumen content: 99 (3% RSD) at 0.1 µg/g. Liver: 105 (2% RSD) at 0.05 µg/g	Holstege et al. 1991
Fats and oils	Sample mixing with light petroleum and extraction five times with light petroleum-saturated acetonitrile. Chlorpyrifos isolation using C_{18} SPE followed by solvent exchange to acetone for analysis.	GC/FPD	<0.08 µg/g (0.08 ppm, w/w)	85–97 at 0.16–0.5 µg/g (ppm, w/w)	Gillespie and Walters 1991
Peppermint oil (chlorpyrifos, TCP)	Chlorpyrifos: Oil application to silica gel column and elution with 3% water-saturated diethyl ether in hexane followed by volume adjustment. TCP: Oil dissolution in benzene: pentane (2:3) and extraction with 0.5% sodium carbonate. Aqueous phase washing with chloroform, acidification and extraction with chloroform. Further extract purification via acidic alumina column chromatography. Trimethysilyl derivative formation.	GC/FPD (chlorpyrifos); GC/ECD (TCP)	0.1 ppm (µg/g, w/w) for chlorpyrifos; 0.5 ppm for TCP	73–104 for chlorpyrifos over concentration range 0.11–10 ppm; 70 to 101% at 0.5–1.0 ppm	Inman et al. 1981

Table 6-2. Analytical Methods for Determining Chlorpyrifos and Transformation Products in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Chicken muscle, skin	Homogenization of weighed tissue once with acetonitrile and twice with 70% acetonitrile/water followed by filtration. Filtrate extraction with zinc acetate/water, filtration and filtrate extraction with dichloromethane. Solvent exchange to hexane and Florisil clean-up.	GC/NPD	2.5 µg/kg (ppb w/w) for muscle; 2.2 µg/kg (ppb, w/w) for skin	Muscle: 91.9 at 6.6 µg/kg (6.6 ppb, w/w) Skin: 105 at 19 µg/kg (ppb, w/w)	Lino and Noronha da Silveira 1994
Bovine milk, tissues (muscle, liver, heart, kidney, brain, spleen, omental fat)	Fat: Sample dissolution in hexane, water removal and extraction with acetonitrile. Extract volume reduction, dilution with aqueous sodium sulfate and back-extraction with hexane. Water removal from extract, concentration and clean-up using silicic acid column chromatography. Tissue: Sample blending with Celite and acetone. Acetone removal and aqueous phase extraction with hexane; clean-up as for fat. Milk: Milk combined with activated Florisil. Application of mixture to Florisil column and elution with 10% (v/v) methylene chloride in hexane.	GC/ECD	0.002 ppm (w/w) for tissues and 0.005 ppm (w/v) for milk	Tissue: 75–100 at 0.012 ppm; Milk: 84 at 0.05 ppm	Claborn et al. 1968

Table 6-2. Analytical Methods for Determining Chlorpyrifos and Transformation Products in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Bovine milk, tissues (muscle, liver, heart, kidney, brain, spleen, omental fat); chlorpyrifos oxygen analog	Fat: Sample dissolution in hexane, water removal and extraction with acetonitrile. Extract volume reduction, dilution with aqueous sodium sulfate and back-extraction with hexane. Water removal from extract, concentration and clean-up using silicic acid column chromatography. Tissue: Sample blending with Celite and acetone. Acetone removal and aqueous phase extraction with hexane; clean-up as for fat. Milk: Milk combined with silicic acid followed by water removal and elution with hexane. Application of mixture to silicic acid column and elution with water-saturated methylene chloride.	GC/ECD	0.1 ppm (w/w) for fat and muscle; 0.025 ppm (w/v) for milk	70 and 92 for muscle and fat, respectively, at 0.1 ppm; 80 from milk at 0.025 ppm	Ivey and Claborn 1968
Bovine tissue (muscle, liver, kidney, fat); TCP	Tissue homogenization with methanol, filtration and mixing with acidified water containing NaCl. TCP extraction with benzene. TCP isolation using alumina column chromatography and derivatization to trimethylsilyl derivative. Total TCP (free plus conjugated) also examined after alkaline hydrolysis (any chlorpyrifos also converted to TCP).	GC/ECD	<0.05 ppm (w/w)	81–89 without hydrolysis; 86–101 with hydrolysis	Dishburger et al. 1977
Bovine fat	Tissue extraction and sweep co-distillation to isolate analytes. Extract clean-up using activated Florisil followed by extract volume reduction.	GC/NPD	No data	92 (5% RSD) at 0.4 mg/kg (ppm, w/w)	Brown et al. 1987

Table 6-2. Analytical Methods for Determining Chlorpyrifos and Transformation Products in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Butter fat, potatoes	Dispersion of homogenized sample with pelletized diatomaceous earth (Hydromatrix), packing into high pressure extraction cell, and extraction with supercritical carbon dioxide. Collection of extracts from fatty samples into a flask and clean-up using GPC and Florisil adsorption chromatography. Extracts from non-fatty samples trapped onto a Florisil column. Chlorpyrifos elution with acetone.	GC/NPD	<0.06 ppm (w/w)	Butter fat: 90 (0.06–0.6 ppm) potatoes: 97 at 0.120 ppm	Hopper and King 1991
Lettuce, strawberries, and tomatoes	Sample homogenization with acetone followed by filtration; pesticide extraction into organic phase by shaking with petroleum ether and methylene chloride. Water removal from extract and organic phase volume reduction in presence of petroleum ether and then acetone to remove methylene chloride.	GC/NPD (AOAC Method 985.22)	No data	No data	Helrich 1990b
Cucumbers, lettuce, radishes, strawberries, tomatoes, witloof chicory	Extraction of homogenized sample with acetone. Analytes recovered via back extraction with methylene chloride followed by water removal and clean-up using activated carbon-silica gel.	GC/NPD (German Pesticides Commission Method S8)	0.05 mg/kg (0.05 ppm, w/w) at 0.5 mg/kg	>70	Thier and Zeumer 1987a

Table 6-2. Analytical Methods for Determining Chlorpyrifos and Transformation Products in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Potatoes, lettuce, citrus fruit	Extraction of homogenized plant material with acetone and saturation of this extract with NaCl and dilution with methylene chloride. Clean-up of organic phase using GPC and silica gel column chromatography.	GC/FPD (German Pesticide Commission Method S19)	No data	>70	Thier and Zeumer 1987b
Non-fatty foods (chlorpyrifos and chlorpyrifos oxygen analog.)	Sample homogenization with acetone followed by filtration. Residues partitioned into methylene chloride and petroleum ether after addition of NaCl. Alternatively, acetone solution passage through Hydromatrix (diatomaceous earth) and residue elution with methylene chloride.	GC/FPD, GC/HECD, GC/NPD (US FDA PAM1 Method 302)	Approximately 20 ppb (w/w, µg/kg) depending on analytical system used	>80	FDA 1994a
Dates (chlorpyrifos and oxygen analog)	Extraction of homogenized sample with benzene. Application of extract to silica gel column and elution with benzene to collect chlorpyrifos and then with acetone to recover the oxygen analog.	GC/NPD	0.01 ppm (w/w, mg/kg) for chlorpyrifos; 0.05 ppm for oxygen analog	93 for chlorpyrifos and 84 for oxygen analog over concentration range 0.01–2.0 ppm	Mansour 1985

[a] Unless otherwise specified, method is for chlorpyrifos. If method was applied to transformation products, these are indicated in parentheses with the matrix studied.

AOAC = Association of Official Analytical Chemists; ECD = electron capture detector; EPA = Environmental Protection Agency; FPD = Flame photometric detector; GC = gas chromatography; GPC = gel permeation chromatography; HPLC = high performance liquid chromatography; MS = mass spectrometry; NCIMS = negative ion chemical ionization mass spectrometry; NPD = nitrogen phosphorus detector (thermionic); OSHA = Occupational Safety and Health Administration; RSD = relative standard deviation; SD = standard deviation; SPE = solid phase extraction; TCP = 3,5,6-trichloropyridinol; TLC = thin layer chromatography; UV = ultraviolet absorbance detection; v/v = volume/volume; w/v = weight/volume

6.2 ENVIRONMENTAL SAMPLES

Methods for the determination of chlorpyrifos and environmental transformation products are shown in Table 6-2.

The analytical methods for chlorpyrifos in air are based on GC with some form of selective detection. For air matrices, collection methods rely on the entrapment of chlorpyrifos onto a polymeric material, such as XAD or polyurethane foam, as the air is pulled through the sorbent (EPA 1988c; Fenske et al.1990; OSHA 1986). The analyte is subsequently recovered from the sorbent through solvent extraction. Losses of chlorpyrifos can occur during Soxhlet extraction or extract concentration using Kudema-Danish devices as a result of the boiling chips used (Hsu et al. 1988). Thus, it is very important that the performance of any method be verified prior to its application in a study. The proper use of field control samples is also very important. Reported LODs were as low as sub parts per trillion (EPA 1988c). Although chlorpyrifos can be converted to its oxygen analog (thiophosphate to phosphate) under normal environmental conditions (see Chapter 5), none of the methods surveyed indicated that this conversion was problematic for the determination of chlorpyrifos in air.

In the case of water, soils, and wastes, sample preparation is based on liquid/liquid extractions (EPA 1986c, 1986d, 1992b, 1992c; Sherma and Slobodien 1984), solid phase extraction (SPE) (Bogus et al.1991; Johnson et al. 1991; Lacorte et al. 1993; Mattem et al. 1991), or Soxhlet extractions (EPA 1986c, 1986d). Humic material in natural waters can reduce recoveries of chlorpyrifos in SPE-based sample preparation (Johnson et al. 1991). The decreased recovery is hypothesized to be the result of inefficient trapping of the chlorpyrifos/humic material complex. EPA Method 507 for the determination of nitrogen- and phosphorus-containing pesticides in drinking water (EPA 1991) should be applicable to chlorpyrifos but has not been validated for this compound. Soxhlet extractions are commonly employed in methods used to study chlorpyrifos residues in carpet dust and on surfaces sampled using wiping approaches (Fenske et al. 1990; Lewis et al. 1994). Supercritical fluid extraction (SFE) has shown promise for the recovery of chlorpyrifos from environmental solids (Lopez-Avila et al. 1991; Miles and Randall 1992). Chlorpyrifos in sample extracts is typically determined using GC, although thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) have also been employed (Bogus et al. 1990; Sherma and Slobodien 1984). Sherma and Slobodien (1984) also used TLC to quantify the chlorpyrifos transformation product TCP in drinking water. Gerhart and Cortes (1990) have reported a method for chlorpyrifos that used direct

injection of well water into a GC retention gap. Reported lower LODs for chlorpyrifos ranged from 5 ppt (w/v) for surface water (Mattem et al. 1991) to 3 ppm (w/w) for soils and sludges (EPA 1986c).

The determination of chlorpyrifos and its transformation products, especially chlorpyrifos oxygen analog and TCP in foods has received considerable attention. Foods are generally divided into fatty (animal products, oils) and non-fatty types (produce). Chlorpyrifos is fairly non-polar and thus tends to partition into fat. This dictates that slightly different methods be used for the extraction of fatty and non-fatty samples. In general, chlorpyrifos, chlorpyrifos oxygen analog, and TCP are extracted from fatty foods using petroleum ether (Hunt et al. 1969), methylene chloride/acetone (Leoni et al. 1992), methanol/methylene chloride (Holstege et al. 1991) acetonitrile (Clabom et al. 1968), or methanol (Dishburger et al. 1977). The sample or initial extracts are usually acidified followed by additional extraction steps to recover TCP (Dishburger et al. 1977; Inman et al. 1981). Non-fatty samples are most often extracted with acetone (FDA 1994a; Helrich 1990b; Thier and Zeumer 1987a, 1987b), although the use of benzene has also been reported (Mansour 1985). Supercritical fluid extraction has been successfully used to recover chlorpyrifos from potatoes and butter fat (Hopper and King 1991) and grass (Cortes et al. 1991).

The determinative step for chlorpyrifos, chlorpyrifos oxygen analog, and TCP is usually GC in conjunction with selective detection such as flame photometric detection (FPD), nitrogen phosphorus thermionic detection (NPD), or electron capture detection (ECD). Depending on the original sample matrix, additional clean-up can be required to remove fats or other material that can interfere with the chromatography (Walters 1990) or with detection (FDA 1994a). In addition, natural sample constituents, such as large amounts of sulfur-containing compounds in cauliflower, onions and broccoli, can increase the FPD background detector signal and make the method less sensitive (Lee and Wylie 1991). Common approaches to further extract purification include SPE (Gillespie and Walters 1991; Leoni et al. 1992; Thier and Zeumer 1987a, 1987b), gel permeation chromatography (GPC) (FDA 1994a; Holstege et al. 1991; Thier and Zeumer 1987b), Florisil column chromatography (Brown et al. 1987; Clabom et al. 1968; FDA 1994a; Hopper and King 1991; Leoni et al. 1992), sweep co-distillation (Luke and Richards 1984) and HPLC (Gillespie and Walters 1986, 1989). The adequate recovery of the desired compound must be validated for the fractionation technique to be used. For example, SPE cartridges from different vendors or production lots have been shown to affect retention and recovery (Gillespie and Walters 1991). Chlorpyrifos oxygen analog has been found to be hydrolyzed by activated silica (Braun 1974). Florisil can also give rise to poor recoveries

of chlorpyrifos oxygen analog (FDA 1994a, 1994b; Leoni et al. 1992). The FDA method for fatty foods or cornposited food (Method 304) can be applied with limited success to chlorpyrifos (variable recovery) but not at all to the oxygen analog (FDA 1994b).

TLC has been used to separate chlorpyrifos and TCP (Judge et al. 1993) and to screen for 170 commonly used pesticides, including chlorpyrifos (Erdmann et al. 1990). Additional analytical techniques that have been applied to chlorpyrifos include GC with atomic emission detection (Lee and Wylie 1991), GC with pulsed positive ion/negative ion chemical ionization mass spectrometry (Stan and Kellner 1989), simultaneous analysis on two GC columns with both ECD and electrolytic conductivity detectors (Hopper 1991), and two-dimensional GC with simultaneous detection by ECD, NPD, and FPD (Stan and Heil 1991).

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 chlorpyrifos 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 chlorpyrifos.

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. Chlorpyrifos and TCP can serve as biomarkers of exposure. TCP will be present at much greater concentrations relative to chlorpyrifos, so it is a better and more sensitive marker of exposure (He 1993; WHO 1975). The method for TCP in urine published by Bartels and Kastl (1992) should be adequately sensitive to study

background concentrations in the general population because they measured low concentrations in control urine from presumably unexposed individuals. A LOD of 0.5 ng/mL (0.5 ppb, w/v) for TCP in urine was stated. The methods of Nolan et al. (1989) and Jitsunari et al. (1989) for TCP in blood and urine claim an LOD of 10 ppb with a reproducibility of 4% at 100 ppb. Chlorpyrifos oxon was not detected in serum and urine of poisoned persons, presumably because of the rapid rate of hydrolysis of the oxon relative to its rate of formation from chlorpyrifos (Drevenkar et al. 1993). The metabolites DEP and DETP can serve as markers of exposure to chlorpyrifos but can also be present as a result of exposure to organophosphorus compounds that have the same phosphate moiety. Thus, they are not specific. Exposure to organophosphorus pesticides also results in decreases in whole blood and erythrocyte acetylcholinesterase activities (Drevenkar et al. 1993; He 1993) and are not specific to exposure to chlorpyrifos. The best marker of exposure to chlorpyrifos appears to be TCP, for which there are adequate methods; therefore, no new methods for TCP are needed.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods are available for the determination of chlorpyrifos in air at sub-ppb concentrations (EPA 1988c; Fenske et al. 1990; OSHA 1986) and are adequate to estimate potential exposures of the general population. No methods were found for chlorpyrifos oxon in air. It has been reported that the oxon is more toxic than the parent compound (Drevenkar et al. 1993), but it does not persist (Walia et al. 1988). No additional methods are needed.

The predominant route of exposure to chlorpyrifos is through contact with contaminated environmental matrices such as food and water. Methods for the determination of chlorpyrifos in water, wastes, soils, and foods are available that have LODs in the ppb and sub-ppb range (e.g., EPA 1992c; FDA 1994a; Gerhart and Cortes 1990; Gillespie and Walters 1991; Mansour 1985; Mattem et al. 1991). Assuming an oral MRL of 0.003 mg/kg/day (Chapter 2), 2 L/day water consumption and a 70-kg person, this converts to a needed method LOD of 0.105 ppm (w/v) in drinking water. Reported LODs in water are 2 ppb (Sherma and Slobodien 1984), 0.9 ppb (Gerhart and Cortes 1990), 0.1 ppb (Lacorte et al. 1993), 0.005 ppb (Mattem et al. 1991), and 5 ppb (Bogus et al. 1990). These methods are sufficiently sensitive to detect concentrations at or below the MRL. Method reproducibilities range from 4 to 16% and will be adequate for most measurements. If 2 kg/day food consumption is assumed, method LODs of 0.105 ppm or 105 ppb (w/w) are needed. The methods of Hunt et al. (1969), Leoni et al. (1992), Lino and Noronha da Silveira (1994), Clabom et al. (1968), Ivey and Clabom (1968), Dishburger et al. (1977), Hopper and King (1991), Thier and Zeumer (1987a), FDA (1994a), and

Mansour (1985) claim method LODs that range from 2 to 100 ppb and are sufficiently sensitive to detect concentrations at or below the MRL. No reproducibility information was available. No additional methods for chlorpyrifos in foods are needed.

Methods are also available for the determination of the oxon in some foods (tissue and produce) (FDA 1994a; Ivey and Claborn 1968; Mansour 1985) at the sub-ppm level. Chlorpyrifos and its oxon are quickly hydrolyzed to TCP; some methods exist for the determination of TCP in drinking water (Sherma and Slobodien 1984), peppermint oil (Gillespie and Walters 1991), and bovine tissue (Dishburger et al. 1977).

6.3.2 Ongoing Studies

Researchers at North Dakota State University (Fargo) and at the University of Maine, Department of Food Science, have been working on immunochemical-based methods for the determination of chlorpyrifos.

Researchers at the U.S. Department of Agriculture in Beltsville, Maryland; at the University of Florida (Gainesville) Department of Food Science and Nutrition; and at the University of Puerto Rico (Mayaguez), Crop Protection, are working on fate and transport of chlorpyrifos in the environment and will be developing methods as needed to define the processes and to develop models to predict fate and transport.

Researchers at National Taiwan University (Taipei) are studying the degradation of chlorpyrifos residues in meat and poultry as a function of cooking methods for modeling purposes and might need to develop some methods.