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 dichlorvos, its metabolites, and other biomarkers of exposure and effect to dichlorvos. 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

Analytical methods for the determination of dichlorvos in human biological samples are listed in Table 6-l.

Dichlorvos can be recovered from biological media through extraction with an organic solvent (Nordgren 1981; Tewari and Harpalani 1977; Tewari et al. 1974), through the use of solid phase extraction (SPE) (Kawasaki et al. 1992; Liu et al. 1989), or through a clean-up of the matrix with no additional isolation steps (Unni et al. 1992). Determination is via gas chromatography (GC) with mass spectrometry (MS), electron capture (ECD), nitrogen-phosphorus (NPD), flame photometric (PPD), or flame ionization (FID) detectors, high performance liquid chromatography (HPLC) with spectrophotometric detection, or thin layer chromatography (TLC). Limits of detection are generally in the sub-ppm range. Reported recoveries for dichlorvos range from 74 to 85% based on measurements of dichlorvos made immediately after addition of the compound to the tissue or plasma.

Dichlorvos is formed in viva from trichlorfon (metrifonate; (o,o-dimethyl-(1 -hydroxy-2,2,2-trichloroethyl) phosphonate), a drug used to treat schistosomiasis (Nordgren 1981; Unni et al. 1992). In addition, the pesticides naled (o,o-dimethyl-o-(1,2-dibromo-2,2-dichloroethyl)phosphate) and trichlorfon can decompose to dichlorvos during sample preparation (see Section 6.2). In light of these

Table 6-1. Analytical Methods for Determining Dichlorvos and Transformation Products in Biological Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Plasma	Dilution with saline, addition of deuterated internal standards, and extraction with hexane. Hexane washed with saturated NaCl solution, dried, solvent exchange to chloroform.	GC/MS-SIM	No data	No data	Nordgren 1981
Plasma	Addition of HCl, centrifugation, filtration.	HPLC/UV	40 ng/mL (40 ppb, w/v)	85 (8% RSD) at 0.15 µg/mL (0.15 ppm, w/v)	Unni et al. 1992
Liver, stomach fluid (autopsy)	Acidification of minced liver or stomach fluid with phosphoric acid, steam distillation, extraction with hexane.	TLC	No data	No data	Tewari et al. 1974
Stomach contents	Equilibration of sample at 60 °C for 30 minutes after addition of HCl and acetone. Extraction of cooled and filtered mixture with chloroform and solvent exchange to acetone.	TLC	500 ppb (500 ng/mL, w/v)	Stomach contents: 74 at 500 µg/mL (500 ppm, w/v) Liver: No data	Tewari and Harpalani 1977
Visceral tissue (stomach, intestine, liver)	Mincing of tissue followed by extraction with diethyl ether, evaporation of solvent and redissolution in ethanol.	TLC (visualization by spraying with 2% NaOH followed by 0.5% orcinol)	2 µg/g (2 ppm, w/w)	90	Mali et al. 1995

Table 6-1. Analytical Methods for Determining Dichlorvos and Transformation Products in Biological Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Plasma, urine	Isolation via C_{18} SPE after dilution of sample with water followed by elution with chloroform:isopropanol (9:1) and solvent exchange to acetonitrile.	GC/FID	approx 400 ppb (400 ng/mL, w/v)	No data	Liu et al. 1989
Serum	Dilution of sample with pH 7 phosphate buffer followed by SPE, elution with *n*-hexane:diethyl ether (8:2, v/v) followed by solvent exchange to methanol:water (70:30, v/v).	HPLC/APCI-MS (SIM)	2 ppb (ng/mL, w/v)	80 at 2 µg/mL (2 ppm, w/v)	Kawasaki et al. 1992
Urine (dichloroethanol)	Adjustment of pH to 3.9 with acetic acid, incubation with β-glucuronidase at 37 °C for 20 hours, and extraction with ether.	GC/ECD	No data	90 at 1.22 µg/mL (1.22 ppm, w/v)	Hutson and Hoadley 1972b
Urine (dimethyl phosphate)	Removal of inorganic phosphate with $Ca(OH)_2$, centrifugation, removal of impurities via cation exchange, formation of pentafluorobenzyl derivative.	GC/FPD	approx 0.02 ppm (0.02 µg/mL, w/v)	149 (9% RSD) at 0.50 ppm (0.50 µg/mL, w/v)	Takamiya 1994

[a] Unless otherwise indicated, the target analyte was dichlorvos.

APCI = atmospheric pressure chemical ionization; ECD = electron capture detector; FID = flame ionization detector; FPD = flame photometric detector; GC = gas chromatography; HPLC = high performance liquid chromatography; MS = mass spectrometry; RSD = relative standard deviation; SIM = selected ion monitoring; SPE = solid phase extraction; TLC = thin layer chromatography; UV = ultraviolet absorbance detector; v/v= volume:volume; w/v = weight:volume;w/w = weight:weight

transformations, caution must be used in the interpretation of data from samples found to contain dichlorvos. It is quite possible that the measured dichlorvos did not arise from exposure to dichlorvos, but rather from exposure to trichlorfon or naled.

6.2 ENVIRONMENTAL SAMPLES

Analytical methods for the determination of dichlorvos in environmental samples are listed in Table 6-2.

Most of the methods for the determination of dichlorvos in air are based on the interaction of the vapor with an adsorbent as air is drawn through a sorbent tube with a sampling pump. Early methods used potassium nitrate for adsorption followed by recovery of dichlorvos via elution with hexane (Bryant and Minett 1978), or dissolution in water of the salt containing adsorbed dichlorvos (Heuser and Scudamore 1966). The dichlorvos in the resulting solution was then determined. The relatively high water solubility of dichlorvos allowed for its collection in water as an air sample was bubbled through a Drechsel bottle (Elgar and Steer 1972). Quantitation was often based on the ability of the desorbed sample to inhibit the enzyme cholinesterase (Elgar and Steer 1972; Heuser and Scudamore 1966). More recent methods use polymeric sorbents such as polyurethane foam (PUP) (EPA 1988d), SPE disks (Markell et al. 1994), XAD (Brouwer et al. 1994; OSHA 1986) or Tenax TA (Roinestad et al. 1993). Analyte recovery was via extraction of the polymeric material with organic solvent, and determination typically by GC. With very few exceptions, detection was achieved using the nitrogen/ phosphorus thermionic detector (NPD), the flame photometric detector (FPD) in the phosphorus mode, or the electron capture detector. Confirmation was obtained using MS. Limits of detection were often in the sub-ppb and sub-ppt range (OSHA 1986; Roinestad et al. 1993).

Methods for the recovery of dichlorvos from water, soil, and wastes are based on liquid/liquid extraction (ASTM 1994; EPA 1991b, 1992a, 1992b; Kadokami et al. 1991) or SPE (DiCorcia et al. 1993; Wang and Huang 1994) followed by extract volume reduction or solvent exchange followed by volume reduction. Supercritical fluid extraction (SFE) also holds promise for the extraction of dichlorvos from soils (Lopez-Avila et al. 1990; Snyder et al. 1992) or as a means to recover dichlorvos from an SPE cartridge (Barnabas et al. 1994). The water solubility of dichlorvos can often result in poor recoveries from aqueous matrices (EPA 1992b); salt is often added during extraction to favor dichlorvos partition into the organic phase (ASTM 1994; EPA 1991b; Kadokami et al. 1991).

Table 6-2. Analytical Methods for Determining Dichlorvos in Environmental Samples

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Pesticide formulations (sand/sugar base fly bait and 4 pounds/gallon emulsifiable concentrates)	Sand/sugar base: Dispersion with diatomaceous earth and elution with chloroform followed by volume reduction. Emulsifiable concentrates:dissolution of known mass of sample to give approx. 1 g DDVP/100 mL chloroform.	IR absorbance (Method 964.04)	No data	No data	AOAC 1990
Pesticide formulations (0.5% spray solution and 1% cattle spray in hydrocarbon solvent)	None for sample; reference solvent preparation by extraction of DDVP from sample with 0.5N NaOH and water removal using sodium sulfate.	IR absorbance (Method 966.07)	No data	No data	AOAC 1990
Air	Known volume of air drawn through polyurethane foam (1–5 L/minute) followed by extraction with 5% diethyl ether in hexane and extract volume reduction.	GC/ECD (EPA Method TO-10)	No data (depends on volume)	72 (13% RSD) at 0.22 $\mu g/m^3$ and 0.9 m^3 sample volume	EPA 1988d
Air	Known volume of air drawn through SPE disk containing styrene-divinylbenzene copolymer. DDVP recovery via extraction with ethyl acetate.	GC/NPD	No data (depends on volume)	97.8 at 450 ng/m^3 (49.5 ppt, v/v)	Markell et al. 1994
Air	Known volume of air drawn through XAD-2 adsorbent followed by extraction with toluene.	GC/FPD (OSHA Method 62)	0.0019 mg/m^3 (0.21 ppb, v/v) based on 480 L air volume	97.4 (10% RSD) at 0.11 ppm	OSHA 1986
Air (personal air)	Known volume of air drawn through cartridge containing XAD-2 and polyurethane foam followed by extraction with toluene.	GC/NPD or GC/FID	No data	94 at 9.9 and 479 µg and 45% and 95% relative humidity (98% retention of 0.6 µm particles)	Brouwer et al. 1994

Table 6-2. Analytical Methods for Determining Dichlorvos in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air, dust	**Air:** Adsorption of DDVP in 1 m^3 of air onto Tenax TA via an air sampling pump followed by desorption with acetone. **Dust:** Extraction of 1 g homogenized dust (from vacuum cleaner bag) with acetone.	GC/MS	Air: 1 ng/m^3 (0.11 ppt, v/v) Dust: 50 ng/g (50 ppb, w/w)	Air: 94 (10.4% RSD) at 0.1 ppb (v/v). Dust: 111 (3.7% RSD) at 50 ng/g (50 ppb)	Roinestad et al. 1993
Drinking water, groundwater	Adjustment of pH to 7, addition of NaCl and extraction with methylene chloride. Removal of water with sodium sulfate and extract volume reduction.	GC/NPD (ASTM Method D5475)	Approx. 0.5 µg/L (0.5 ppb, w/v)	102 (8.8% RSD) at 1 µ/L	ASTM 1994
Drinking water, groundwater	Adjustment of pH to 7, addition of NaCl and extraction with methylene chloride. Removal of water with sodium sulfate and extract volume reduction.	GC/NPD (EPA Method 507)	2.5 µg/L (2.5 ppb, w/v)	86–100 (15% RSD) at 25 µg/L	EPA 1991b
Groundwater	Addition of sodium chloride to water and extraction with methylene chloride. Water removal using sodium sulfate and solvent exchange to acetone.	GC/MS (SIM)	0.017 µg/L (0.017 ppb, w/v)	99.4 (1.9% RSD) at 0.1 µg/L (0.1 ppb, w/v)	Kadokami et al. 1991
Urban precipitation	Filtration of precipitation and passage through a column packed with XAD-2 resin. Elution from XAD using dichloromethane followed by removal of water from dichloromethane with anhydrous sodium sulfate and volume reduction to 1 mL using Kuderna-Danish concentrator and nitrogen stream. Addition of internal standards just prior to analysis.	GC/MS (SIM)	0.015 µg/L (ppb)	84 (16% RSD) at 0.01 µg/l; 93 (9.1% RSD) at 0.1 µg/L	Haraguchi et al. 1995
Water	Adsorption of DDVP onto graphitized carbon black, elution in reverse direction using methylene chloride/methanol (80:20) and volume reduction.	HPLC/UV	22 ng/L (22 ppt, w/v)	85 (10% RSD) at 1–4 µg/L (ppb, w/v)	Di Corcia et al. 1993
Water	Adsorption of DDVP onto SPE (C_{18}, Florisil) and elution with ethyl acetate.	GC/FPD	No data	98.6 (2.8% RSD) from C_{18} at 0.21 ppm	Wang and Huang 1994

Table 6-2. Analytical Methods for Determining Dichlorvos in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Waste water	Adjustment of pH to 7 and extraction with methylene chloride, water removal, and solvent exchange to hexane.	GC/NPD, GC/FPD, or GC/MS (EPA Method 622)	0.1 µg/L (0.1 ppb, w/v)	72 (7.7% RSD) over concentration range 15–517 µg/L	EPA 1992a
Water, soil, sludges, and waste	Liquid/liquid extraction of water at pH = 7 using methylene chloride. Extraction of solid samples using methylene chloride/acetone (1:1). Column chromatography clean-up if needed.	GC/FPD or GC/NPD (EPA Method 8141A)	8 µg/L (8 ppb, w/v) for water to 40 mg/kg (ppm, w/w) for non- water-miscible waste	Water: 79 (11% RSD) at 14.3 µg/L (14.3 ppb, w/v). Soil: 13 (9% RSD) at 475 µg/kg (0.475 ppb, w/v)	EPA 1992b
Non-fatty foods	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)	Approx. 20 ppb (w/w, µg/kg) depending on analytical system used.	>80	FDA 1994a
Vegetables and fruits; brown rice	**Vegetables and fruits:** Sample blended with acetone, filtered, and blended with a fresh aliquot of acetone; filtrates were combined, subjected to volume reduction, mixed with Celite 545, filtered and rinsed with acetone: water. Cleanup using liquid/liquid partition and volume reduction. **Brown rice:** Ground sample blended with acetonitrile, filtered, and extracted with another aliquot of acetonitrile; extracts combined and extracted with hexane, acetonitrile volume reduced and taken to liquid/liquid partition for cleanup as for vegetables and fruits.	GC/FPD	No data	76–113 (%RSD range 1–10%) at 0.05 ppm.	Nakamura et al. 1994

Table 6-2. Analytical Methods for Determining Dichlorvos in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Produce, wheat, soybeans	**Non-fatty samples (<2% fat):** homogenization with sodium sulfate and ethyl acetate; solvent evaporation, redissolution in acetone. **Fatty samples (>5% fat):** Preparation as for non-fatty samples with the addition of a hexane/ acetonitrile partitioning step.	GC/FPD; GC/MS	No data	61–111 (%RSD 0.1–18%) at 0.5 and 1 ppm	Miyahara et al. 1994
Potatoes	Sample blended and combined with Hydromatrix, addition of internal standards, and SFE.	GC/ITMS	6 ng/g	72 (12% RSD) at 0.5 µg/g (0.5 ppm)	Lehotay and Eller 1995
Cauliflower	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.	GC/NPD (German Pesticide Commission Method S17)	Approx. 0.1 mg/kg (ppm, w/v)	>80	Thier and Zeumer 1987
Various produce	Extraction of finely chopped sample with acetonitrile or ethyl acetate. DDVP extraction into chloroform or clean-up using column chromatography.	GC/FPD	No data	87–94 at 0.5 mg/kg (0.5 ppm, w/v)	Ministry of Agriculture, Fisheries, and Food 1977
Wheat	Homogenization of grain with methanol and clean-up on a charcoal column.	GC/NPD	0.005 ppm	92 (6% RSD) at 0.25 ppm	Crisp and Tarrant 1971
Lettuce	Homogenization of sample and extraction with acetone:dichloromethane:petroleum ether (1:1:1) followed by solvent exchange to acetone.	SFC/NPD	Approx. 25 µg/kg (25 ppb, w/w)	86 at 50–100 µg/kg	Zegers et al. 1994
Various crops	Adsorption of homogenized sample onto Florisil to obtain free-flowing powder. Extraction in glass column with ethyl acetate or methylene chloride:acetone (9:1).	GC/NPD	5 µg/kg (5 ppb, w/w)	84–92 at 0.01–0.5 mg/kg (ppm, w/w)	Kadenczki et al. 1992
Wine	Adsorption onto C_{18} SPE cartridge, washing with 20% ethanol in water and elution with ethyl acetate.	GC/ECD/NPD	0.01 mg/L (0.01 ppm, w/v)	93 (4% RSD) at 0.2 mg/L (0.2 ppm, w/v)	Holland et al. 1994

Table 6-2. Analytical Methods for Determining Dichlorvos in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Various produce	Dispersion of homogenized sample with anhydrous sodium sulfate followed by extraction with ethyl acetate, water removal, and volume reduction.	GC/FPD, GC/MS	Approx. 10 µg/kg (10 ppb, w/w)	83 (6.9% RSD) at 0.2–0.3 mg/kg (ppm, w/w)	Agüera et al. 1993
Milk (whole, skim, chocolate, infant formula)	Sample extracted with 1:4 acetone: acetonitrile followed by centrifugation. Extraction of precipitate twice more with acetone: acetonitrile. Extraction of supernatant from centrifugation combined with extracts followed by back-extraction into dichloromethane. Water removal and evaporation of solvent, redissolution in acetonitrile followed by SPE and solvent exchange to acetone.	GC/FPD	No data	Mean of 54 (18% RSD) at 0.02 ppm.	Erney 1995
Various produce, swine/cattle liver	Maceration of sample with anhydrous sodium sulfate and extraction with ethyl acetate followed by GPC clean-up.	GC/NPD	No data	>90	Roos et al. 1987
Honey	Sample extracted with acetonitrile: water (2:1, v/v), filtered, and extracted with hexane; volume reduction, cleanup using Florisil, evaporation to dryness, and redissolution in hexane.	GC/NPD	0.030 ng/g (ppb)	83 (4.3% RSD) at 1 µg/g (1 ppm)	García et al. 1995
Bovine liver, rumen content	Homogenization with sodium sulfate and extraction with methanol: methylene chloride (1:9), solvent evaporation, redissolution in hexane:ethylacetate (60:40), GPC clean-up.	GC/FPD	0.01–0.05 µg/g (ppm, w/w) using 5 g samples	Rumen content: 81 (2% RSD) at 1 µg/g Liver: 74 (6% RSD) at 0.5 µg/g	Holstege et al. 1991

Table 6-2. Analytical Methods for Determining Dichlorvos in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Pig tissue (brain, liver, muscle, kidney, fat), blood and urine (DDVP, desmethyldichlorvos, dichloroacetaldehyde, dichloroethanol, dichloroacetic acid)	**DDVP:** lean tissue–homogenization/extraction with sodium sulfate and ethyl acetate; water removal from extract; fat–extraction with diethyl ether followed by solvent exchange to hexane-saturated acetonitrile, washing with hexane, and solvent exchange to ethyl acetate. **Blood and urine:** dilution with ethanol and extraction with ethyl acetate followed by water removal using anhydrous sodium sulfate.	GC/NPD (DDVP and methylated desmethyl-dichlorvos); GC/ECD for others	0.05–0.1 ppm (mg/kg, w/w)	Recovery from brain, liver, muscle, kidney 86–100 for all analytes at concentrations greater than 0.2 ppm	Schultz et al. 1971
Pig tissue (brain, liver, muscle, kidney, fat), blood and urine (DDVP, desmethyldichlorvos, dichloroacetaldehyde, dichloroethanol, dichloroacetic acid) (continued)	**Dichloroacetaldehyde:** Tissue and blood–Treatment of macerated sample or blood with sodium sulfate and sulfuric acid followed by extraction with diethyl ether. **Dichloroethanol:** Lean tissue and blood–Hydrolysis with 3 N sulfuric acid followed by protein precipitation, removal, and extraction with ethyl ether:benzene (1:1) and water removal using sodium sulfate. Derivatization with trifluoroacetic anhydride. Fat–Hydrolysis with 3 N sulfuric acid over steam bath and processing as for lean tissue and blood. **Dichloroacetic acid and desmethyl-dichlorvos:** Tissue and liquid samples–Homogenization, hydrolysis, extraction with ethyl ether, drying, formation of methyl esters.		No data		
Milk, eggs, body tissues	**Milk:** dispersion of milk with silicic acid, application to a chromatographic column packed with sodium sulfate and silicic acid, elution with methylene chloride:hexane (3:2), concentration and solvent exchange to hexane.	GC/FPD	Milk: 0.003 ppm;	77–97 at 0.01 ppm for all matrices except liver; poor recovery from liver	Ivey and Claborn 1969

Table 6-2. Analytical Methods for Determining Dichlorvos in Environmental Samples (continued)

Sample matrix[a]	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
	Fat and chicken skin: homogenization with sodium sulfate with heat to liquify fat and extraction with hexane. Back-extraction into acetonitrile and solvent exchange to hexane. Clean-up using silicic acid column chromatography.		Tissues: 0.002 ppm		
Milk, eggs, body tissues (continued)	**Muscle:** homogenization with sodium sulfate, extraction with acetonitrile, and filtration. Extraction of acetonitrile with hexane to remove fat and solvent exchange to hexane. Clean-up using silicic acid column chromatography. **Blood and eggs:** blending of sample with acetonitrile and sodium sulfate, solvent exchange to hexane. Clean-up of egg extract with silicic acid chromatography. Drying and volume reduction of blood hexane extract.				

[a] Unless otherwise indicated, the target analyte was dichlorvos.

ASTM = American Society for Testing and Materials; DDVP = dichlorvos; ECD = electron capture detector; EPA = Environmental Protection Agency; FID = flame ionization detector; FPD = flame photometric detector; GC = gas chromatography; GPC = gel permeation chromatography; HECD = Hall electrolytic conductivity detector; HPLC = high performance liquid chromatography; IR = infrared absorbance spectroscopy; ITMS = ion trap mass spectrometry; MS = mass spectrometry; NPD = nitrogen phosphorus detector; OSHA = Occupational Safety and Health Administration; RSD = relative standard deviation; SFE = supercritical fluid extraction; SFC = supercritical fluid chromatography; SIM = selected ion monitoring; SPE = solid phase extraction; UV = ultraviolet absorbance detection; v/v = volume/volume; w/v = weight/volume; w/w = weight/weight;

The pH of liquid samples is often adjusted to 7 prior to extraction to minimize the hydrolysis of dichlorvos during extraction (ASTM 1994; EPA 1991b, 1992a, 1992b).

The determination of dichlorvos in the resulting extracts is typically by GC with selective detection such as NPD, FPD, or MS, although HPLC with ultraviolet (UV) absorbance detection (DiCorcia et al. 1993) and TLC have also been reported (Askew et al. 1969; Shevchuk et al. 1987). Limits of detection range from low parts per trillion (ppt) (ng/L) for relatively clean water (ground, drinking water) to the mgkg (ppm) range for non-water-miscible wastes.

Electrochemical sensors have been developed and applied in a research setting. Potentiometric sensors (Imato and Ishibashi 1995; Ivnitskii and Rishpon 1994) and amperometric sensors (Hartely and Hart 1994; Martorell et al. 1994) based on immobilized acetyl- or butyrylcholinesterase have been described. Inhibition of the enzyme activity in proportion to the concentration of organophosphorus pesticides, including dichlorvos, in water is the basis of their operations. Sensitivities as low as sub-micromolar concentrations of dichlorvos in water have been demonstrated. However, they are not specific to dichlorvos and have not undergone testing for ruggedness in environmental use.

Significant losses of dichlorvos can occur during preparation of samples for analysis. Some types of boiling chips have been found to facilitate the decomposition of dichlorvos during extract volume reductions (Hsu et al. 1988). The volatility of dichlorvos can also result in losses during volume reductions of extracts (Holland 1977; Lartiges and Garrigues 1993) so care must be taken to avoid taking extracts to dryness during solvent exchange operations. Loss of dichlorvos can also result from improper storage of soil samples (Snyder et al. 1992) prior to extraction; samples should be kept cold and processed as soon as possible. The storage of SPE cartridges onto which dichlorvos has been adsorbed, as after collection of dichlorvos from water, can also result in losses (Lacorte et al. 1995). It is very important that appropriate control samples be used and that the method be validated prior to its use in a critical study.

Dichlorvos can be produced from other chemicals during sample preparation and analysis. Trichlorfon rearranges and is dechlorinated in acidic, neutral, or basic media to form dichlorvos and hydrochloric acid (EPA 1992b). In addition, naled can be converted to dichlorvos during sample workup (EPA 1992b). The analyst must be aware that such transformations can lead to incorrect or misleading results for dichlorvos quantitations. High temperatures in the GC injector or oven can facilitate the

transformation of naled and trichlorfon to dichlorvos (EPA 1992b; Yamashita et al. 1991) and lead to incorrect quantitative results for dichlorvos. The use of cool on-column injection can minimize injector-related transformations of trichlorfon (Yamashita et al. 1991), but transformation on-column can still occur and can be minimized through the use of short, thin-film columns to facilitate elution at lower temperatures (FDA 1994a).

Dichlorvos residues have been determined in a variety of foods including produce, grain, meats, milk, honey, and wine. Dichlorvos is most easily recovered from low-moisture foods, such as grain, by homogenization with an organic solvent (Crisp and Tarrant 1971). For non-fatty, high moisture foods, the sample is typically homogenized in the presence of a salt such as sodium sulfate and an organic solvent (Agiiera et al. 1993; FDA 1994a; Ministry of Agriculture, Fisheries and Food 1977; Roos et al. 1987; Thier and Zeumer 1987; Zegers et al. 1994). The salt serves to absorb water and to favor the partition of dichlorvos into the organic phase. In some cases, homogenized sample is mixed with Florisil, as for produce in Kadenczki et al. (1992), or silicic acid, as for milk in Ivey and Clabom (1969) to obtain a free-flowing powder that is packed into a column and eluted with organic solvent to recover dichlorvos. Column chromatography (gel permeation, adsorption) is often used to remove impurities that could interfere with analysis. For fatty foods such as meats, additional chromatographic or solvent partition clean-up operations are needed to remove the fats that can foul chromatographic systems. Careful use of controls and method validation is indicated because of the sample losses that can occur during clean-up. For example, significant losses of dichlorvos occur during clean-up steps in FDA PAM1 Method 304 for fatty foods (FDA 199413). Emey (1995) hypothesizes that loss of dichlorvos during sample workup of skim milk arises from lack of a fatty “keeper” and subsequent volatilization losses of the analyte. The transformation products of dichlorvos, including desmethyldichlorvos, dichloroacetaldehyde, dichloroethanol, and dichloroacetic acid have also been recovered from pork and other pig tissues and fluids (Schultz et al. 1971). In this case, the chemical derivatives of the transformation products are formed before chromatographic analysis. The same concerns about chemical transformations during sample preparation discussed above also apply to foods.

Dichlorvos is most often determined in the extracts using GC with selective detection, although one method was reported that employed supercritical fluid chromatography (SFC) (Zegers et al. 1994). Limits of detection for dichlorvos in produce were reported to be in the ppm to low-ppb range and those in animal samples were reported to be in the high-ppb to low-ppb range.

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 dichlorvos 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 dichlorvos.

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 to dichlorvos can occur from environmental exposure as a result of contacting air (inhalation), water (ingestion), food (ingestion), or dermal contact. Methods exist for the determination of dichlorvos in blood and tissues; limits of detection in plasma have been reported to be 40 ppb with 8% relative standard deviation (RSD) (Unni et al. 1992) and 2 ppb (Kawasaki et al. 1992). Their utility is limited, however, because of the short half-life of dichlorvos in these media (Blair et al. 1975; Unni et al. 1992). Dimethyl phosphate is more persistent in urine (Das et al. 1983; Takamiya 1994), but is not specific to dichlorvos; any organophosphate that contains a dimethyl phosphate moiety will produce dimethyl phosphate upon hydrolysis. Dimethyl phosphate can be detected in urine down to concentrations of 0.02 ppm, although a high (149%) recovery was reported at a concentration of 0.50 ppm (Takamiya 1994). The bias at the limit of detection is unclear. Additional information would make the method more meaningful. Additional transformation products include dichloroacetaldehyde, dichlorethanol, and dichloracetic acid (Schultz et al. 1971), which are specific to dichlorvos exposure. Dichloroethanol has been detected in human urine after treatment with more glucuronidase followed by GC (Hutson and Hoadley 1972b). However, an endogenous interferant resulted in a large measurement error and limited application of the method to high exposures. Additional method work

could improve that situation. Exposures to naled and trichlorphon, two organophosphates that are converted in the body to dichlorvos, would also have to be ruled out before a definitive determination of dichlorvos exposure could be made.

The most sensitive biomarker for exposure to dichlorvos is inhibition of the activity of serum cholinesterase. This enzyme is related to the target for dichlorvos toxicity, neural acetylcholinesterase. Neural and erythrocyte acetylcholinesterase are produced by the same gene and a reasonable correlation exists in animal studies between neural and erythrocyte acetylcholinesterase inhibition resulting from organophosphate exposure. Volunteers who consumed 0.033 mg/kg/day dichlorvos over a 21-day period demonstrated a 30% inhibition of serum cholinesterase activity without effect on erythrocyte acetylcholinesterase activity or clinical signs of organophosphate neurotoxicity (Boyer et al. 1977).

Serum cholinesterase and erythrocyte acetylcholinesterase are measured by the spectrophotometric method of Ellman (Ellman et al. 1961). Blood samples are centrifuged and aliquots of serum are used for assay. Erythrocyte acetylcholinesterase can be determined by lysing the pellet in hypotonic solution and washing the erythrocyte membranes where the enzyme is located. Samples are added to buffer containing the substrate acetylthiocholine and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). The product of the reaction, thiocholine, reacts with the DTNB to give a yellow product which is measured spectrophotometrically. Kits are available from a number of manufacturers to perform these assays using autoanalyzer equipment.

Normal ranges for enzyme activity have been established. Dichlorvos inhibits serum cholinesterase at levels that have no effect on erythrocyte acetylcholinesterase in most species, including humans (Hayes 1982). Given the variability in normal activities in humans (especially for serum cholinesterase), confirmation of exposure is best established by repeated determinations demonstrating an increase in activity to a constant level after exposure has ceased. Information on cholinesterase activities can be combined with the measurement of other transformation products to increase the certainty that exposure to dichlorvos has occurred.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. If an MRL of 0.00006 ppm (0.0005 mg/m^3) for chronic inhalation exposure is assumed, an analytical method for dichlorvos in air must have a limit of detection of

0.00006 ppm (0.0005 mg/m^3) or less. Limits of detection for dichlorvos in air of 1 ng/m^3 (0.11 ppt) (Roinestad et al. 1993) and 0.0019 mg/m^3 (OSHA 1986) have been reported. The Roinestad method is sufficiently sensitive to permit determination of potential inhalation exposures below the MRL. No new methods are needed.

An oral MRL for chronic exposure to dichlorvos has been established as 0.0005 mg/kg/day which corresponds to a dose of 0.035 mg/day for a 70-kg person. If a 2 L/day consumption of water is assumed, this corresponds to a required limit of detection (LOD) of 0.0175 mg/L (0.0175 ppm, weight per volume [w/v]) for drinking water. Most of the methods for drinking water report LODs in the low-ppb range (ASTM 1994; DiCorcia et al. 1993; EPA 1991b) and are adequate. No new methods for drinking water are needed. If a 2 kg/day consumption of food is assumed, this corresponds to a needed LOD in food of 0.0175 mg/kg (0.0175 ppm, weight per weight [w/w]). The FDA method for dichlorvos in non-fatty foods (FDA 1994a) has an LOD of 20 ppb and is just outside of the adequate range. The methods of Crisp and Tarrant (1971) for wheat (LOD=0.005 ppm) and Kadenczki et al. (1992) for dichlorvos on various crops (LOD=5 ppb) are adequate. However, methods for dichlorvos in fatty foods are more problematic. The method of Schultz et al. (1971) for dichlorvos in pig tissue has an LOD of 0.05-0.1 ppm and is not adequate. The FDA method for fatty foods requires additional column chromatography clean-up and is not satisfactory for dichlorvos (FDA 1994b). Thus, additional methods for fatty foods are needed. Virtually no information was found on methods for environmental transformation products. Methods are needed for these compounds in foods.

6.3.2 Ongoing Studies

No ongoing research was found in which new methods for detecting dichlorvos are being developed.