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

Few methods are available for the determination of chlorfenvinphos in biological samples. Some methods which may be applicable to biological media are summarized in Table 6-1. Most methods involve an extraction step followed by one or more purification and fractionation procedures, then analysis, usually by gas chromatography (GC). Two detection methods are commonly used, nitrogen-phosphorus detection (NPD) (Thier and Zeumer 1987; Wagner et al. 1990) and flame photometric detection (FPD) (Ivey et al. 1973). Both of these methods are specific for phosphorus-containing compounds and are very sensitive (low- to sub-ppb levels). Recovery, where reported, is very good (>80%).

Several cautions should be noted. First, the stability of chlorfenvinphos in biological media is unknown. The cold-storage stability (5 to –20 EC) of chlorfenvinphos in crops and soil has been reported (Kawar et al. 1973). However, enzymes present in biological media may reduce levels of organophosphate pesticides (Singh et al. 1986). Second, it is difficult to eliminate or reduce interfering compounds and maintain acceptable recovery of chlorfenvinphos. Quality control procedures are recommended to assure that the method performance is acceptable. Third, other organophosphorus pesticides may co-elute with chlorfenvinphos (Sasaki et al. 1987), so a confirmatory method is recommended A few methods are available for measuring metabolites of chlorfenvinphos. Chlorfenvinphos undergoes biotransformation to a variety of polar metabolites including diethyl phosphate. Methods for measurement of dialkyl phosphates involve extraction from urine using an ion exchange resin and derivatization prior to GC analysis (Bradway et al. 1981; Lores and Bradway 1977). The

Table 6-1. Analytical Methods for Determining Chlorfenvinphos in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human milk, cervical fluid, sperm fluid	Solvent extraction; clean-up on Florisil	GC/NPD	0.040 µg/kg	No data	Wagner et al. 1990; Thier and Zeumer 1987
Rat liver, muscle, whole blood	Solvent extration; clean-up	GC/thermionic detection	0.10–0.02 ppm	92 (muscle, liver)	Hladká et al. 1975
Rat liver, blood	Solvent extraction	TLC/enzyme-inhibition detection	sub-nanogram levels	94–96 (blood); 84–89 (liver)	Vitorović 1982
Cattle and chicken fat, skin, muscle, liver, heart, kidney, feces	Fat, skin: isolation by filtration through celite; solvent partition Tissues, feces: solvent extraction; solvent partition All: clean-up on sodium sulfate/silicic acid column	GC/FPD	0.001 ppm	83–100	Ivey et al. 1973

GC = gas chromatography; FPD = flame photometric detection; NPD = nitrogen/phosphorus detection; TLC = thin layer chromatography

performance of the methods is variable; extraction is not always complete, and GC interferences often present problems.

6.2 ENVIRONMENTAL SAMPLES

Representative analytical methods for determining chlorfenvinphos in environmental samples are summarized in Table 6-2. Methods involve solvent extraction, purification and fractionation, and gas chromatographic analysis. Although most methods for measuring chlorfenvinphos in environmental samples involve GC coupled with specific detectors (including MS), other methods are available, including high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection (Bagon and Warwick 1982; Schlett 1991), and thin layer chromatography (TLC) (Roberts and Stoydin 1976).

Methods for determining chlorfenvinphos in aqueous samples include solvent extraction (EPA 1992b; Wan et al. 1994) or isolation using solid phase extraction (SPE) (Schlett 1991). Further clean-up of the extract may not be required prior to analysis by HPLC (Schlett 1991) or GC (EPA 1992b; Wan et al. 1994). For GC analysis, confirmation using a second column is recommended (EPA 1992b; Wan et al. 1994). Detection limits are in the sub-ppb range; recovery was not reported.

Similarly, methods for determining chlorfenvinphos in sediments, solid wastes, and soils use a solvent extraction procedure. A variety of clean-up procedures are used, including Florisil column purification (Beynon et al. 1966), solvent partition (Miles et al. 1979; Williams 1975b), and gel permeation chromatography (Wan et al. 1994). Extracts are analyzed by GC with electron capture detection (ECD) (Beynon et al. 1966; Edwards et al. 1968) or phosphorus-specific detectors (Wan et al. 1994; Williams 1975b). Detection limits are in the low-ppb range (1–20); recovery is excellent ($95%).

A chemiluminscence assay has been developed that should be a useful screening tool for environmental media. It is not as sensitive as GC methods, but is inexpensive, fast, and may be used as a portable detection system (Moris et al. 1995). Capillary electrophoresis is a relatively new technique for environmental analysis. It is rapid, but does not yet achieve detection limits needed for environmental analysis (Süsse and Müller 1995).

Methods for determining chlorfenvinphos on a variety of foods and crops have been reported. Most involve solvent extraction followed by clean-up using adsorption column techniques (FDA 1979; Kadenczki et al.

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Workplace air	Collection on filters/Tenax traps, desorption	HPLC/UV	2 µg/m^3 [a]	No data[b]	Bagon and Warwick 1982
Drinking and surface water	Filtration; solid-phase extraction (SPE)	HPLC/UV	≈0.025 µg/L	No data	Schlett 1991
Drinking and surface water	On-line enrichment (SPE)	Thermospray LC/MS	15 ng/L	52	Sennert et al. 1995
Waste water (EPA Method 1657)	Solvent extraction; optional clean-up using GPC and/or SPE	Capillary GC/FPD; confirmation on second GC column	2 ng/L	No data	EPA 1992b
Water and sediments	Water: solvent extraction Sediments: solvent extraction; clean-up by GPC	Dual column/dual detector: capillary GC/NPD, FPD	0.01 µg/L (water); 1 µg/kg (soil, sediment)	99 (water); 97 (soil); 96 (sediment)	Wan et al. 1994
Solid wastes (EPA Method 8141A)	Solvent extraction; optional cleanup using Florisil column or GPC	Capillary GC/NPD	No data	No data	(SW-846) EPA 1992a
Soil	Samples are tumbled with solvent; optional clean-up on Florisil column	GC/ECD	0.01 ppm	95	Beynon et al. 1966
Soil	Ultrasonic extraction; solvent partition	GC	No data	95 ave.	Miles et al. 1979
Soil	Solvent extraction	GC/ECD	<0.02 ppm	95–115	Edwards et al. 1968
Pesticide formulations	Solvent extraction	GC/FID	Not applicable	92–97 for mg quantities	Paterson 1970

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Animal tissue, milk	Solvent extraction; clean-up on Florisil column	GC/thermoionic detection	0.001 ppm (milk); 0.005 ppm (tissue)	73–87 (beef fat); 84–105 (milk)	FDA 1979
Fruits and vegetables	Solvent extraction	Capillary GC/FPD; confirmation by capillary GC/MS	10 µg/kg	90.5	Agüera et al. 1993
Vegetables	Solvent extraction and partition	GC	0.02 mg/kg	85–97 ave.	Frank et al. 1990
Foods	Solvent extraction; column clean-up	capillary GC/FPD; confirmation by GC/MS or GC/FPD	≈9 ppb (trans isomer), ≈7 ppb (cis isomer)	89–97 (trans isomer); 72–101 (cis isomer)	Leoni et al. 1992
Fruits and vegetables	Prepared food is adsorbed onto Florisil, extracted with solvent	Capillary GC/PFD or NPD	0.01 mg/kg	80–89	Kadenczki et al. 1992
Fruit (apples)	Soxhlet extraction	Capillary GC/NPD	0.099 µg/mL	98	Barrio et al. 1994
Crops (lipid-containing)	Solvent extraction; cleanup by solvent partitioning	Capillary GC/FPD	1 ppb	79	Nakamura et al. 1994
Milk	Solvent extraction; clean-up by solvent partitioning	GC/FPD or thermoionic detector; confirmation by TLC	≥0.01 mg/kg	81–96	Stijve 1984
Pharmaceuticals	Disolution in solvent, filtration; GPC clean-up	Capillary GC/ELCD; confirmation by second column or MS	<0.05 mg/kg	97–110 (all pesticides)	Heikes and Craun 1992

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Cosmetics	Solvent extraction utilizing adsorption columns; GPC clean-up	GC/FPD	≤0.2 mg/kg	98-108	Specht 1990

[a] Based on instrumental detection limits; value will vary with volume of air sampled.
[b] Desorption efficiency from Tenax is 100%.

GC = gas chromatography; ELCD = electrolytic conductivity detection; FID = flame ionization detection; FPD = flame photometric detection; GPC = gel permeation chromatography; HPLC = high performance liquid chromatography; LC = liquid chromatography; MS = mass spectrometry; NPD = nitrogen/phosphorous detection; SPE = solid-phase extraction; TLC = thin layer chromatography; UV = visible/ultraviolet detection

1992; Leoni et al. 1992) or solvent partitioning (Frank et al. 1990; Stijve 1984). Chlorfenvinphos is determined by GC with phosphorus-specific detectors (flame photometric [FPD]; and nitrogen-phosphorus [NPD]) (Agüera et al. 1993; Leoni et al. 1992; Kadenczki et al. 1992; Stijve 1984). Chlorfenvinphos in sample extracts is confirmed using GC with MS (Agüera et al. 1993; Leoni et al. 1992) or TLC (Stijve 1984). Detection limits are in the low-ppb range; recovery is acceptable (>80%).

A summary of methods for determination of chlorfenvinphos environmental degradation products is shown in Table 6-3. The breakdown products 2,4-dichloroacetophenone, 1-(2,4-dichlorophenyl)-1-ethan-1-ol, and 2,4-dichlorophenacyl chloride can be determined in soil and earthworms using solvent extraction and GC/ECD (Edwards et al. 1968). Detection limits are approximately 0.05 ppm and recovery is excellent (95–115%). The hydrolysis product 2,2',4'-trichloroacetophenone has been determined in corn extracts using GC/ECD and GC/FPD. Detection limits are 0.02 ppm (ECD) and 0.002 (FPD), and recovery is excellent (Beroza and Bowman 1966). Free and conjugated degradation products have been determined in soils and crops by GC/ECD (Beynon et al. 1968). Free products are extracted with solvent; conjugated products are hydrolyzed with sulfuric acid. Recovery is acceptable (80–100% for soils, 50–90% for crops); detection limits range from 0.01 to 0.2 ppm for soils (varies with compound) and from 0.005 to 0.05 ppm for crops. Degradation products, including soil-bound (polar) compounds, were determined by GC/ECD of soil extracts. The polar compounds were methylated prior to GC analysis. Recovery is acceptable (65–105%); detection limits are approximately 0.02 ppm.

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 chlorfenvinphos 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 chlorfenvinphos.

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

Table 6-3. Analytical Methods for Determining Biomarkers for Chlorfenvinphos

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Acetylcholinesterase in erythrocytes			No data	No data	Zwiener and Ginsburg 1988
Free tryptophan in plasma		Spectrophotometry	No data	No data	Dudka and Szczepaniak 1993
Urine levels of desethyl chlorfenvinphos metabolite	Solvent extraction; methyation	GC/FPD	0.002 ppm	97%	Hunter et al. 1972
Blood levels of chlorfenvinphos	Headspace	GC/FID;GC/ thermionic detection	No data	No data	Klys 1985

GC = gas chromatography; FID = flame ionization detection

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. Few methods are available for measuring exposure to chlorfenvinphos. These are summarized in Table 6-4. Blood levels of chlorfenvinphos were determined in a case of human poisoning (Klys 1985); however, sensitivity and reliability were not reported. Chlorfenvinphos is metabolized in the body to esters of phosphoric acid, and methods are available for determining urine levels of these metabolites (Bradway et al. 1981; Lores and Bradway 1977). However, these phosphate compounds are not specific for chlorfenvinphos, but are common to all organophosphate pesticides. Decreased levels of acetylcholinesterase in plasma or erythrocytes have been reported to be indicative of chlorfenvinphos poisoning (Zwiener and Ginsberg 1988). Again, this assay is not specific for chlorfenvinphos. An increase in the level of tryptophan after exposure to chlorfenvinphos has been reported in rats (Dudka and Szczepaniak 1993). Good precision and accuracy were reported for the method; however, specific values were not given. It is not known if the available analytical methods will be sensitive enough to measure chlorfenvinphos levels in body tissues and fluids of the background population. Since chlorfenvinphos is apparently not produced or imported into this country, it should not be necessary to determine these background levels. It would be helpful to have methods which would permit assessment of the severity of exposure of a highly exposed population.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods are available for determining chlorfenvinphos in water, soils and sediments, and foods (Table 6-2). A summary of available methods for determining the environmental degradation products of chlorfenvinphos in soils (Beynon et al. 1968; Rouchaud et al. 1988), crops (Beynon et al. 1968; Beroza and Bowman 1966), and worms (Edwards et al. 1968) is shown in Table 6-3. Sensitive methods (sub-ppb levels) are available for determining chlorfenvinphos in water; however, better information is needed regarding the recovery and precision of the methods (EPA 1992b; Schlett 1991; Wan et al. 1994). Methods for determining chlorfenvinphos in soils and sediments are sensitive (low ppb levels) and good recovery ($95%) has been reported (Beynon et al. 1966; Edwards et al. 1968; Wan et al. 1994). Methods for measuring chlorfenvinphos in some foods are sensitive (low ppb levels) with acceptable recovery (72–105%) (Agüera et al. 1993; FDA 1979; Frank et al. 1990; Kadenczki et al. 1992; Leoni et al. 1992; Stijve 1984). Available methods have

Table 6-4. Analytical Methods for Determining Environmental Degradation Products of Chlorfenvinphos

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil, worms	Solvent extraction	GC/ECD	≈0.05 ppm	No data	Edwards et al. 1968
Soil	Solvent extraction; clean-up on alumina	GC/ECD	0.01–0.2 ppm	80–100	Beynon et al. 1968
Crops	Maceration with solvent; clean-up on alumina. Hydrolysis of conjugates are hydrolyzed with sulfuric acid/heat; solvent extraction; clean-up on alumina	GC/ECD	0.005–0.15 ppm	50–90	Beynon et al. 1968
Soil	Solvent extraction; solvent partition; clean-up by TLC. Soil bound compounds: extraction at basic pH; pH adjustment and solvent extraction; clean-up by TLC; methylation	GC/ECD; confirmation by GC/MS	≈0.02 mg/kg	65–105	Rouchaud et al. 1988
Corn	Sample is blended with solvent	GC/ECD; GC/FPD	0.02 ppm (ECD); 0.002 ppm (FPD)	94–101	Beroza and Bowman 1966

GC = gas chromatography; ECD = electron capture detection; FPD = flame photometric detection; MS = mass spectrometry; TLC = thin layer chromatography

sufficient sensitivity for measuring chlorfenvinphos in water, soils and sediments, and foods at background levels. Information on the precision of these methods would be helpful. No methods are available for measuring chlorfenvinphos in ambient air. Given its low volatility, chlorfenvinphos is not likely to be detected in ambient air. Methods are available for monitoring occupational exposure to chlorfenvinphos (Bagon and Warwick 1982). Research investigating the relationship between levels of chlorfenvinphos measured in water, soils and sediments, and food and health effects would be helpful.

6.3.2 Ongoing Studies

No ongoing studies involving chlorfenvinphos were located.