7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring pyrethrins and pyrethroids, its metabolites, and other biomarkers of exposure and effect to pyrethrins and pyrethroids. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

7.1 BIOLOGICAL MATERIALS

Exposure to pyrethrins and pyrethroids is most commonly evaluated by the analysis of urine and blood using gas chromatography (GC) combined with electron capture detection (ECD), flame ionization detection (FID), or mass spectrometry (MS) and high performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detector. Recovery is generally high, and sensitivity is in the parts per billion (ppb) range.

A simple and rapid method for the isolation of synthetic pyrethroids using a solid phase extraction method is described by Junting and Chichang (1991). A similar method that employs HPLC for analysis was used to quantify pyrethrins in plasma by Wintersteiger et al. (1994). This method eliminates time-consuming repeated extractions with organic solvents and centrifugations without losing the efficiency of recovery.

Pyrethrins and pyrethroids are extensively metabolized by the cleavage of the ester linkage, oxidation, and conjugation. Metabolites formed are less lipophilic, and are rapidly and easily excreted in the urine. Exposure to pyrethrins and pyrethroids can be monitored by the detection of these excreted metabolites. Pyrethroids such as cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, fenvalerate, phenothrin, and permethrin can be metabolized resulting in the formation of halosubstituted chrysanthemic and

3-phenoxybenzoic acids (Angrer and Ritter 1997; Yao et al. 1992). The rapid metabolism of the pyrethroids makes it useful to monitor for pyrethroid metabolites in the urine of exposed individuals rather than monitoring the actual concentration of the pyrethroid itself in blood (Leng et al. 1999a). Also, it has been demonstrated that storing frozen urine samples for up to a year results in no further degradation of the metabolites (Leng et al. 1999a). In contrast, pyrethroids stored in plasma are susceptible to continued degradation (Leng et al. 1999a).

Methods for analyzing pyrethrins and pyrethroids as well as the metabolites in biological samples are shown in Table 7-1.

7.2 ENVIRONMENTAL SAMPLES

Concerns about contamination of environmental media, plants, and animals with pyrethrins have led to the need for more rapid, sensitive, and selective methods of analysis. As with biological samples, the most common methods of analysis are GC combined with ECD, FID, or flame photometric detection (FPD) and HPLC coupled with UV detector. Thermal conductivity detectors, thermionic detectors, and nitrogen phosphorus detectors have also been used in conjunction with GC.

Pyrethrins and pyrethroids are nonpolar compounds and nonsystemic in plants; thus, the extraction procedures in environmental samples are simpler than those used for organophosphate and carbamate insecticides. Generally, the samples are homogenized with a nonpolar solvent, such as hexane or benzene, or a binary solvent mixture, such as hexane-acetone, hexane-isopropanol, or light petroleum-diethyl ether. The pyrethrins and pyrethroids, along with a wide variety of other lipophilic substances are co-extracted during this procedure. The resulting solution is dried with anhydrous sodium sulfate. If there are too many co-extracted compounds, separation by liquid-liquid partition or column chromatography is performed. Samples with a low water content, such as tea, tobacco, and straw, are usually extracted with a binary solvent mixture, as are moist vegetables and fruits. Soil samples are first ground and filtered to remove large particles and stones, and then are extracted with acetone-hexane, methanol, acetone, or acetonitrile. Pyrethrins and pyrethroids are extracted from water samples with hexane, methylene chloride, or acetonitrile with subsequent drying with anhydrous sodium sulfate.

Table 7-1. Analytical Methods for Determining Pyrethrins and Pyrethroids in Biological Materials

Sample matrix	Analyte	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood, milk	Deltamethrin, cyhalothrin cypermethrin, flumethrin	Acidify with 1 N HCl; extract twice with CH_3CN and filter; extract filtrate with hexane and discard the hexane phase; remove CH_3CN under a stream of N_2 and heat to dryness; cleanup on a silica gel column; dissolve in CH_3CN and filter through a 0.45 µm pore cellulose filter	LC/UV	1.0 µg/kg	78–91	Bissacot and Vassilieff 1997a, 1997b
Plasma	Pyrethrins	Dilute centrifuged plasma with water; cleanup on a solid phase extraction column; elute with methanol	RPHPLC/UV	0.167 mg/L	70–72	Wintersteiger et al. 1994
Plasma	Cyfluthrin, cypermethrin, permethrin	Precipitation of proteins followed by liquid-liquid extraction	GC/ECD	5 µg/L	No data	Leng et al. 1999a
Plasma, urine	Fenopropathrin, permethrin, cypermethrin, fenvalerate, deltamethrin	Mix samples with 70% methanol; pour on to Sep-Pak C_{18} columns pretreated with chloroform, methanol, methanol/water, and water; wash with water; elute with chloroform; evaporate to dryness under stream of N_2; redissolve in ethanol	GC/FID	2 mg/L	81–93 90–102	Junting and Chuichang 1991
Urine	Cis- and trans-3-(2,2-dichloro-vinyl)-2,2-dimethyl-cycloprane-1-carboxylic acid; 3-phenoxyben-zoic acid; fluoro-phenoxybenzoic acid	Liquid-liquid extraction followed by methylation of the free acid metabolites	GC/MS	0.5 µg/L	No data	Leng et al. 1999b

Table 7-1. Analytical Methods for Determining Pyrethrins and Pyrethroids in Biological Materials

Sample matrix	Analyte	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Cis- and trans-3-(2,2-dichloro-vinyl)-2,2-dimethyl-cycloprane-1-carboxylic acid; cis-3-(2,2-dibro-movinyl)-2,2-dimethylcy-cloprane-1-car-boxylic acid; 3-phenoxy-benzoic acid; 4-fluoro-3-phenoxy-ben-zoic acid	Acidify with AcOH; add concentrated H_2SO_4; heat at 90 °C for 1 hour; cleanup on C_{18} column; elute with methanol into a vial containing concen-trated H_2SO_4; complete derivati-zation on water bath for 1 hour; extract with hexane	GC/MS	0.3–0.5 µg/L	90–98	Angerer and Ritter 1997
Urine	Dibromovinyl-dimethylcyclo-propane car-boxylic acid, 3-phenoxy-benzyl-hydroxy-ethyl acetate, 3-phenoxyben-zoic acid	Adjust pH of sample to 6.5; extract with hexane; evaporate to dryness; redissolve in methanol	RPHPLC/UV	No data	95	Yao et al. 1992
Urine	Deltamethrin, fenvalerate	Extract with hexane; concentrate and cleanup on Florisil column; elute with benzene; dry on anhydrous Na_2SO_4	GC/ECD	0.2 µg/L	92–95.3	Yi-Qun et al. 1994

AcOH = acetic acid; CH_3CN = acetonitrile; ECD = electron capture detector; FID = flame ionization detector; GC = gas chromatography; HCl = hydrochloric acid; H_2SO_4 = sulfuric acid; LC = liquid chromatography; MS = mass spectrometry; N_2 = nitrogen; Na_2SO_4 = sodium sulfate; RPHPLC = reverse phase high performance liquid chromatography; UV = ultra-violet detection

In the analysis of pyrethrins, the total residues of the six active compounds are often analyzed for, but in the analysis of pyrethroids, the individual compounds are usually quantified (Chen and Wang 1996). An extensive review of the chromatographic methods employed for the determination of pyrethrins and pyrethroids in foods, crops, and environmental media has been published (Chen and Wang 1996). Many pyrethroids such as bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, fenvalerate, and permethrin possess one or more halogenated atoms which are sensitive to ECD. Often, derivitization is used to create a sensitive group for pyrethroids that do not possess halogenated atoms (allethrin, resmethrin, phenothrin, and tetramethrin, for example), or to improve the sensitivity and peak tailing situations in some halogenated pyrethroids (Chen and Wang 1996). Consequently, GC/ECD is the most popular analytical approach for analyzing pyrethroids in environmental samples.

A detection limit of 0.1 μg/m^3 and a mean recovery of 100.15% has been reported for the analysis of cypermethrin in air using GC/ECD (Pomorska 1999), while permethrin and resmethrin had detection limits in the ng/m^3 and very good recoveries with a GC/MS method (Roinestad et al. 1993). This is comparable to methods used for the determination of other pyrethroids in air using GC (EMMI 1997). The analysis of pyrethrins and pyrethroids in water is also accomplished through the use of GC and HPLC. Detection limits in the ppb (μg/L) range have been achieved (EMMI 1997). A method for the analysis of selected pyrethroids in soils has been described by Alawi et al. (1990) that utilizes GC equipped with a nitrogen phosphorus detector (NPD).

Methods for analyzing pyrethrins in environmental samples are shown in Table 7-2.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of pyrethrins and pyrethroids are available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (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 pyrethrins.

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

Table 7-2. Analytical Methods for Determining Pyrethrins and Pyrethroids in Environmental Samples

Sample matrix	Analyte	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Allethrin, fenvalerate, pyrethrin I, resmethrin	Air samples collected on a sorbent cartridge; extract with 5% diethyl ether in hexane	GC/ECD; GC/NPD; GC/FPD; HPLC/UV	0.1–50 µg/m^3	No data	ASTM D4861 EMMI 1997
Air	Cypermethrin	Air samples collected on a sorbent cartridge; extract with acetone	GC/ECD	0.1 µg/m^3	100.15 (mean recovery)	Pomorska 1999
Air	Resmethrin, permethrin	Air samples collected on filter paper or Tenax tubes; extract with acetone	GC/MS	1 ng/m^3 (permethrin); 10 ng/m^3 (resmethrin)	109.5–110.9 (permethrin); 84.6 (resmethrin)	Roinestad et al. 1993
Air (dust)	Resmethrin, permethrin	Dust samples are homogenized in a blender or food processor; extract with acetone	GC/MS	50 ng/g (permethrin); 100 ng/g (resmethrin)	94.8–124.4 (permethrin); 82.6 (resmethrin)	Roinestad et al. 1993
Air	Pyrethrums	Air samples collected with sampling pump equipped with filter; extract with CH_3CN	HPLC/UV	0.020 mg/m^3	No data	NIOSH 5008 EMMI 1997
Fats, oils, milk, cheese, fish	Allethrin, bifenthrin, deltamethrin, esfenvalerate, fenvalerate, permethrin, tetramethrin, tralomethrin	Dissolve fat in petroleum ether; extract with CH_3CN; dilute with water; clean up on Florisil column; extract with petroleum ether/ethyl ether	GC/ECD	No data	No data	FDA 211.1; FDA 231.1 EMMI 1997
Fatty and non fatty foods	Allethrin, bifenthrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, fenvalerate, fluvalinate, permethrin, tetramethrin, tralomethrin	Dissolve fat in petroleum ether; extract with CH_3CN; dilute with water; cleanup on Florisil column; elute with a series of eluants—methylene chloride, hexane, and CH_3CN	GLC/ECD	No data	No data	FDA 252 EMMI 1997

Table 7-2. Analytical Methods for Determining Pyrethrins and Pyrethroids in Environmental Samples

Sample matrix	Analyte	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Non-fatty foods	Allethrin, bifenthrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, fenvalerate, fluvalinate, permethrin, tetramethrin, tralomethrin	Extract with CH_3CN or CH_3CN/water mixture; dilute with water; extract with petroleum ether; cleanup on Florisil column; elute with petroleum ether/ethyl ether	GC/FPD	No data	No data	FDA 232.1 EMMI 1997
Non-fatty food	Bifenthrin, cyfluthrin, deltamethrin, esfenvalerate, fenvalerate, flucythrinate, fluvalinate, permethrin	Blend with acetone and filter; extract with petroleum ether and methylene chloride; concen-trate to remove methylene chloride	GC/ECD; GC/FPD	No data	No data	FDA 232.4 EMMI 1997
Non-fatty food	Deltamethrin, fenpropathrin, tralomethrin	Blend with acetone and filter; extract with methylene chloride; cleanup on a column containing charcoal, MgO, and Celite 545; elute with CH_3CN-benzene	GC/FPD; GC/TSD	No data	No data	FDA 232.3 EMMI 1997
Fruits, vegetables, grains	Bifenthrin, cyhalothrin, cypermethrin, deltamethrin, fenpropathrin, fenvalerate, fluvalinate, permethrin	Homogenize in CH_3CN and filter; extract with hexane; wash with 4% NaCl; dry over anhydrous Na_2SO_4; evaporate to dryness; redis-solve in hexane; extract with CH_3CN; evaporate to dryness; redissolve in hexane; cleanup on Florisil column; elute with a mixture of petroleum ether and ethyl ether.	GC/ECD	No data	>70	Pang et al. 1997

Table 7-2. Analytical Methods for Determining Pyrethrins and Pyrethroids in Environmental Samples

Sample matrix	Analyte	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Ground-water, drinking water	Permethrin	Extract with methylene chloride; dry over anhydrous Na_2SO_4; concen-trate; add methyl tert-butyl ether	GC/ECD	0.5 μg/L	No data	EMSLC 508 EMMI 1997
Waste water, municipal and industrial	Allethrin, cyfluthrin, fenvalerate, phenothrin, pyrethrins I, pyrethrins II, resmethrin, tetramethrin	Saturate sample with NaCl; extract with CH_3CN; concentrate extract	HPLC/UV	2 μg/L	No data	EAD 1660 EMMI 1997
Waste water, industrial	Resmethrin	Extract with methylene chloride; dry over anhydrous Na_2SO_4; concentrate	GC/FID	36 μg/L	No data	EMSLC 616 EMMI 1997
Soil	Cypermethrin, permethrin, cyfluthrin, fluvalinate, deltamethrin	Extract with acetone saturated with sodium chloride; dry with Na_2SO_4	GC/NPD	0.004–0.012 mg/kg	114	Alawi et al. 1990
Pet shampoo	Pyrethrins I, pyrethrins II, tetramethrin	Dilute with water and add Celite 545; add to a Celite column; elute with petroleum ether; filter through 0.5 μm filter	HPLC/UV GC/FID	No data	No data	EPA-B EMMI 1997

Table 7-2. Analytical Methods for Determining Pyrethrins and Pyrethroids in Environmental Samples

Sample matrix	Analyte	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Pesticide formulation	Pyrethrins I, pyrethrins II	Extract with petroleum ether; filter; evaporate the filtrate to <1 mL; add 0.5 N alcoholic NaOH and reflux gently; concentrate; add Filter-Cel and 10% $BaCl_2$ filter; neutralize with H_2SO_4 using phenolphthalein; extract with petroleum ether; extract with 0.1 N NaOH; add Deniges reagent and let stand in darkness; add alcohol and precipitate HgCl with NaCl solution; filter; add dilute HCl; add chloroform and ICl solution	Titration	No data	No data	AOAC 936.05 EMMI 1997
Pesticide formulation	Permethrin	Dissolve in methyl isobutyl ketone	GC/FID	No data	No data	AOAC 986.03 EMMI 1997
Pesticide formulation	Deltamethrin	Dissolve (and sonicate) in isooctane-1,4-dioxane; filter	HPLC/UV	No data	No data	AOAC 991.03 EMMI 1997
Pesticide formulation	Allethrin	Add ethylenediamine; swirl; let stand; wash with pyridine; add thymophthalein indicator; titrate with 0.1 N NaOMe	Titration	No data	No data	AOAC 953.05 EMMI 1997
Pesticide formulation	Pyrethrums	Dilute with acetone; add dicyclohexyl phthalate (internal standard)	GC/FID	No data	No data	AOAC 982.02 EMMI 1997

Table 7-2. Analytical Methods for Determining Pyrethrins and Pyrethroids in Environmental Samples

Sample matrix	Analyte	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Pesticide formulation	Pyrethrins I, pyrethrins II	Add sample to a column packed, bottom to top, with anhydrous Na_2SO_4, Florisil, and anhydrous Na_2SO_4; wash with hexane; elute with acetone; evaporate to dryness; dissolve residue in carbon disulfide; dry over anhydrous Na_2SO_4	GC/FID	No data	No data	EPA-B EMMI 1997
Aerosol formulation	Resmethrin	Cool can in freezer overnight; punch holes in can to relieve pressure; open can and warm to room temperature; remove remaining volatiles by placing in a water bath; dissolve residue in benzene	GC/TCD	No data	No data	PMD-Res EMMI 1997
Aerosol formulation	Resmethrin	Cool can in freezer overnight; punch holes in can to relieve pressure; open can and warm to room temperature; remove remaining volatiles by placing in a water bath; dissolve residue in methanol	RPHPLC/ UV	No data	No data	PMD-Res EPA-B EMMI 1997

BaCl = barium chloride; CH_3CN = acetonitrile; ECD = electron capture detector; FID = flame ionization detector; FPD = flame photometric detector; GC = gas chromatography; HCl = hydrochloric acid; HgCl = mercuric chloride; HPLC = high performance liquid chromatography; H_2SO_4 = sulfuric acid; ICl =iodine monochloride; MgO = magnesium oxide; NaCl = sodium chloride; NaOH = sodium hydroxide; NaOMe = sodium methoxide; Na_2SO_4 = sodium sulfate; NPD = nitrogen phosphorus detector; RPHPLC = reverse phase high performance liquid chromatography; TCD = thermal conductivity detector; TSD = thermionic detector; UV = ultra-violet detection

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.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Methods for detecting and quantifying pyrethrins and pyrethroids in blood (Bissacot and Vassilief 1997a, 1997b), plasma (Junting and Chuichang 1991; Wintersteiger et al. 1994), and urine (Junting and Chuichang 1991; Yi-Qun et al. 1994) are available. Chromatographic techniques, such as GC and HPLC, were used to isolate pyrethrins and their degradation products. ECD, FID, UV, and MS were coupled with the separation techniques to detect these compounds. Sensitivity was high (blood: 1 µg/kg; plasma: 2–0.17 µg/kg; urine: 0.2–0.5 µg/L). These methods can accurately detect pyrethrins at background concentrations in blood, plasma, and urine. Methods are available to characterize the metabolites of selected pyrethroids in urine (Angrer and Ritter 1997; Leng et al. 1999a; Yi-Qun et al. 1994). The sensitivity is high and the recovery is good. The existing methods for detecting the pyrethrins in biological samples seem to be adequate.

Effect. Other than the clinical signs of Type I and Type II pyrethroid poisoning discussed in Section 3.8.2, there are no known biomarkers of effect for pyrethrins and pyrethroids. It is important to note that while these effects are characteristic signs of pyrethroid poisoning, they are not exclusive to pyrethroid poisoning. In humans, pyrethroids are rapidly metabolized by esterase, mainly in the liver, and it may be possible to correlate carboxyesterase activity with pyrethroid induced adverse effects (Leng et al. 1999b). However, human plasma contains very little carboxyesterases and the liver is not accessible for routine measurements. As a parameter of carboxyesterase activity for Type II pyrethroids, the production of cyanic acid in human lymphocytes was measured (Leng et al. 1999b). Initial findings indicate that the determination of carboxyesterase activity in lymphocytes may potentially be used as a marker for individual susceptibility.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods are available to measure pyrethrins in air (EMMI 1997; Pomorska 1999), foods (EMMI 1997), water (EMMI 1997), waste water (EMMI 1997), soil (Alawi et al. 1990), and in formulations (methods by American Society for Testing Materials [ASTM], NIOSH, Food and Drug

Administration [FDA], EPA, Environmental Measurements Laboratory Center [EMLC], Engineering and Analysis Division [EAD], and AOAC). Sensitivity and recovery are not mentioned for several of the methods.

7.3.2 Ongoing Studies

Z. Elrassi, of Oklahoma State University, is conducting research on the development of high performance capillary electrophoresis (HPCE) and capillary electrochromatography (CEC) methods for the rapid, sensitive, and efficient separation of pesticides and their metabolites (CRIS 2001). B.W. Blair, of the Canadian Food Inspection Agency, Food Inspection Directorate, Lab Services Division, is developing a rapid, simple, and inexpensive immunological assay technology for low molecular weight contaminants of significance in food safety testing, as a tool for monitoring contaminants in foods and other environmental media (CRIS 2001).

In a study sponsored by the National Institute of Environmental Health Sciences at University of California, Davis, research is being performed to develop and validate single compounds, as well as a class selective immunoassay for urinary metabolites of hazardous compounds such as pyrethroids for use as biomarkers of internal exposure to theses compounds (CRISP 2001).