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Analysis of Pesticides in Water Samples

Central Water Commission,
Dept. of Water Resources, RD & GR

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PREFACE

Rivers are the main source of water in India, and are particularly used for agricultural irrigation and drinking water supply. Pesticides have played a major role in achieving the maximum crop production to meet the food demand of increasing population but maximum usage and accumulation of pesticide residues is highly detrimental to aquatic and other ecosystem. As most of the rivers pass through agricultural fields, they are subject to contamination with the different pesticides used for crop protection. The continuous consumption of contaminated water can pose severe health threats to local residents of this area

Central Water Commission has been playing a major role in the water quality monitoring of river water since year 1963 and at present, is observing water quality at 658 key locations (625 on HO network and 33 WQSS) covering major river basins of India. As per Guidelines of Water Quality Monitoring, 2017 available in CPCB website, pesticide analysis of river water samples also necessary and frequency of pesticide analysis is given as once in a year during pre-monsoon.

This book titled as "Analysis of pesticides in Water Samples" is to provide brief knowledge about pesticides and its classification, fate and effects of pesticides, sample collection, containers, sample storage, preservation and transportation, methods involving for the pesticides analysis.

Also, in this book it is briefed about extraction process and various methods available for extraction processes for pesticide analysis. This book may be utilized as a guidance document for water sampling, handling and analysis for pesticides in the water quality laboratories of CWC.

I would like to place on record my appreciation of Shri Reading Shimray, Chief Engineer (P&DO), CWC; Shri Pankaj Kumar Sharma, Director, RDC-2, CWC and his team for excellently bringing out this book.

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Introduction

The term "pesticide" is a composite term that includes all chemicals that are used to kill or control pests. These are a broad class of bioactive compounds used in crop protection, food preservation, and human health. In agriculture, this includes herbicides (weeds), insecticides (insects), fungicides (fungi), nematocides (nematodes), and rodenticides (vertebrate poisons). Several hundreds of pesticides of different chemical nature are currently used for agricultural purposes all over the world (Sneha et al. 2012). They differ from other chemical substances because they are spread deliberately into the environment. Exposure to the pesticides through the most important routes of uptake (oral, dermal, and inhalation) depends on the physicochemical characteristics of the pesticide and the nature of the contact, varying with the edge, lifestyle, and working conditions. The level of pesticides in different environmental compartments—such as water, agricultural foods, and products of animal origin—has became a relevant issue.

Unlike other contaminants, pesticides may affect both workers and the general population as a result of the consumption of contaminated food and water, domestic use, and proximity to agricultural settings. Information about actual human exposure to pesticides has important uses, including informing risk assessments, helping predict the potential consequences of exposures, and developing exposure criteria for regulations and other public policy guidance.

Rivers are the main source of water in India, and are particularly used for agricultural irrigation and drinking water supply. Modern agriculture practices reveal an increase in use of pesticides to meet the food demand of increasing population which results in contamination of the environment. Pesticides have played a major role in achieving the maximum crop production but maximum usage and accumulation of pesticide residues is highly detrimental to aquatic and other ecosystem. As most of the rivers pass through agricultural fields, they are subject to contamination with the different pesticides used for crop protection. Pesticide residues in drinking water have become a major challenge over the last few years.

The continuous consumption of contaminated water can pose severe health threats to local residents of this area (Indira Devi et al. 2017).

Classification of Pesticides

Pesticides differ in their physical, chemical and identical properties from one class to other. Therefore, it is worthy to classify them based their properties and study under their respective groups. Presently, there are three most popular method of pesticides classification (Drum et al. 1980).

i. Classification based on the mode of entry
ii. Classification based on pesticide function and the pest organism they kill,
iii. Classification based on the chemical composition of the pesticide.

The most common and useful method of classifying pesticide is based on their chemical composition and nature of active ingredients.

Classification Based on Chemical Composition of Pesticides

It is such kind of classification that gives the clue about the efficacy, physical and chemical properties of the respective pesticides. The information on chemical and physical characteristics of pesticides is very useful in determining the mode of application, precautions that need to be taken during application and the application rates. Based on the chemical composition, pesticides are classified into four main groups namely;

1. Organochlorines,
2. Organophosphorus,
3. Carbamates and
4. Pyrethrin and pyrethroids (Buchel et al. 1983).

In general, modern pesticides are organic chemicals. They include pesticides of both synthetic and plant origin. However, some inorganic compound is also used as pesticides. Insecticides are important pesticides that can be further classified into several sub-classes. The sub-classification of insecticides is given in Fig. 1

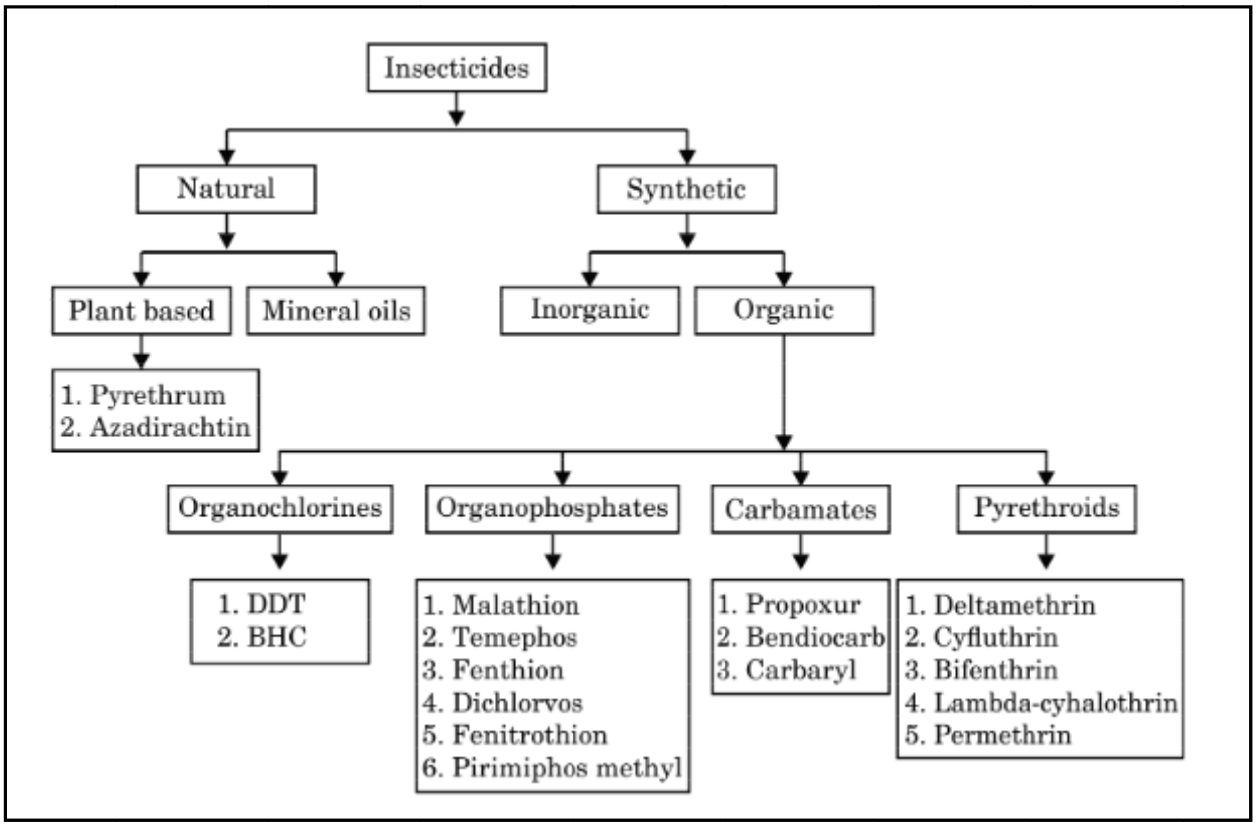
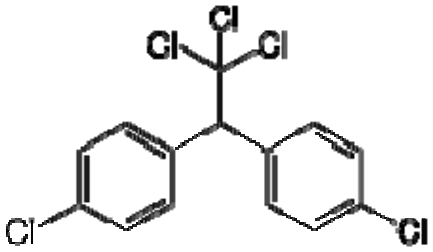


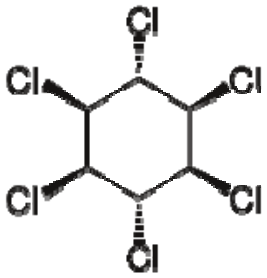
Figure 1: Classification of Insecticides

Organochlorine

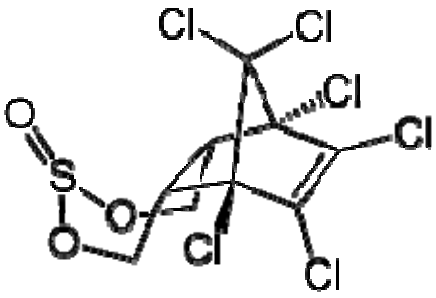
Organochlorines pesticides (also known as chlorinated hydrocarbons) are organic compounds attached with five or more chlorine atoms. They represent the one of the first group of pesticides ever synthesized and used in agriculture and in public health. Most of them were widely used as insecticides for the control of a wide range of insects, and they have a long-term residual effect in the environment. These insecticides may disrupt the nervous system of the insects leading to convulsions and paralysis followed by eventual death. Most common examples of these pesticides includes: DDT, lindane, endosulfan, aldrin, dieldrin and chlordane (I. C. Yadav et al. 2017).



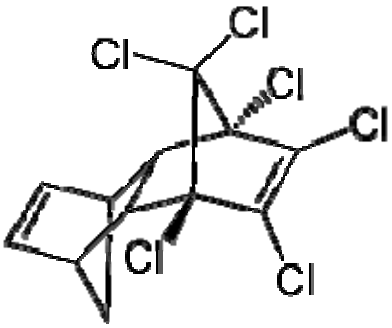
DDT (Dichlorodiphenyltrichloroethane**)**



Lindane (γ- Hexachlorocyclohexane (HCH)**)**



Endosulfan

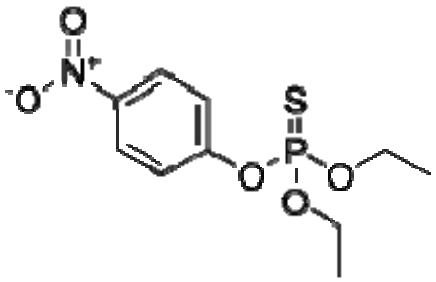


Aldrin

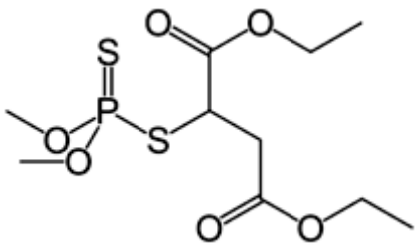
Organophosphates

Organophosphate pesticides are considered to be one of the broad spectrum pesticides which control wide range of pests due to their multiple functions. They are characterized with stomach poison, contact poison and fumigant poison leading to nerve poisons. These pesticides are also biodegradable, cause minimum environmental pollution and are slow pest resistance [Martin et al. 1968]. Organophosphorus insecticides are more toxic to vertebrates and invertebrates as cholinesterase inhibitors leading to a permanent overlay of acetylcholine neurotransmitter across a synapse. As a result, nervous impulses fail to move across the synapse causing a rapid twitching of voluntary muscles, hence, leading to

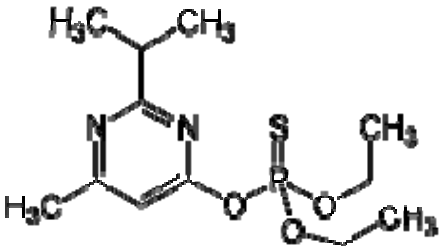
paralysis and death. Some of the widely used organophosphorus insecticides include parathion, malathion, diazinon and Chlorpyriphos.



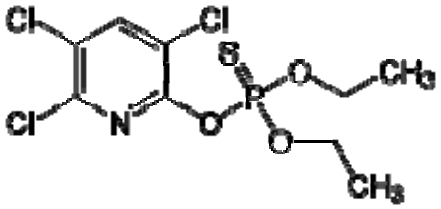
Parathion



Malathion



Diazinon

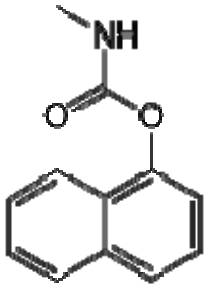


Chlorpyriphos

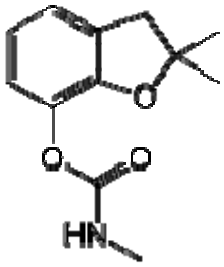
Carbamates

Structurally, Carbamates are similar to organophosphates. However, they differ in their origin. Organophosphates are derivatives of phosphoric acid, while carbamates derived from carbamic acid. The working principal of carbamate pesticides is similar to organophosphate pesticides by affecting the transmission of nerve signals resulting in the death of the pest by poisoning (Drum et al. 1980). Sometimes, they are also used as stomach and contact poisons as well as fumigant. They can be easily degraded under natural environment with minimum

environmental pollution. Some of the widely used insecticides under this group include carbaryl, carbofuran, propoxur and aminocarb.



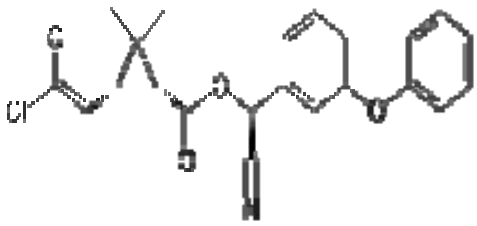
Carbaryl



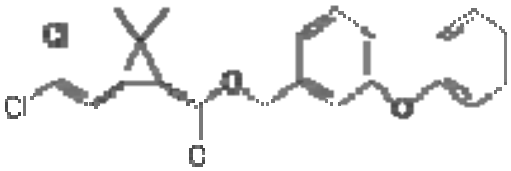
Carbofuran

Synthetic pyrethroids (I. C. Yadav et al. 2017)

Synthetic pyrethroid pesticides are group of organic pesticide that can be synthesized by duplicating the structure of natural pyrethrins. Relatively, they are more stable with longer residual effects than natural pyrethrins. Pyrethrins are grinded to produce active components. The major active components are pyrethrin I and pyrethrin II plus smaller amounts of the related cinerins and jasmolins. Synthetic-pyrethroid pesticides are highly toxic to insects and fish but slightly toxic to mammals and birds. Most of synthetic insecticides are non-persistent, and got broken easily on exposure to light. They are considered to be amongst the safest insecticides for use in food. Cypermethrin and Permethrin are the most used synthetic-pyrethroid pesticides.



Cypermethrin



Permethrin

Fate and effects of pesticides

The extensive use of pesticides harms the soil, air, food, surface and ground waters, and quality causing serious impacts on the environment and on human health. Nowadays, the contamination of water by pesticides is a very alarming ecological problem, especially in regions of intensive agriculture where leakage of these highly toxic substances into the water supplies may cause adverse effects on human and animal health. Several hundred pesticides of different chemical nature are currently used for agricultural purposes all over the world (Sneha et al. 2012).

Factors affecting pesticide toxicity in aquatic systems

The ecological impacts of pesticides in water are determined by the following criteria are given at Table 1.

Table 1: Factors for determination of ecological impacts of pesticides in water (Ongley et al. 1996).

Toxicity	Mammalian and non-mammalian toxicity usually expressed as LD50 ("Lethal Dose": concentration of the pesticide which will kill half the test organisms over a specified test period). The lower the LD50, the greater the toxicity; values of 0-10 are extremely toxic (OMAF, 1991).
	Drinking water and food guidelines are determined using a risk-based assessment. Generally, Risk = Exposure (amount and/or duration) × Toxicity.
	Toxic response (effect) can be acute (death) or chronic (an effect that does not cause death over the test period but which causes observable effects in the test organism such as cancers and tumours, reproductive failure, growth inhibition, teratogenic effects, etc.).
Persistence:	Measured as half-life (time required for the ambient concentration to decrease by 50%). Persistence is determined by biotic and abiotic degradational processes. Biotic processes are biodegradation and metabolism; abiotic processes are mainly

	hydrolysis, photolysis, and oxidation (Calamari et al, 1993). Modern pesticides tend to have short half lives that reflect the period over which the pest needs to be controlled.
Degradates:	The degradational process may lead to formation of "degradates" which may have greater, equal or lesser toxicity than the parent compound. As an example, DDT degrades to DDD and DDE.
Fate (Environmental):	The environmental fate (behavior) of a pesticide is affected by the natural affinity of the chemical for one of four environmental compartments (Calamari et al, 1993): solid matter (mineral matter and particulate organic carbon), liquid (solubility in surface and soil water), gaseous form (volatilization), and biota. This behavior is often referred to as "partitioning" and involves, respectively, the determination of the soil sorption coefficient (K_{OC}); solubility; Henry's Constant (H); and the n-octanol/water partition coefficient (K_{OW}). These parameters are well known for pesticides and are used to predict the environmental fate of the pesticide.

An additional factor can be the presence of impurities in the pesticide formulation but that are not part of the active ingredient. A recent example is the case of 3-trifluoromethy-l-4-nitrophenol (TFM), a lampricide used in the Lakes for many years for the control of the sea lamprey. Although the environmental fate of TFM has been well known for many years, recent research by Munkittrick et al. (1994) has found that TFM formulation includes one or more highly potent impurities that impact on the hormonal system of fish and cause liver disease.

Effect of Pesticides

Despite beneficial results of using pesticides in agriculture and public health sector, their use also invite deleterious environmental and public health effects. Pesticides hold a unique position among environmental contaminants due to their high biological activity and toxicity. Most pesticides do not distinguish between pests and other similar incidental life form. They are potentially harmful to humans, animals, other living organisms, and the environment if used incorrectly . It

is estimated that about 5000 – 20,000 people died and about 500,000 to 1 million people get poisoned every year by pesticides (FAO/WHO. 2000). At least half of the intoxicated and 75% of those who die due to pesticide is agricultural workers. The rest is being poisoned due to eating of contaminated food.

The World Health Organization (WHO) states, "The toxicity of a pesticide depends on its function and other factors. For example, insecticides tend to be more toxic to humans than herbicides."

Human health effects of pesticides

Human health effects are caused by:

Skin contact:	Handling of pesticide products
Inhalation:	Breathing of dust or spray
Ingestion:	Pesticides consumed as a contaminant on/in food or in water.

Farm workers have special risks associated with inhalation and skin contact during preparation and application of pesticides to crops. However, for the majority of the population, a principal vector is through ingestion of food that is contaminated by pesticides. Degradation of water quality by pesticide runoff has two principal human health impacts. The first is the consumption of fish and shellfish that are contaminated by pesticides; this can be a particular problem for subsistence fish economies that lie downstream of major agricultural areas. The second is the direct consumption of pesticide-contaminated water. Many health and environmental protection agencies have established "acceptable daily intake" (ADI) values which indicate the maximum allowable daily ingestion over a person's lifetime without appreciable risk to the individual (Ongley et al. 1996).

Table 2: General symptoms of Pesticide Poisoning (I. C. Yadav et al. 2017).

Mild poisoning	*Moderate poisoning*	*Moderate poisoning*
Any of the following: • Irritation of the nose, throat, eyes or skin • Headache • Dizziness • Loss of appetite • Thirst • Nausea • Diarrhea • Sweating • Weakness or fatigue • Restlessness • Nervousness • Changes in mood • Insomnia	Any of the mild symptoms, plus any of the following: • Vomiting • Excessive salivation • Coughing • Feeling of constriction in throat and chest • Abdominal cramps • Blurring of vision • Rapid pulse • Excessive perspiration • Profound weakness • Trembling • Muscular incoordination • Mental confusion	Any of the mild symptoms, plus any of the following: • Inability to breathe • Extra phlegm or mucous in the airways • Small or pinpoint pupils • Chemical burns on the skin • Increased rate of breathing • Loss of reflexes • Uncontrollable muscular twitching • Unconsciousness • Death

Ecological effects of pesticides (Ongley et al. 1996).

Pesticides are included in a broad range of organic micro pollutants that have ecological impacts. Different categories of pesticides have different types of effects on living organisms, therefore generalization is difficult. Although terrestrial impacts by pesticides do occur, the principal pathway that causes ecological impacts is that of water contaminated by pesticide runoff. The two principal mechanisms are bioconcentration and biomagnification.

Bioconcentration: This is the movement of a chemical from the surrounding medium into an organism. The primary "sink" for some pesticides is fatty tissue ("lipids"). Some pesticides, such as DDT, are "lipophilic", meaning that they are soluble in, and accumulate in, fatty tissue such as edible fish tissue and human fatty tissue. Other pesticides such as glyphosate are metabolized and excreted.

Biomagnification: This term describes the increasing concentration of a chemical as food energy is transformed within the food chain. As smaller organisms are eaten by larger organisms, the concentration of pesticides and other chemicals are increasingly magnified in tissue and other organs. Very high concentrations can be observed in top predators, including man.

The ecological effects of pesticides (and other organic contaminants) are varied and are often inter-related. Effects at the organism or ecological level are usually considered to be an early warning indicator of potential human health impacts. The major types of effects are listed below and will vary depending on the organism under investigation and the type of pesticide. Different pesticides have markedly different effects on aquatic life which makes generalization very difficult. The important point is that many of these effects are chronic (not lethal), are often not noticed by casual observers, yet have consequences for the entire food chain.

- Death of the organism.
- Cancers, tumors and lesions on fish and animals.
- Reproductive inhibition or failure.
- Suppression of immune system.
- Disruption of endocrine (hormonal) system.
- Cellular and DNA damage.
- Teratogenic effects (physical deformities such as hooked beaks on birds).
- Poor fish health marked by low red to white blood cell ratio, excessive slime on fish scales and gills, etc.
- Intergenerational effects (effects are not apparent until subsequent generations of the organism).
- Other physiological effects such as egg shell thinning.

These effects are not necessarily caused solely by exposure to pesticides or other organic contaminants, but may be associated with a combination of environmental stresses such as eutrophication and pathogens. These associated stresses need not be large to have a synergistic effect with organic micro pollutants.

Pesticides for Surface Water Quality Monitoring as per CPCB Guidelines

Pesticides included for analysis in the Guidelines on Water Quality Monitoring, 2017 by CPCB for Surface Waters (CPCB, 2017)

- Alachlor
- Atrazine
- Aldrin/Dieldrin
- α-, β-, γ- and δ- Hexachlorocyclohexane (HCH).
- Butachlor
- Chloropyiphos
- 2,4-Dichlorophenoxyacetic Acid
- DDT (o,p and pp isomers of DDT, DDE and DDT)
- Endosulfan (α-, β-)
- Ethion
- Isoproturon
- Malathion
- Methyl Parathion
- Monochrotophos
- Photate.

Sampling and Sample Preservation for Pesticides in Water

To assess the extent of contamination of water, effective and properly designed analytical methods having sufficient sensitivity and accuracy are needed. To collect a representative water sample for pesticide analysis, all sampling parameters must be selected properly. This refers predominantly to the selection of the appropriate sampling site, sampling technique and volume of the water sample. To prevent the sample matrix from any undesirable alterations during its transport from the sampling site to an analytical laboratory, physical (e.g. temperature, light intensity) and chemical/biochemical (e.g. pH, microbial growth) conditions must be under control, i.e. the sample must be preserved carefully (Igor Liska et al. 2006).

Sample Collection

The objective of sampling is to collect a portion of material from an environmental compartment (water, sediment or biota) small enough in volume to be conveniently transported and handled in the laboratory, while still accurately retaining is representatively. This requires that the sample will be handled and, if necessary, treated in such a way that no significant changes in composition occur that may hamper proper analysis.

The overall accuracy of trace-level measurements of target constituents in environmental samples is determined through the optimized combination of sample collection and analytical determination steps. It is noted that, the sample collection is having crucial impact on the final measurement. Typically, sampling procedures vary with the characteristics and concentrations of the target analytes and the nature of the matrix or matrices of interest. In other words, each sampling or pre-concentration procedures should be selected to accommodate the compatibility between target compound, matrix, and instrumental method.

Samples that flow freely are collected as grab samples using automatic sampling equipment. A grab sample is taken at a selected location and time, and then analyzed for pesticides. They are usually taken from the middle of the flowing water (main) stream 30 cm below the water surface. The collection of grab samples is appropriate when it is desired (i) to characterize water quality at a particular time and location, (ii) to provide information about minima and maxima and (iii) to analyze parameters which can be subject to change.

Containers to hold samples should be adequate, perfectly clean, dry, and free of contaminants that may interfere with the analysis. Note that these containers should not be rinsed with water from location as this could leave a pollutant film in the interior of the container. It is recommended to collect the water samples directly in the containers in which they will be transferred to and stored in the laboratory until their extraction and analysis.

Sample Containers

The type of sample container used is of utmost importance. Test sample containers and document that they are free of analytes of interest, especially when sampling and analyzing of very low analytes. Containers typically made of plastic or glass, but one material may be preferred over the other.

Hydrophobic pesticides can be easily adsorbed on polymer surfaces. From an aqueous sample containing organic compounds that are stored in a common plastic bottle, majority percentage of these compounds can be adsorbed within 24 h. Therefore, the storage of water samples in plastic containers must be avoided and the use of glass vessels only is recommended. Trace levels of some pesticides may sorb onto the walls of glass containers. Thus, hard glass (pyrex or equivalent) containers are preferred for pesticide samples. Some analytes (eg. Bromine-containing compounds and some pesticides, and polynuclear aromatic compounds) are light-sensitive: collect them in amber-colored glass containers to minimize photo-degradation. Container caps, typically plastic, also can be a problem. Don not use caps with paper liners. Use foiled or Polytetrafluoroethlylene (PTFE) liners.

Therefore, Amber colored Glass bottle fitted with a screw cap lined with PTFE are preferred for Pesticide water samples. The containers must be identified with site sampling information, name of the sample collector, date, time and analysis to perform. If possible, it is best to use pre-printed labels with most of this information. It is recommended that containers intended to hold samples come pre-labeled with type of analysis and site and station information from the laboratory.

Sample Volume

Sample volume depends on the number of analyses to be performed and on the technical requirements of a particular analysis. The volume of the water sample required for a single analysis of pesticides usually ranges from tens of milliliters up to 1–2 L, depending on the methodology applied. Traditional gas chromatography (GC) or high-performance liquid chromatography (HPLC) methods, in which an aliquot of the extract of the water sample is analyzed, require processing of a

large volume of water in order to reach the desired sensitivity. Always collect enough sample volume in the appropriate container in order to comply with sample handling, storage, and preservation requirements (Igor Liska et al. 2006).

Water samples for the analysis of pesticides are collected and stored in 1 L glass amber bottles previously treated to eliminate all organic pollutants. Sample containers must be scrupulously clean so that they do not contaminate the samples placed in them. Table 3 provides general information on appropriate types of sample container and the recommended procedures for cleaning them when water samples are to be used for the pesticide analysis and herbicides analysis.

Table 3: Recommended pre-treatment of water sample containers [UNEP/WHO 1996]

Parameter	Matrix	Recommended container	Washing procedure
Organochlorine, Organophosphorus, And PCBs	Water	1 L glass (amber) with Teflon-line cap	Rinse three times with tap water, once with chromic acid*, three times with organic free water, twice with washing acetone, once with special grade acetone**, twice with pesticide grade hexane and dry (uncapped) in a hot air oven at 360°C
Pentachlorophenol phenolics Penoxy acid herbicdes	Water	1 L glass (amber) with Teflon-line cap	Rinse three times with tap water, once with chromic acid*, three times with organic-free water, twice with washing acetone, once with special grade acetone**, twice with pesticide grade hexand and dry (uncapped) in a hot air oven at 360°C.

* Chromic Acid – 35 mL saturated $Na_2Cr_2O_7$ per liter reagent grade conc. H_2SO_4.

** Special grade acetone - pesticide grade when GC analysis to be performed, UV grade for LC analysis

Sample Labeling

Use labels to prevent sample misidentification. Gummed paper labels or tags generally are adequate. The labels must be water resistant and should clearly display. Include at least the following information: a unique identification sample number, sample type, name of the collector, date and time of collection, place of collection, and sample preservative. Also include date and time of preservation for comparison to date and time of collection. Affix tags or self-adhesive labels to sample containers before, or at the time of sample collection (APHA, 1060B). labels may make as given below in Table 4.

Table 4: Water Sample Details: Pesticides analysis

1.	Unique identification sample number	
2.	Sample type	
3.	Name of the collector	
4.	Date collection,	
5.	Name of Site	
6.	River	
7.	Division	
8.	Sample preservative	

Sample Storage & Transportation

Before selecting samples, the possibilities for shipping these materials to the analytical laboratory –no matter if domestic or abroad –have to be carefully evaluated. Especially biota may have a short period of expiration and may need cooling or freezing during transport or storage. Therefore, the timely accessibility and availability of fridges, freezers, icepacks, thermos bottles, etc. has to be decided and agreed in advance.

Deliver samples to laboratory as soon as practicable after collection, typically within 2 days. If shorter sample holding times are required, make special

arrangements to ensure timely delivery to the laboratory. If samples are shipped by a commercial carrier, include the waybill number in the sample documentation. (APHA, 1060B).

Sample bottles should be placed in a box for transport to the laboratory. Sturdy, insulated wooden or plastic boxes will protect samples from sunlight, prevent the breakage of sample bottles, and should allow a temperature of ≤ 6°C to be attained and maintained during transport.

Sample Preservation

Preservation of the sample during transport and storage depends on the type of pesticides to be analyzed. It has to prevent any potential means of losses of analytes from the liquid phase, such as transfer to the gaseous phase (evaporation), transfer to the solid phase (adsorption on the container walls), hydrolysis, photodegradation and biodegradation. Keep the samples as cool as possible without freezing. Preferably pack samples in crushed or cubed ice or commercial ice substitutes before shipment. Avoid using dry ice because it will freeze samples and may cause glass containers to break. Any case, the storage of the water sample at ≤ 6°C until extraction, minimization of the volume of the gaseous phase in the container and the use of gas-tight caps is recommended.

Many modern polar pesticides can easily hydrolyse when the pH reaches a certain critical value. Hence it is necessary to maintain the pH at a desired value using a buffer solution or, usually in the case of acidic compounds, simply by acidifying the water sample. Keeping the samples in the dark and using amber-glass sample containers when available should prevent photolysis of the analytes.

Residual chlorine should be reduced at the sampling site by adding 0.008% of sodium sulfite ($Na_2S_2O_3$) (this may be added as a solid with stirring or shaking until dissolved) to water sample. It is very important that the sample be dechlorinated prior to adding acid to lower the pH of the sample. Adding sodium sulfite and H_2SO_4 or NaOH to the sample bottles prior to shipping to the sampling site is not permitted. Adjusting sample pH should be used at the sampling site to

retard the microbiological degradation of some analytes in water (https://flenviro.com/FDEP_Holding_Times_and_Preservations.pdf).

Most standardized methods recommend reducing the time of storage of the water sample to a minimum and performing the extraction as soon as possible. Storage periods that are usually suggested range from 7 to 14 days. Preservation & Maximum holding time of samples for pesticide analysis is given at Table 5.

Table 5: Preservation & Maximum holding time of Samples for Pesticide Analysis

Parameter	Preservation	Maximum Holding Time
Organo-Chlorine Pesticides (APHA 6630)/ Acidic Herbicides (APHA 6640)/Organo-phosphorous pesticides	Refrigerated at ≤ 6°C, kept in the dark and pH 5 – 9 with H_2SO_4 or NaOH	• Extraction within 7 days • Analysis of sample extract within 40 days after extraction
Carbamate Pesticides (APHA 6610)	Refrigerated at ≤ 6°C, kept in the dark and pH 3-8 with H_2SO_4 or NaOH	• Extraction within 7 days • Analysis of sample extract within 40 days after extraction

Methods for Pesticide Analysis

The reference methods for identification and quantification of trace organic constituents in water generally involve isolation and concentration of the organic compounds from a sample by solvent or gas extraction, separation of the components, and the identification and the quantification of the compounds with a detector.

The Reference analysis methods and procedure for Pesticides analysis are given by EPA (Method 1699: Pesticides in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS, December 2007) and NEERI (Guidance Manual for Drinking Water Quality Monitoring and Assessment, 2007) including sample

preparation, extraction, and analysis are given as Annexure-I and Annexure-II respectively.

American Public Health Association (APHA) published revised and enlarged 23rd edition under the title "Standards methods for the Examination of Water and Waste Water which contains 45 sections with significant technical/editorial revisions. The analytical methods for pesticide analysis are covered in APHA 23rd edition Part: 6000.

APHA 23 edition Part: 6000

6010 Introduction	Page 6-1 to 6-6
6020 Quality Assurance and quality Control	Page 6-6 to 6-16
6410 Extractable Base	Page 6-73 to 6-87
6610 Carbamate Pesticides	Page 6-112 to 6-120
6630 Organochlorine Pesticides	Page 6-121 to 6-134
6640 Acidic Herbicide Compounds	Page 6-135 to 6-146
6651 Glyphosate Herbicides	Page 6-146 to 6-148

Gas chromatography is widely used in water analysis as it provides a quick and relatively inexpensive way of determining many organic compounds, particularly pesticides, polychlorinated biphenyls (PCBs) and volatile organic compounds (VOCs). It is, however, a sophisticated analytical technique in that it often requires an experienced operator to interpret and evaluate the results of analyses.

Gas chromatography (GC), like all chromatography, is a separation technique; that is to say that it is a method of separating mixtures of chemicals rather than analysing them. However, virtually all commercially available gas chromatographs include a detector which can, in conjunction with the known chromatographic behaviour of the chemical being determined, both identify and quantify many different chemical species.

There are actually two gas chromatography techniques, gas-solid chromatography (GSC), in which the stationary phase is a solid, and gas-liquid chromatography (GLC) in which the stationary phase is a liquid held as a thin layer upon a solid. Of the two techniques, the latter tends to be the one that is used in water analysis.

Extraction and Clean-Up in Pesticide Analysis (FSSAI manual, 2015)

It is hardly possible to analyze all pesticides present in water; therefore the most important substances have to be controlled first. Because of the extremely low analyte concentration levels and complex matrix composition, usually it is necessary to pre-concentrate the selected groups of pesticides and clean-up the samples from interfering components, especially in the presence of humus and fatty acids or suspended matter in surface waters. There are various methods of pesticide isolation from environmental water samples.

The major reasons for the preparation of water samples prior to pesticide analysis are

- Pre-concentration of analytes in order to enrich the sample with traces of the analytes;
- Removal or reduction of compounds that interfere in the separation and/or detection steps;
- Transfer of analytes into a homogeneous liquid matrix amenable to chromatographic analysis.

Extraction

Extraction means separation of pesticide residues from the matrix by using solvent. The extraction procedure should be such that it quantitatively removes pesticides form matrix (high efficiency), does not cause chemical change in pesticide and use inexpensive and easily cleaned apparatus.

Choice of extraction method

The main objective behind employing a particular method for a specific substrate is to bring the solvent to close proximity of the pesticide residues for sufficient period so that pesticide residues get solubilised in the solvent. The

choice of method depends on the type of substrate. The substrates in pesticide residue analysis could be liquids like water, fruit juices, body fluids (urine, blood etc.) and solids like soil, flesh, green plant materials (leaves, fruit etc.), dry fodder, grains etc.

For Liquid substrates

1. **Partitioning**: Samples like water, body fluids, and juices are extracted by partitioning with water immiscible solvent. The addition of sodium chloride in aqueous samples improves the extraction efficiency by reducing the solubility of pesticide in water. It also prevents the emulsion formation, which is frequently encountered during partitioning.

Choice of solvent

The choice of solvent for extraction depends on the a) nature of the substrate and b) the type of pesticide to be extracted. However, the solvent should satisfy the following conditions.

- Should have high solubility for the pesticide and least solubility for co-extractives.
- Should not change the pesticide chemically or react with it.
- Economical
- Low boiling.
- Easily separated from the substrate.
- Compatible to the method of final determination.

Choice of solvent depending on type of substrate

The solvent for extraction of pesticide in for aqueous substrate: Water immiscible solvent like hexane, petroleum ether, benzene, dichloromethane, chloroform, ethyl acetate, etc.

Choice of solvent depending on nature of pesticides

The pesticide molecules can be broadly divided into two group's namely non-ionic and ionic type. The non-ionic pesticides also differ in their polarity. For

nonionic type of pesticides, organic solvents with varying polarity depending on the polarity of pesticide molecules are employed.

1. **Use of absorbent:** The pesticide residues from aqueous samples can be extracted by passing the sample through solid adsorbents packed in glass column. The adsorbents have high affinity for pesticide molecules, therefore, they are held up on the absorbent whereas water passes out. The solid adsorbents are then extracted with organic solvent. The solid adsorbents normally used for removal of pesticide from aqueous samples are given below:

Solid adsorbents	Liquid coated or bonded on inert solids
1. Activated charcoal 2. Polyurethane foam 3. Cellulose triacetate 4. Molecular sieves 5. Ion exchange resins 6. Magnsium Sulphate 7. Silica gel (activated) 8. Akynuba activated (acidic, basic and neutral) 9. Florisil and Extrulet	1. Carbowax 4000 coated on chromosorb 2. Undecane coated on chromosorb W. 3. RPC-18-HPLC column material 4. RPC-C-8-HPLC column material

Various methods have been developed for the extraction of pesticides in water such as liquid-liquid extraction (LLE), solid-phase extraction (SPE), solid-phase micro extraction (SPME), and stir bar sorption extraction (SBSE).

Recent techniques of extraction

Liquid-Liquid Extraction (LLE) and Solid Phase Extraction (SPE) are the most frequently applied sample-handling techniques in the determination of pesticides in water. The theoretical background of LLE and SPE is based on similar principles of the interphase distribution of a solute in a two-phase system. The distribution of a solute in such a two-phase system (liquid–liquid or liquid–solid) is governed by the affinities of the solute to these phases.

Traditional sample preparation methods (liquid-liquid extraction, Soxhlet extraction, etc.) are laborious, time consuming, expensive, requires large amounts of organic solvents and usually involve many steps, leading to loss of some analyte quantity. As a result, modern sample preparation procedures, such as accelerated solvent extraction (ASE), supercritical fluid extraction (SFE), microwave assisted extraction (MAE), solid phase extraction (SPE), solid phase microextraction (SPME), matrix solid phase dispersion (MSPD) extraction and QuEChERS (quick, easy, cheap, effective, rugged and safe), have been developed to overcome the drawbacks of the traditional approaches.

Solid Phase Extraction (SPE)

SPE is one of the most commonly used sorbent techniques in analyzing pesticide residues. Solid phase extraction technique is based on the concept of selective retention by the device for the analyte, in this case the pesticide. SPE can be made to work on either the batch or column mode.

SPE procedures comprise two-step transfer of the solute. First, the solute is sorbed from water on the solid surface of a sorbent or in the stationary phase bound to this surface and, subsequently, the solute is eluted with an appropriate solvent (or thermally desorbed into the gas chromatograph). Here, the choice of a suitable sorbent (i.e. the selection of strength of interactions responsible for binding the solute on the solid phase) and the choice of eluting solvent or thermal desorption conditions (i.e. the energy needed for breaking of the bonds) must be properly tuned to obtain a quantitative preconcentration.

Stir-Bar Sorptive Extraction (SBSE)

SBSE was introduced in 1999 by Pat Sandra's group to overcome some of the limits of the existing techniques, in particular in the recovery of medium-to-high volatility analytes when sampled in liquid phase with polydimethylsiloxane-open tubular traps (PDMS-OTT); further aim was to improve the limited recovery achievable in ultra-trace analysis with solid-phase micro extraction (SPME), especially under un-favorable phase ratios when working with small volumes of sorptive material (in general PDMS) coating the fused-silica fibre. SBSE was first

developed for sampling in liquid phase and is based upon sorption of the investigated analytes or fraction onto a very thick film of PDMS coated onto a glass-coated magnetic stir bar (commercially known as Twister, Gerstel GmbH, and Mulheim, Germany). Sampling is done by directly introducing the SBSE device into the aqueous sample; in the original experiments, the analytes sampled for a given time were recovered by thermal desorption and then on-line transferred to a gas chromatography (GC) or GC–mass spectrometry (MS) system for analysis. Later, liquid desorption in combination with high performance liquid chromatography (HPLC) also was applied, mainly for analytes not analyzable by GC.

Clean-up Techniques

Cleanup refers to a step or series of steps in the analytical procedure in which the bulk of the potentially interfering co-extractives are removed by physical or chemical methods. During extraction, the solvent comes in contact with the substrate matrix, to enable extraction of the pesticide along with some of the constituents of the substrate matrix also get solubilized. The extract not only contains pesticide residues but also other constituents, which are called co-extractives (FSSAI manual, 2015).

The removal of interfering co- extractives from extract is called clean up. The co-extractive generally extracted along with pesticide from various substrates are moisture, colored pigment like chlorophyll, xanthophylls and anthocyanins, colourless compounds like oil, fat and waxes etc. When dry substrate is extracted with water immiscible solvent, it contains traces of moisture, which can be removed by passing the extract through anhydrous sodium sulfate. High moisture containing substrate are extracted with water miscible solvent, the extract contains lot of water and water soluble compounds, the extract is concentrated to remove organic solvent, the aqueous phase is diluted with saturated sodium chloride solution and then extracted with water immiscible solvent just like water samples. After removal of moisture, the other coextractives are removed by using various separation techniques.

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ANNEXURE-I

Method 1699: Pesticides in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS

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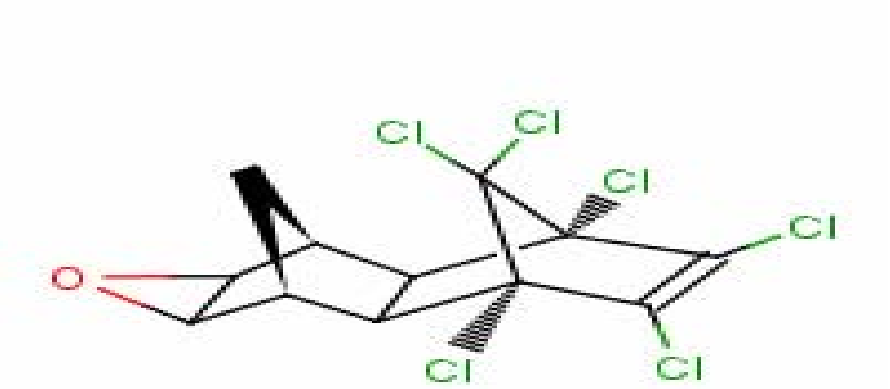

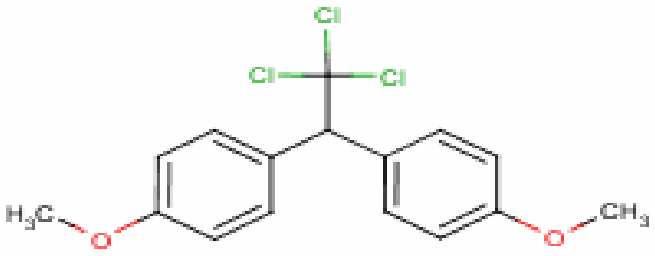

U.S. Environmental Protection Agency
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Office of Science and Technology
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EPA-821-R-08-001
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Introduction

EPA Method 1699 determines organochlorine, organophosphorus, triazine, and pyrethroid pesticides in environmental samples by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) using isotope dilution and internal standard quantitation techniques. This method has been developed for use with aqueous, solid, tissue and biosolids matrices.

Disclaimer

This method has been reviewed by the Engineering and Analytical Support Branch of the Engineering and Analysis Division (EAD) in OST. The method is available for general use, but has not been published in 40 CFR Part 136. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Method 1699: Pesticides in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS

1.0 Scope and Application

1.1 Method 1699 is for determination of selected organochlorine, organo-phosphorus, triazine, and pyrethroid pesticides in multi-media environmental samples by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS).

1.2 This Method was developed for use in EPA's Clean Water Act (CWA) programs; other applications are possible. It is based on existing EPA methods (Reference 1) and procedures developed at Axys Analytical Services (Reference 2).

1.3 The analytes that may be measured by this method and their corresponding Chemical Abstracts Service Registry Numbers (CASRNs) and ambient water quality criteria are listed in Table 1.

1.4 The detection limits and quantitation levels in this Method are usually dependent on the level of interferences rather than instrumental limitations. The method detection limits (MDLs; 40 CFR 136, appendix B) and minimum levels of quantitation (MLs; 68 FR 11790) in Table 1 are the levels at which pesticides can be determined in the absence of interferences.

1.5 This Method is restricted for use by analysts experienced in HRGC/HRMS or under the close supervision of such qualified persons. Each laboratory that uses this Method must demonstrate the ability to generate acceptable results using the procedure in Section 9.2.

1.6 This method is performance-based which means that you may modify the method to improve performance (e.g., to overcome interferences or improve the accuracy or precision of the results) provided that you meet all performance requirements in this method. These requirements for establishing equivalency of a modification are in Section 9.1.2. For Clean Water Act (CWA) uses, additional flexibility is described at 40 CFR 136.6. Modifications not in the scope of Part 136.6 or in Section 9 of this method may require prior review and approval.

2.0 Summary of Method

Flow charts that summarize procedures for sample preparation, extraction, and analysis are given in Figure 1 for aqueous and solid samples, Figure 2 for multi-phase samples, and Figure 3 for tissue samples.

2.1 Extraction (Section 12)

2.1.1 Aqueous samples (samples containing less than one percent solids) – Stable isotopically labeled analogs of the pesticides are spiked into a 1-L sample. The sample is extracted at neutral pH with methylene chloride using separatory funnel extraction (SFE) or continuous liquid/liquid extraction (CLLE).

2.1.2 Solid, semi-solid, and multi-phase samples (excluding municipal sludge and tissue) – The labeled compounds are spiked into a sample containing 10 g (dry weight) of solids. Samples containing multiple phases are pressure filtered and any aqueous liquid is discarded. Coarse solids are ground or homogenized. Any non-aqueous liquid from multi-phase samples is combined with the solids and extracted with methylene chloride, methylene chloride:hexane (1:1) or acetone:hexane (1:1) in a Soxhlet extractor or with toluene in a Soxhlet/Dean-Stark (SDS) extractor (Reference 3).

2.1.3 Municipal sludges are homogenized, spiked with labeled compounds, and Soxhlet extracted with dichloromethane.

2.1.4 Fish and other tissue – A 20-g aliquot of sample is homogenized, and a 10-g aliquot is spiked with the labeled compounds. The sample is mixed with anhydrous sodium sulfate, allowed to dry for 30 minutes minimum, and extracted for 18 - 24 hours using methylene chloride in a Soxhlet extractor. The extract is evaporated to dryness, and the lipid content is determined.

2.2 Concentration (Section 12)

2.2.1 Extracts are macro-concentrated using rotary evaporation, a heating mantle, or a Kuderna-Danish evaporator.

2.2.2 Extracts to be injected into the HRGC/HRMS are concentrated to a final volume of 20 μL using nitrogen evaporation (blowdown).

2.3 Cleanup (Section 13)

2.3.1 Extracts of aqueous, solid or mixed phase samples are cleaned up using an aminopropyl SPE column followed by a microsilica column.

2.3.2 Extracts may be further cleaned up using gel permeation chromatography (GPC) or solid-phase cartridge techniques.

2.3.3 Extracts in which the organo-chlorine pesticides only are to be determined may be further cleaned up using silica gel, Florisil, or alumina chromatography.

2.4 Determination by GC/HRMS – Immediately prior to injection, a labeled injection internal standard is added to each extract and an aliquot of the extract is injected into the gas chromatograph (GC). The analytes are separated by the GC and detected by a high-resolution (≥8,000) mass spectrometer. Two exact m/z's for each pesticide are monitored throughout a pre-determined retention time window.

2.5 An individual pesticide is identified by comparing the GC retention time and ion-abundance ratio of two exact m/z's with the corresponding retention time of an authentic standard and the theoretical or acquired ion-abundance ratio of the two exact m/z's.

2.6 Quantitative analysis is performed in one of two ways using selected ion current profile (SICP) areas:

2.6.1 For pesticides for which a labeled analog is available, the GC/HRMS is multi-point calibrated and the concentration is determined using the isotope dilution technique.

2.6.2 Pesticides for which a labeled analog is not available are determined using the internal standard technique. The labeled compounds are used as internal standards, affording recovery correction for all pesticides.

2.7 The quality of the analysis is assured through reproducible calibration and testing of the extraction, cleanup, and GC/MS systems.

3.0 Definitions and units of measure

Definitions and units of measure are given in the glossary at the end of this Method.

4.0 Interferences

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts, elevated baselines, and/or lock-mass suppression causing misinterpretation of chromatograms. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Where possible, reagents are cleaned by extraction or solvent rinse.

4.2 Proper cleaning of glassware is extremely important, because glassware may not only contaminate the samples but may also remove the analytes of interest by adsorption on the glass surface.

4.2.1 Glassware should be rinsed with solvent and washed with a detergent solution as soon after use as is practical. Sonication of glassware containing a detergent

solution for approximately 30 seconds may aid in cleaning. Glassware with removable parts, particularly separatory funnels with fluoropolymer stopcocks, must be disassembled prior to detergent washing.

4.2.2 After detergent washing, glassware should be rinsed immediately, first with methanol, then with hot tap water. The tap water rinse is followed by another methanol rinse, then acetone, and then methylene chloride.

4.2.3 Baking of glassware in a kiln or other high temperature furnace (300 - 500°C) may be warranted after particularly dirty samples are encountered. The kiln or furnace should be vented to prevent laboratory contamination by pesticide vapors. Baking should be minimized, as repeated baking of glassware may cause active sites on the glass surface that may irreversibly adsorb pesticides. Volumetric ware should not be baked at high temperature.

4.2.4 After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

4.2.5 Immediately prior to use, the Soxhlet apparatus should be pre-extracted for approximately 3 hours and the extraction apparatus should be rinsed with the extraction solvent.

4.3 All materials used in the analysis must be demonstrated to be free from interferences by running reference matrix method blanks (Section 9.5) initially and with each sample batch (samples started through the extraction process on a given 12-hour shift, to a maximum of 20 samples).

4.3.1 The reference matrix must simulate, as closely as possible, the sample matrix under test. Ideally, the reference matrix should not contain the pesticides in detectable amounts, but should contain potential interferents in the concentrations expected to be found in the samples to be analyzed.

4.3.2 When a reference matrix that simulates the sample matrix under test is not available, reagent water (Section 7.6.1) can be used to simulate water samples; playground sand (Section 7.6.2) or white quartz sand (Section 7.3.2) can be used to simulate soils; filter paper (Section 7.6.3) can be used to simulate papers and similar materials; and corn oil (Section 7.6.4) can be used to simulate tissues.

4.4 Interferences co-extracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. Interfering compounds may be present at concentrations several orders of magnitude higher than the pesticides. The most frequently encountered interferences are chlorinated biphenyls, chlorinated and brominated dibenzodioxins and dibenzofurans, methoxy biphenyls, hydroxydiphenyl ethers, benzylphenyl ethers, brominated diphenyl ethers, polynuclear aromatics, and polychlorinated naphthalenes. Because very low levels of pesticides are measured by this Method, elimination of interferences is essential. The cleanup steps given in Section 13

can be used to reduce or eliminate these interferences and thereby permit reliable determination of the pesticides at the levels shown in Table 1.

4.5 Each piece of reusable glassware should be numbered to associate that glassware with the processing of a particular sample. This will assist the laboratory in tracking possible sources of contamination for individual samples, identifying glassware associated with highly contaminated samples that may require extra cleaning, and determining when glassware should be discarded.

4.6 Organic acids and other substances make it difficult to extract and clean up biosolids (sewage sludge) samples. The exact procedures to be used are dependent on the analytes to be determined. If all analytes in this Method are to be determined, gel permeation chromatography (GPC), the amino-propyl SPE column, and the layered alumina/Florisil column have been found effective. For the organo-chlorine pesticides, sequential extraction with acetonitrile and methylene chloride followed by back extraction with sodium sulfate-saturated water has been found effective. An anthropogenic isolation column (Section 13.6; see Section 7.5.2 for column details), GPC (Section 13.2), high performance liquid chromatography (HPLC; Section 13.5), Florisil (Section 13.7), and alumina (Section 13.8) are additional steps that may be employed to minimize interferences in the sludge matrix.

4.7 The natural lipid content of tissue can interfere in the analysis of tissue samples for measurement of pesticides. The lipid contents of different species and portions of tissue can vary widely. Lipids are soluble to varying degrees in various organic solvents and may be present in sufficient quantity to overwhelm the column chromatographic cleanup procedures used for sample extracts. Lipids must be removed by the anthropogenic isolation column procedure in Section 13.6, followed by GPC (Section 13.2).

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical used in this Method has not been precisely determined; however, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

5.1.1 Some pesticides, most notably 4,4'-DDT and 4,4'-DDD, have been tentatively classified as known or suspected human or mammalian carcinogens. Pure standards of the pesticides should be handled only by highly trained personnel thoroughly familiar with handling and cautionary procedures and the associated risks.

5.1.2 It is recommended that the laboratory purchase dilute standard solutions of the analytes in this Method. However, if primary solutions are prepared, they must be prepared in a hood, and a NIOSH/MESA approved toxic gas respirator must be worn when high concentrations are handled.

5.2 This Method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this Method. A reference file of material safety data sheets (MSDSs) should also be made available to all personnel involved in these analyses. It is also suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this Method and that the results of this monitoring be made available to the analyst. Additional information on laboratory safety can be found in References 4-7. The references and bibliography at the end of Reference 6 are particularly comprehensive in dealing with the general subject of laboratory safety.

5.3 The pure pesticides and samples suspected to contain high concentrations of these compounds are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. Each laboratory must develop a strict safety program for handling these compounds. The practices in Reference 8 for handling chlorinated dibenzo-*p*-dioxins and dibenzofurans (CDDs/CDFs) are also recommended for handling pesticides.

5.3.1 Facility – When finely divided samples (dusts, soils, dry chemicals) are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak tight or in a fume hood demonstrated to have adequate air flow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in the case of an accident.

5.3.2 Protective equipment – Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters. Eye protection (preferably full face shields) must be worn while working with exposed samples or pure analytical standards. Latex gloves are commonly used to reduce exposure of the hands. When handling samples suspected or known to contain high concentrations of the pesticides, an additional set of gloves can also be worn beneath the latex gloves.

5.3.3 Training – Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.4 Personal hygiene – Hands and forearms should be washed thoroughly after each operation involving high concentrations of the pesticides, and before breaks (coffee, lunch, and shift).

5.3.5 Confinement – Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Effluent vapors – The effluent of the sample splitter from the gas chromatograph (GC) and from roughing pumps on the mass spectrometer (MS) should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high-boiling alcohols to condense pesticide vapors.

5.3.7 Waste handling – Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel should be trained in the safe handling of waste.

5.3.8 Decontamination

5.3.8.1 Decontamination of personnel – Use any mild soap with plenty of scrubbing action.

5.3.8.2 Glassware, tools, and surfaces – Chlorothene NU Solvent is a less toxic solvent that should be effective in removing pesticides. Satisfactory cleaning may be accomplished by rinsing with Chlorothene, then washing with any detergent and water. If glassware is first rinsed with solvent, the wash water may be disposed of in the sewer. Given the cost of disposal, it is prudent to minimize solvent wastes.

5.3.9 Laundry – Clothing known to be contaminated should be collected in plastic bags. Persons that convey the bags and launder clothing should be advised of the hazard and trained in proper handling. Clothing may be put into a washer without contact if the launderer knows of the potential problem. The washer should be run through a cycle before being used again for other clothing.

5.3.10 Wipe tests – A useful method of determining cleanliness of work surfaces and tools is to perform a wipe test of the surface suspected of being contaminated.

5.3.10.1 Using a piece of filter paper moistened with Chlorothene or other solvent, wipe an area approximately 10 x 10 cm.

5.3.10.2 Extract and analyze the wipe by GC with an electron capture detector (ECD) or by this Method.

5.3.10.3 Using the area wiped (e.g., 10 x 10 cm = 0.01 m^2), calculate the concentration in $\mu g/m^2$. A concentration less than 1 $\mu g/m^2$ indicates acceptable cleanliness; anything higher warrants further cleaning. More than 100 $\mu g/m^2$ constitutes an acute hazard and requires prompt cleaning before further use of the equipment or work space, and indicates that unacceptable work practices have been employed.

5.4 Biosolids samples may contain high concentrations of biohazards, and must be handled with gloves and opened in a hood or biological safety cabinet to prevent exposure. Laboratory staff should know and observe the safety procedures required in a microbiology laboratory that handles pathogenic organisms when handling biosolids samples.

6.0 Apparatus and materials

Note: *Brand names, suppliers, and part numbers are for illustration purposes only and no endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here. Meeting the performance requirements of this Method is the responsibility of the laboratory.*

6.1 Sampling equipment for discrete or composite sampling

6.1.1 Sample bottles and caps

6.1.1.1 Liquid samples (waters, sludges and similar materials containing 5 percent solids or less) – Sample bottle, amber glass, 1.1-L minimum, with screw cap.

6.1.1.2 Solid samples (soils, sediments, sludges, paper pulps, filter cake, compost, and similar materials that contain more than 5 percent solids) – Sample bottle, wide mouth, amber glass, 500-mL minimum.

6.1.1.3 If amber bottles are not available, samples must be protected from light.

6.1.1.4 Bottle caps – Threaded to fit sample bottles. Caps must be lined with fluoropolymer.

6.1.1.5 Cleaning

6.1.1.5.1 Bottles are detergent water washed, then solvent rinsed before use.

6.1.1.5.2 Liners are detergent water washed and rinsed with reagent water (Section 7.6.1).

6.1.2 Compositing equipment – Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Only glass or fluoropolymer tubing must be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing must be thoroughly rinsed with methanol, followed by repeated rinsing with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

6.2 Equipment for glassware cleaning

Note: *If blanks from bottles or other glassware or with fewer cleaning steps than required in this Method show no detectable pesticide contamination, unnecessary cleaning steps and equipment may be eliminated.*

6.2.1 Laboratory sink with overhead fume hood

6.2.2 Kiln – Capable of reaching 450°C within 2 hours and maintaining 450 - 500°C within ∀10°C, with temperature controller and safety switch (Cress Manufacturing Co, Santa Fe Springs, CA, B31H, X31TS, or equivalent). See the precautions in Section 4.2.3.

6.2.3 Aluminum foil – solvent rinsed or baked in a kiln. If baked in a kiln, heavy-duty aluminum foil is required, as thinner foil will become brittle and unusable.

6.3 Equipment for sample preparation

6.3.1 Laboratory fume hood of sufficient size to contain the sample preparation equipment listed below.

6.3.2 Glove box (optional)

6.3.3 Tissue homogenizer – VirTis Model 45 Macro homogenizer (American Scientific Products H-3515, or equivalent) with stainless steel Macro-shaft and Turbo-shear blade.

6.3.4 Meat grinder – Hobart, or equivalent, with 3- to 5-mm holes in inner plate.

6.3.5 Equipment for determining percent moisture

6.3.5.1 Oven – Capable of maintaining a temperature of 110 ∀5°C

6.3.5.2 Desiccator

6.3.6 Balances

6.3.6.1 Analytical – Capable of weighing 0.1 mg

6.3.6.2 Top loading – Capable of weighing 10 mg

6.4 Extraction apparatus

6.4.1 Water and solid samples

6.4.1.1 pH meter, with combination glass electrode

6.4.1.2 pH paper, wide range (Hydrion Papers, or equivalent)

6.4.1.3 Graduated cylinder, glass, 1-L capacity and Erlenmeyer Flask, glass, 1-L capacity

6.4.1.4 Liquid/liquid extraction – Separatory funnels, 250-, 500-, and 2000-mL, with fluoropolymer stopcocks

6.4.1.5 Solid-phase extraction

6.4.1.5.1 1-L filtration apparatus, including glass funnel, frit support, clamp, adapter, stopper, filtration flask, and vacuum tubing (Figure 4). For wastewater samples, the apparatus should accept 90 or 144 mm disks. For drinking water or other samples containing low solids, smaller disks may be used.

6.4.1.5.2 Vacuum source – Capable of maintaining 25 in. Hg, equipped with shutoff valve and vacuum gauge

6.4.1.5.3 Glass-fiber filter – Whatman GMF 150 (or equivalent), 1 micron pore size, to fit filtration apparatus in Section 6.4.1.5.1

6.4.1.5.4 Solid-phase extraction disk containing octadecyl (C_{18}) bonded silica uniformly enmeshed in an inert matrix – Fisher Scientific 14-378F (or equivalent), to fit filtration apparatus in Section 6.4.1.5.1

6.4.1.6 Continuous liquid/liquid extraction (CLLE) – Fluoropolymer or glass connecting joints and stopcocks without lubrication, 1.5-2 L capacity (Hershberg-Wolf Extractor, Cal-Glass, Costa Mesa, California, 1000 mL or 2000 mL, or equivalent)

6.4.2 Soxhlet/Dean-Stark (SDS) extractor (Figure 5 and Reference 3) for filters and solid/sludge samples

6.4.2.1 Soxhlet – 50-mm ID, 200-mL capacity with 500-mL flask (Cal-Glass LG-6900, or equivalent, except substitute 500-mL round-bottom flask for 300-mL flat-bottom flask)

6.4.2.2 Thimble – 43 H 123 to fit Soxhlet (Cal-Glass LG-6901-122, or equivalent)

6.4.2.3 Moisture trap – Dean Stark or Barret with fluoropolymer stopcock, to fit Soxhlet

6.4.2.4 Heating mantle – Hemispherical, to fit 500-mL round-bottom flask (Cal-Glass LG-8801-112, or equivalent)

6.4.2.5 Variable transformer – Powerstat (or equivalent), 110-volt, 10-amp

6.4.3 Beakers – 400- to 500-mL

6.4.4 Spatulas – Stainless steel

6.5 Filtration apparatus

6.5.1 Pyrex glass wool – Solvent-extracted using a Soxhlet or SDS extractor for 3 hours minimum

6.5.2 Glass funnel – 125- to 250-mL

6.5.3 Glass-fiber filter paper – Whatman GF/D (or equivalent), to fit glass funnel in Section 6.5.2.

6.5.4 Drying column – 15- to 20-mm ID Pyrex chromatographic column equipped with coarse-glass frit or glass-wool plug

6.5.5 Buchner funnel – 15-cm

6.5.6 Glass-fiber filter paper for Buchner funnel above

6.5.7 Filtration flasks – glass, 1.5- to 2.0-L, with side arm

6.5.8 Pressure filtration apparatus – Millipore YT30 142 HW, or equivalent

6.6 Centrifuge apparatus

6.6.1 Centrifuge – Capable of rotating 500-mL centrifuge bottles or 15-mL centrifuge tubes at 5,000 rpm minimum

6.6.2 Centrifuge bottles – 500-mL, with screw-caps, to fit centrifuge

6.6.3 Centrifuge tubes – 12- to 15-mL, with screw-caps, to fit centrifuge

6.7 Cleanup apparatus

6.7.1 Automated gel permeation chromatograph (Analytical Biochemical Labs, Inc, Columbia, MO, Model GPC Autoprep 1002, or equivalent)

6.7.1.1 Column – 600-700 mm long H 25 mm ID glass, packed with 70 g of 200-400 mesh SX-3 Bio-beads (Bio-Rad Laboratories, Richmond, CA, or equivalent)

6.7.1.2 Syringe – 10-mL, with Luer fitting

6.7.1.3 Syringe filter holder – stainless steel, and glass-fiber or fluoropolymer filters (Gelman 4310, or equivalent)

6.7.1.4 UV detectors – 254-nm, preparative or semi-preparative flow cell (Isco, Inc., Type 6; Schmadzu, 5-mm path length; Beckman-Altex 152W, 8-µL micro-prep flow cell, 2-mm path; Pharmacia UV-1, 3-mm flow cell; LDC Milton-Roy UV-3, monitor #1203; or equivalent)

6.7.2 Reverse-phase high-performance liquid chromatograph (Reference 9)

6.7.2.1 Pump – Perkin-Elmer Series 410, or equivalent

6.7.2.2 Injector – Perkin-Elmer ISS-100 Autosampler, or equivalent

6.7.2.3 6-Port switching valve – Valco N60, or equivalent

6.7.2.4 Column – Hypercarb, 100 x 4.6 mm, 5 Φm particle size, Keystone Scientific, or equivalent

6.7.2.5 Detector – Altex 110A (or equivalent) operated at 0.02 AUFS at 235 nm

6.7.2.6 Fraction collector – Isco Foxy II, or equivalent

6.7.3 Pipets, precleaned

6.7.3.1 Disposable, Pasteur, 150-mm long x 5-mm ID (Fisher Scientific 13-678-6A, or equivalent)

6.7.3.2 Disposable, serological, 50-mL (8- to 10- mm ID)

6.7.4 Glass chromatographic columns

6.7.4.1 150-mm long x 8-mm ID, (Kontes K-420155, or equivalent) with coarse-glass frit or glass-wool plug and 250-mL reservoir

6.7.4.2 200-mm long x 15-mm ID, with coarse-glass frit or glass-wool plug and 250-mL reservoir

6.7.4.3 300-mm long x 22-mm ID, with coarse-glass frit, 300-mL reservoir, and glass or fluoropolymer stopcock

6.7.5 Oven – For baking and storage of adsorbents, capable of maintaining a constant temperature (∀ 5°C) in the range of 105-250°C

6.7.6 System for solid-phase extraction

6.7.6.1 Vac-Elute Manifold (Analytichem International, or equivalent)

6.7.6.2 Vacuum trap: Made from 500-mL sidearm flask fitted with single-hole rubber stopper and glass tubing

6.7.6.3 Rack for holding 50-mL volumetric flasks in the manifold

6.8 Concentration apparatus

6.8.1 Rotary evaporator – Buchi/Brinkman-American Scientific No. E5045-10 or equivalent, equipped with a variable temperature water bath

6.8.1.1 Vacuum source for rotary evaporator equipped with vacuum gauge and with shutoff valve at the evaporator

6.8.1.2 A recirculating water pump and chiller are recommended. Use of tap water for cooling the evaporator wastes large volumes of water and can lead to inconsistent performance as water temperatures and pressures vary.

6.8.1.3 Round-bottom flask – 100-mL and 500-mL or larger, with ground-glass fitting compatible with the rotary evaporator

6.8.2 Kuderna-Danish (K-D) concentrator

6.8.2.1 Concentrator tube – 10-mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

6.8.2.2 Evaporation flask – 500-mL (Kontes K-570001-0500, or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012 or equivalent)

6.8.2.3 Snyder column – Three-ball macro (Kontes K-503000-0232, or equivalent)

6.8.2.4 Boiling chips

6.8.2.4.1 Glass or silicon carbide – Approximately 10/40 mesh, extracted with methylene chloride and baked at 450°C for one hour minimum

6.8.2.4.2 Fluoropolymer (optional) – Extracted with methylene chloride

6.8.2.5 Water bath – Heated, with concentric ring cover, capable of maintaining a temperature within ∀ 2°C, installed in a fume hood

6.8.3 Nitrogen evaporation apparatus – Equipped with water bath controlled in the range of 30 - 60°C (N-Evap, Organomation Associates, Inc., South Berlin, MA, or equivalent), installed in a fume hood

6.8.4 Sample vials

6.8.4.1 Amber glass, 2- to 5-mL with fluoropolymer-lined screw-cap

6.8.4.2 Glass, 0.3-mL, conical, with fluoropolymer-lined screw or crimp cap

6.9 Gas chromatograph – Must have splitless or on-column injection port for capillary column, temperature program with isothermal hold, and must meet all of the performance specifications in Section 10.

6.9.1 GC column – 60 ∀ 5-m long x 0.25 ∀ 0.02-mm ID; 0.10-µm film DB-17, or equivalent

6.9.1.1 The column must meet the following minimum retention time and resolution criteria, and must be adjusted or replaced when these criteria are not met:

6.9.1.1.1 The retention time for methoxychlor must be greater than 39 minutes.

6.9.1.1.2 trans-chlordane and trans-nonachlor (or the labeled analogs) must be uniquely resolved to a valley height less than 10 percent of the shorter of the two peaks.

6.9.1.2 Endrin and DDT breakdown – The column must meet the endrin/DDT breakdown criteria in Section 10.6.2.3. Some GC injectors may be unable to meet requirements for endrin and DDT breakdown. This problem can be minimized by operating the injector at 200 - 205 °C, using a Pyrex (not quartz) methyl silicone deactivated injector liner, and deactivating the injector with dichlorodimethylsilane. A temperature

programmed injector has also been shown to minimize decomposition of labile substances such as endrin and DDT (Reference 10).

6.10 Mass spectrometer – 28- to 40-eV electron impact ionization, must be capable of selectively monitoring a minimum of 22 exact m/z's minimum at high resolution (greater than 8,000) during a period less than 1.5 seconds, and must meet all of the performance specifications in Section 10.

6.11 GC/MS interface – The mass spectrometer (MS) must be interfaced to the GC such that the end of the capillary column terminates within 1 cm of the ion source but does not intercept the electron or ion beams.

6.12 Data system – Capable of collecting, recording, storing, and processing MS data

6.12.1 Data acquisition – The signal at each exact m/z must be collected repetitively throughout the monitoring period and stored on a mass storage device.

6.12.2 Response factors and multipoint calibrations – The data system must record and maintain lists of response factors (response ratios for isotope dilution) and multipoint calibrations. Computations of relative standard deviation (RSD) are be used to test calibration linearity. Statistics on initial (Section 9.4) and ongoing (Section 15.6.4) performance should be computed and maintained, either on the instrument data system, or on a separate computer system.

7.0 Reagents and standards

7.1 pH adjustment and back-extraction

7.1.1 Potassium hydroxide (KOH) – Dissolve 20 g reagent grade KOH in 100 mL reagent water.

7.1.2 Sulfuric acid (H_2SO_4) – Reagent grade (specific gravity 1.84)

7.1.3 Hydrochloric acid – Reagent grade, 6N

7.1.4 Sodium chloride solution – Prepare at 5% (w/v) solution in reagent water

7.1.4 Sodium sulfate solution – Prepare at 2% (w/v) in reagent water; pH adjust to 8.5 - 9.0 with KOH or H_2SO_4

7.2 Solution and tissue drying, municipal sludge extract back-extraction, and solvent evaporation (blowdown)

7.2.1 Solution drying – Sodium sulfate, reagent grade, granular, anhydrous (Baker 3375, or equivalent), rinsed with methylene chloride (20 mL/g), baked at 400°C for 1 hour minimum, cooled in a desiccator, and stored in a pre-cleaned glass bottle with screw-cap that prevents moisture from entering. If, after heating, the sodium

sulfate develops a noticeable grayish cast (due to the presence of carbon in the crystal matrix), that batch of reagent is not suitable for use and should be discarded. Extraction with methylene chloride (as opposed to simple rinsing) and baking at a lower temperature may produce sodium sulfate that is suitable for use.

7.2.2 Tissue drying – Sodium sulfate, reagent grade, powdered, treated and stored as in Section 7.2.1

7.2.3 Solution for back-extraction of municipal sludge extracts – Sodium sulfate solution: 2% (w/v) in reagent water, pH adjusted to pH 8.5 to 9.0 with KOH or H_2SO_4

7.2.4 Prepurified nitrogen

7.3 Extraction

7.3.1 Solvents – Acetone, toluene, cyclohexane, hexane, methanol, methylene chloride, isooctane, and nonane; distilled in glass, pesticide quality, lot-certified to be free of interferences

7.3.2 White quartz sand, 60/70 mesh – For Soxhlet/Dean-Stark extraction (Aldrich Chemical, Cat. No. 27-437-9, or equivalent). Bake at 450 – 500°C for 4 hours minimum.

7.4 GPC calibration solution – Prepare a solution containing 2.5 mg/mL corn oil, 0.05 mg/mL bis(2-ethylhexyl) phthalate (BEHP), 0.01 mg/mL methoxychlor, 0.002 mg/mL perylene, and 0.008 mg/mL sulfur, or at concentrations appropriate to the response of the detector.

7.5 Adsorbents for sample cleanup

7.5.1 Silica gel

7.5.1.1 Activated silica gel – 100-200 mesh, Supelco 1-3651 (or equivalent), mesh, rinsed with methylene chloride, baked at 180±5 °C for a minimum of 1 hour, cooled in a desiccator, and stored in a precleaned glass bottle with screw-cap that prevents moisture from entering.

7.5.1.1.1 10% deactivated silica – Place 100 g of activated silica gel (Section 7.5.1.1) in a clean glass bottle or jar and add 10 g (or mL) of reagent water. Cap the bottle tightly to prevent moisture from entering or escaping.

7.5.1.1.2 Tumble the bottle for 5 - 10 hours to thoroughly mix the water and silica. Keep bottle tightly sealed when silica is not being removed for use.

7.5.1.2 Acid silica gel (30% w/w) – Thoroughly mix 44 g of concentrated sulfuric acid with 100 g of activated silica gel in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a screw-capped bottle with fluoropolymer-lined cap.

7.5.1.3 Basic silica gel – Thoroughly mix 30 g of 1N sodium hydroxide with 100 g of activated silica gel in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a screw-capped bottle with fluoropolymer-lined cap.

7.5.1.4 Potassium silicate

7.5.1.4.1 Dissolve 56 g of high purity potassium hydroxide (Aldrich, or equivalent) in 300 mL of methanol in a 750- to 1000-mL flat-bottom flask.

7.5.1.4.2 Add 100 g of activated silica gel (Section 7.5.1.1) and a stirring bar, and stir on an explosion-proof hot plate at 60-70°C for 1-2 hours.

7.5.1.4.3 Decant the liquid and rinse the potassium silicate twice with 100-mL portions of methanol, followed by a single rinse with 100 mL of methylene chloride.

7.5.1.4.4 Spread the potassium silicate on solvent-rinsed aluminum foil and dry for 2-4 hours in a hood. Observe the precaution in Section 5.3.2.

7.5.1.4.5 Activate overnight at 200-250°C prior to use.

7.5.2 Anthropogenic isolation column – Pack the column in Section 6.7.4.3 from bottom to top with the following:

7.5.2.1 2 g silica gel (Section 7.5.1.1)

7.5.2.2 2 g potassium silicate (Section 7.5.1.4)

7.5.2.3 2 g granular anhydrous sodium sulfate (Section 7.2.1)

7.5.2.4 10 g acid silica gel (Section 7.5.1.2)

7.5.2.5 2 g granular anhydrous sodium sulfate

7.5.3 Aminopropyl solid-phase extraction (SPE) column – 1 g aminopropyl-bonded silica (Varian NH2, or equivalent).

Note: *Other SPE columns (e.g., C_{18} octadecyl, cyanopropyl) may be used provided the laboratory establishes the elution conditions and meets the requirements in Section 9.2 with the SPE column as an integral part of the analysis.*

7.5.4 Florisil column

7.5.4.1 Florisil – PR grade, 60-100 mesh (U.S. Silica Corp, Berkeley Springs, WV, or equivalent). Alternatively, prepacked Florisil columns may be used. Use the following procedure for Florisil activation and column packing.

7.5.4.2 Fill a clean 1- to 2-L bottle 1/2 to 2/3 full with Florisil and place in an oven at 130-150°C for a minimum of three days to activate the Florisil.

7.5.4.3 Immediately prior to use, dry pack a 300-mm x 22-mm ID glass column (Section 6.7.4.3) bottom to top with 0.5-1.0 cm of warm to hot anhydrous sodium sulfate (Section 7.2.1), 10-10.5 cm of warm to hot activated Florisil (Section 7.5.4.2), and 1-2 cm of warm to hot anhydrous sodium sulfate. Allow the column to cool and pre-elute immediately with 100 mL of n-hexane. Keep column wet with hexane to prevent water from entering.

7.5.4.4 Using the procedure in Section 13.7.3, establish the elution pattern for each carton of Florisil or each lot of Florisil columns received.

7.5.5 Alumina column

7.5.5.1 Alumina – Neutral, Brockman Activity I, 80-200 mesh (Fisher Scientific Certified, or equivalent). Heat for 16 hours at 400 to 450°C. Seal and cool to room temperature. Add 7% (W/W) reagent water and tumble for 1 to 2 hours. Keep bottle tightly sealed.

7.5.5.2 Immediately prior to use, partially fill a 150-mm x 8-mm ID glass column (Section 6.7.4.1) with n-hexane. Pack the column bottom to top with 0.5 - 1 cm of warm to hot anhydrous sodium sulfate (Section 7.2.1), 10 - 10.5 cm alumina (Section 7.5.5.1) and 1 - 1.5 cm of warm to hot anhydrous sodium sulfate. Allow the column to cool and pre-elute immediately with 100 mL of hexane. Keep column wet with hexane to prevent moisture from entering.

7.6 Reference matrices – Matrices in which the pesticides and interfering compounds are not detected by this Method

7.6.1 Reagent water – Bottled water purchased locally, or prepared by passage through activated carbon

7.6.2 High-solids reference matrix – Playground sand or similar material. Prepared by extraction with methylene chloride and/or baking at 450°C for a minimum of 4 hours.

7.6.3 Paper reference matrix – Glass-fiber filter, Gelman type A, or equivalent. Cut paper to simulate the surface area of the paper sample being tested.

7.6.4 Tissue reference matrix – Corn or other vegetable oil.

7.6.5 Other matrices – This Method may be verified on any reference matrix by performing the tests given in Section 9.2. Ideally, the matrix should be free of the pesticides, but in no case must the background level of the pesticides in the reference matrix exceed the minimum levels in Table 1. If low background levels of the pesticides are present in the reference matrix, the spike level of the analytes used in Section 9.2 should be increased to provide a spike-to-background ratio of approximately 5 (Reference 11).

7.7 Standard solutions – Prepare from materials of known purity and composition or purchase as solutions or mixtures with certification to their purity, concentration, and authenticity. If the chemical purity is 98 % or greater, the weight may be used without correction to calculate the concentration of the standard. Observe the safety precautions in Section 5 and the recommendation in Section 5.1.2.

7.7.1 For preparation of stock solutions from neat materials, dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 10 to 20 mg of lindane to three significant figures in a 10-mL ground-glass-stoppered volumetric flask and fill to the mark with nonane. After the compound is completely dissolved, transfer the solution to a clean 15-mL vial with fluoropolymer-lined cap.

7.7.2 When not being used, store standard solutions in the dark at room temperature in screw-capped vials with fluoropolymer-lined caps. Place a mark on the vial at the level of the solution so that solvent loss by evaporation can be detected. Replace the solution if solvent loss has occurred.

7.8 Native (unlabeled) stock solutions

7.8.1 Native stock solution – Prepare to contain the pesticides at the concentrations shown in Table 3, or purchase prepared solutions. If additional pesticides are to be determined, include the additional native compounds in this stock solution.

7.8.2 Stock solutions should be checked for signs of degradation (e.g., discoloration, precipitation) prior to preparing calibration or performance test standards. Reference standards that can be used to determine the accuracy of standard solutions are available from several vendors.

7.9 Labeled compound stock solutions (Table 3)

7.9.1 Labeled pesticide stock solution – Prepare the labeled pesticides in isooctane or nonane at the concentrations in Table 3 or purchase prepared standards. If additional pesticides are to be determined by isotope dilution, include the additional labeled compounds in this stock solution.

7.9.2 Labeled injection internal standard stock solution – Prepare labeled PCB 52 in nonane or isooctane at the concentration shown in Table 3, or purchase a prepared standard.

7.10 Calibration standards – Combine and dilute the solutions in Sections 7.8 and 7.9 to produce the calibration solutions in Table 4 or purchase prepared standards for the CS-1 to CS-6 set of calibration solutions. These solutions permit the relative response (labeled to native) and response factor to be measured as a function of concentration. The CS-4 standard is used for calibration verification (VER).

7.11 Native IPR/OPR standard spiking solution – Used for determining initial precision and recovery (IPR; Section 9.2) and ongoing precision and recovery (OPR; Section 15.6). Dilute the Native stock solution (Section 7.8.1) with acetone to produce the concentrations of the pesticides as shown in Table 3. When 1 mL of this solution is spiked into the IPR (Section 9.2.1) or OPR (Section 15.6) and concentrated to a final volume of 20 µL, the concentration of the pesticides in the final volume will be either 8 or 20 ng/mL (pg/ΦL), as shown in Table 3. Prepare only the amount necessary for each reference matrix with each sample batch.

7.12 Labeled standard spiking solution – This solution is spiked into each sample (Section 9.3) and into the IPR (Section 9.2.1), OPR (Section 15.6), and blank (Section 9.5) to measure recovery. Dilute the Labeled pesticide stock solution (Section 7.9.1) with acetone to produce the concentrations of the labeled compounds shown in Table 3. When 1 mL of this solution is spiked into an IPR, OPR, blank, or sample and concentrated to a final extract volume of 20 µL, the concentration in the final volume will be as shown in Table 3. Prepare only the amount necessary for each reference matrix with each sample batch.

7.13 Endrin/4,4'-DDT breakdown solution – Prepare a solution to contain 100 ng/mL (pg/µL) of DDT and 50 ng/mL (pg/µL) of endrin in isooctane or nonane. This solution is to determine endrin/4,4'-DDT breakdown in Sections 10.6 and 15.5.

7.14 Labeled injection internal standard spiking solution – This solution is added to each concentrated extract prior to injection into the HRGC/HRMS. Dilute the Labeled injection internal standard stock solution (Section 7.9.2) in nonane to produce a concentration of the injection internal standards at 800 ng/mL, as shown in Table 3. When 2 µL of this solution is spiked into a 20 µL extract, the concentration of each injection internal standard will be nominally 80 ng/mL (pg/µL), as shown in Table 3.

Note: *The addition of 2 µL of the Labeled injection internal standard spiking solution to a 20 µL final extract has the effect of diluting the concentration of the components in the extract by 10%.*

Provided all calibration solutions and all extracts undergo this dilution as a result of adding the Labeled injection internal standard spiking solution, the effect of the 10% solution is compensated, and correction for this dilution should not be made.

7.15 QC Check Sample – A QC Check Sample should be obtained from a source independent of the calibration standards. Ideally, this check sample would be a certified Standard Reference Material (SRM) containing the pesticides in known concentrations in a sample matrix similar to the matrix under test. The National Institute of Standards and Technology (NIST) in Gaithersburg, Maryland has SRMs, and the Institute for National Measurement Standards of the National Research Council of Canada in Ottawa has certified reference materials (CRMs), for pesticides in various matrices.

7.16 Stability of solutions – Standard solutions used for quantitative purposes (Sections 7.8 - 7.14) should be assayed periodically (e.g., every 6 months) against SRMs from NIST (if available), or certified reference materials from a source that will attest to the authenticity and concentration, to assure that the composition and concentrations have not changed.

8.0 Sample collection, preservation, storage, and holding times

8.1 Collect samples in amber glass containers following conventional sampling practices (Reference 12); collect field and trip blanks as necessary to validate the sampling.

8.2 Aqueous samples

8.2.1 Samples that flow freely are collected as grab samples or in refrigerated bottles using automatic sampling equipment. Collect 1-L. If high concentrations of the pesticides are expected, collect a smaller volume (e.g., 100 mL) in addition to the 1-L sample. Do not rinse the bottle with sample before collection.

8.2.2 If residual chlorine is present, add 80 mg sodium thiosulfate per liter of water. Any method suitable for field use may be employed to test for residual chlorine (Reference 9).

8.2.3 Maintain aqueous samples in the dark at <6°C from the time of collection until receipt at the laboratory (see 40 CFR 136.6(e), Table II). If the sample will be frozen, allow room for expansion.

8.2.4 If the sample will not be analyzed within 72 hours, adjust the pH to a range of 5.0 to 9.0 with sodium hydroxide or sulfuric acid solution. Record the volume of acid or base used.

8.3 Solid, mixed-phase, semi-solid, and oily samples, excluding tissue.

8.3.1 Collect samples as grab samples using wide-mouth jars. Collect a sufficient amount of wet material to produce a minimum of 20 g of solids.

8.3.2 Maintain solid, semi-solid, oily, and mixed-phase samples in the dark at <6°C from the time of collection until receipt at the laboratory. Store solid, semi-solid, oily, and mixed-phase samples in the dark at less than -10°C.

8.4 Fish and other tissue samples

8.4.1 Fish may be cleaned, filleted, or processed in other ways in the field, such that the laboratory may expect to receive whole fish, fish fillets, or other tissues for analysis.

8.4.2 Collect fish, wrap in aluminum foil, and maintain at <6°C from the time of collection until receipt at the laboratory, to a maximum time of 24 hours. If a longer transport time is necessary, freeze the sample. Ideally, fish should be frozen upon collection and shipped to the laboratory under dry ice.

8.4.3 Freeze tissue samples upon receipt at the laboratory and maintain in the dark at less than -10°C until prepared. Maintain unused sample in the dark at less than -10°C.

8.4.4 Store sample extracts in the dark at less than -10°C until analyzed.

8.5 Holding times – See 40 CFR 136.3(e) Table II

8.5.1 Aqueous samples – Extract within 7 days of collection, and analyze within 40 days of extraction.

8.5.2 Solid, mixed-phase, semi-solid, tissue, and oily samples – Extract and analyze within 1 year of collection. If a sample is to be stored for more than 14 days, and results are to be reported in solids units, either hermetically seal the sample container or determine the moisture content upon receipt and immediately prior to analysis. Adjust the final concentration based on the original moisture content.

9.0 Quality assurance/quality control

9.1 Each laboratory that uses this Method is required to operate a formal quality assurance program (Reference 14). The minimum requirements of this program consist of an initial demonstration of laboratory capability, analysis of samples spiked with labeled compounds to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the Method.

If the Method is to be applied to a sample matrix other than water (e.g., soils, filter cake, compost, tissue) the most appropriate alternate reference matrix (Sections 7.6.2 - 7.6.5 and 7.15) is substituted for the reagent water matrix (Section 7.6.1) in all performance tests.

9.1.1 The laboratory must make an initial demonstration of the ability to generate acceptable precision and recovery with this Method. This demonstration is given in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, and to overcome matrix interferences, the laboratory is permitted certain options to improve separations or lower the costs of measurements. These options include alternate extraction, concentration, and cleanup procedures, and changes in columns and detectors (see also 40 CFR 136.6). Alternate determinative techniques, such as the substitution of spectroscopic or immuno-assay techniques, and changes that degrade Method performance, are not allowed. If an analytical technique other than the techniques specified in this Method is used, that technique must have a specificity equal to or greater than the specificity of the techniques in this Method for the analytes of interest.

9.1.2.1 Each time a modification is made to this Method, the laboratory is required to repeat the procedure in Section 9.2. If the detection limit of the Method will be affected by the change, the laboratory is required to demonstrate that the MDLs (40 CFR Part 136, Appendix B) are lower than one-third the regulatory compliance level or the MDLs in this Method, whichever are greater. If calibration will be affected by the change, the instrument must be recalibrated per Section 10. Once the modification is demonstrated to produce results equivalent or superior to results produced by this Method as written, that modification may be used routinely thereafter, so long as the other requirements in this Method are met (e.g., labeled compound recovery).

9.1.2.2 The laboratory is required to maintain records of modifications made to this Method. These records include the following, at a minimum:

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) that performed the analyses and modification, and of the quality control officer that witnessed and will verify the analyses and modifications.

9.1.2.2.2 A listing of pollutant(s) measured, by name and CAS Registry number.

9.1.2.2.3 A narrative stating reason(s) for the modifications.

9.1.2.2.4 Results from all quality control (QC) tests comparing the modified method to this Method, including:

a) Calibration (Section 10)
b) Calibration verification (Section 15.3)
c) Initial precision and recovery (Section 9.2)
d) Labeled compound recovery (Section 9.3)

e) Analysis of blanks (Section 9.5)
f) Accuracy assessment (Section 9.4)

9.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:

a) Sample numbers and other identifiers
b) Extraction dates
c) Analysis dates and times
d) Analysis sequence/run chronology
e) Sample weight or volume (Section 11)
f) Extract volume prior to each cleanup step (Section 13)
g) Extract volume after each cleanup step (Section 13)
h) Final extract volume prior to injection (Section 14)
i) Injection volume (Sections 10.3 and 14.3)
j) Dilution data, differentiating between dilution of a sample or extract (Section 17.5)
k) Instrument and operating conditions
l) Column (dimensions, liquid phase, solid support, film thickness, etc)
m) Operating conditions (temperatures, temperature program, flow rates)
n) Detector (type, operating conditions, etc)
o) Chromatograms, printer tapes, and other recordings of raw data
p) Quantitation reports, data system outputs, and other data to link the raw data to the results reported

9.1.2.3 Alternate HRGC columns and column systems – If a column or column system alternate to those specified in this Method is used, that column or column system must meet the requirements in Section 6.9.1.

9.1.3 Analyses of method blanks are required to demonstrate freedom from contamination (Section 4.3). The procedures and criteria for analysis of a method blank are given in Sections 9.5 and 15.7.

9.1.4 The laboratory must spike all samples with labeled compounds to monitor Method performance. This test is described in Section 9.3. When results of these spikes indicate atypical Method performance for samples, the samples are diluted to bring Method performance within acceptable limits. Procedures for dilution are given in Section 17.5.

9.1.5 The laboratory must, on an ongoing basis, demonstrate through calibration verification and the analysis of the ongoing precision and recovery standard (OPR) and

blanks that the analytical system is in control. These procedures are given in Sections 15.1 through 15.7.

9.1.6 The laboratory should maintain records to define the quality of data generated. Development of accuracy statements is described in Sections 9.4 and 15.6.4.

9.2 Initial precision and recovery (IPR) – To establish the ability to generate acceptable precision and recovery, the laboratory must perform the following operations:

9.2.1 For low solids (aqueous) samples, extract, concentrate, and analyze four 1-L aliquots of reagent water spiked with 1 mL each of the Native spiking solution (Section 7.11) and the Labeled spiking solution (Section 7.12), according to the procedures in Sections 11 through 18. For an alternate sample matrix, four aliquots of the alternate reference matrix (Sections 7.6.1 - 7.6.5) are used. All sample processing steps that are to be used for processing samples, including preparation (Section 11), extraction (Section 12), and cleanup (Section 13), must be included in this test.

9.2.2 Using results of the set of four analyses, compute the average percent recovery (X) of the extracts and the relative standard deviation (RSD) of the concentration for each compound, by isotope dilution for pesticides with a labeled analog, and by internal standard for pesticides without a labeled analog and for the labeled compounds.

9.2.3 For each pesticide and labeled compound, compare RSD and X with the corresponding limits for initial precision and recovery in Table 5. If RSD and X for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual RSD exceeds the precision limit or any individual X falls outside the range for recovery, system performance is unacceptable for that compound. Correct the problem and repeat the test (Section 9.2).

9.3 To assess Method performance on the sample matrix, the laboratory must spike all samples with the Labeled spiking solution (Section 7.12).

9.3.1 Analyze each sample according to the procedures in Sections 11 through 18.

9.3.2 Compute the percent recovery of the labeled pesticides using the internal standard method (Section 17.2).

9.3.3 The recovery of each labeled compound must be within the limits in Table 5. If the recovery of any compound falls outside of these limits, Method performance is unacceptable for that compound in that sample. Additional cleanup procedures must then be employed to attempt to bring the recovery within the normal range. If the recovery cannot be brought within the normal range after all cleanup procedures have been employed, water samples are diluted and smaller amounts of soils, sludges, sediments, and other matrices are analyzed per Section 18.

9.4 It is suggested but not required that recovery of labeled compounds from samples be assessed and records maintained.

9.4.1 After the analysis of 30 samples of a given matrix type (water, soil, sludge, pulp, etc.) for which the labeled compounds pass the tests in Section 9.3, compute the average percent recovery (R) and the standard deviation of the percent recovery (S_R) for the labeled compounds only. Express the assessment as a percent recovery interval from R ! $2S_R$ to R + $2S_R$ for each matrix. For example, if R = 90% and S_R = 10% for five analyses of pulp, the recovery interval is expressed as 70 to 110%.

9.4.2 Update the accuracy assessment for each labeled compound in each matrix on a regular basis (e.g., after each five to ten new measurements).

9.5 Method blanks – A reference matrix Method blank is analyzed with each sample batch (Section 4.3) to demonstrate freedom from contamination. The matrix for the Method blank must be similar to the sample matrix for the batch, e.g., a 1-L reagent water blank (Section 7.6.1), high-solids reference matrix blank (Section 7.6.2), paper matrix blank (Section 7.6.3); tissue blank (Section 7.6.4), or alternate reference matrix blank (Section 7.6.5).

9.5.1 Spike 1.0 mL each of the Labeled spiking solution (Section 7.12) into the Method blank, according to the procedures in Sections 11 through 18. Prepare, extract, clean up, and concentrate the Method blank. Analyze the blank immediately after analysis of the OPR (Section 15.6) to demonstrate freedom from contamination.

9.5.2 If any pesticide (Table 1) is found in the blank at greater than the minimum level (Table 1) or one-third the regulatory compliance limit, whichever is greater; or if any potentially interfering compound is found in the blank at the minimum level for each pesticide in Table 1 (assuming a response factor of 1 relative to the quantitation reference in Table 2 for a potentially interfering compound; i.e., a compound not listed in this Method), analysis of samples must be halted until the sample batch is re-extracted and the extracts re-analyzed, and the blank associated with the sample batch shows no evidence of contamination at these levels. All samples must be associated with an uncontaminated Method blank before the results for those samples may be reported or used for permitting or regulatory compliance purposes.

9.6 QC Check Sample – Analyze the QC Check Sample (Section 7.15) periodically to assure the accuracy of calibration standards and the overall reliability of the analytical process. It is suggested that the QC Check Sample be analyzed at least quarterly.

9.7 The specifications contained in this Method can be met if the apparatus used is calibrated properly and then maintained in a calibrated state. The standards used for calibration (Section 10), calibration verification (Section 15.3), and for initial (Section 9.2) and ongoing (Section 15.6) precision and recovery should be identical, so that the most precise results will be obtained. A GC/HRMS instrument will provide the most reproducible

results if dedicated to the settings and conditions required for determination of pesticides by this Method.

9.8 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and spiked samples may be required to determine the accuracy of the analysis when the internal standard method is used.

10.0 Calibration

10.1 Establish the operating conditions necessary to meet the retention times (RTs) and relative retention times (RRTs) for the pesticides in Table 2.

10.1.1 Suggested operating conditions:

GC conditions	
Injector	Split/splitless, 2 min
Carrier gas	Helium @ 200 kPa
Injector temperature	180 - 220°C or temperature programmed
Maximum column temperature	300°C
GC Temperature program	
Initial temperature and hold	50°C for 1 minute
Initial ramp	50 - 180°C @ 10°C per minute
Second hold	180°C for 0 minute
Second ramp	180 - 200°C @ 1.5°C per minute
Third hold	200°C for 2 minutes
Third ramp	200 - 295°C @ 6°C per minute
Final hold	295°C for 1 minutes or until methoxychlor elutes
Interface temperature	290°C
Mass spectrometer conditions	
Source temperature	250°C
Electron energy	35 eV
Trap current	500 - 900 ΦA
Mass resolution	8000
Detector potential	340 - 400 V

10.1.1.1 All portions of the column that connect the GC to the ion source should remain at or above the interface temperature during analysis to preclude condensation of less volatile compounds.

10.1.1.2 The GC conditions may be optimized for compound separation and sensitivity. Once optimized, the same GC conditions must be used for the analysis of all standards, blanks, IPR and OPR standards, and samples.

10.1.2 Retention time calibration for the native and labeled pesticides

10.1.2.1 Inject the CS-4 calibration standard (Section 7.10 and Table 4). Establish the beginning and ending retention times for the scan descriptors in Table 6. Scan descriptors other than those listed in Table 6 may be used provided the MLs in Table 1 are met. Store the retention time (RT) and relative retention time (RRT) for each compound in the data system.

10.1.2.2 The absolute retention time of methoxychlor must exceed 39 minutes on the DB-17 column; otherwise, the GC temperature program must be adjusted and this test repeated until the minimum retention time criterion is met. If a GC column or column system alternate to the DB-17 column is used, a similar minimum retention time specification must be established for the alternate column or column systems so that interferences that may be encountered in environmental samples will be resolved from the analytes of interest. This specification is deemed to be met if the retention time of methoxychlor is greater than 39 minutes on such alternate column.

10.2 Mass spectrometer (MS) resolution

10.2.1 Using PFK (or other reference substance) and a molecular leak, tune the instrument to meet the minimum required resolving power of 8,000 (10% valley) at m/z 280.9825 or other significant PFK fragment in the range of 250 - 300. For each descriptor (Table 6), monitor and record the resolution and exact m/z's of three to five reference peaks covering the mass range of the descriptor. The level of PFK (or other reference substance) metered into the HRMS during analyses should be adjusted so that the amplitude of the most intense selected lock-mass m/z signal (regardless of the descriptor number) does not exceed 10% of the full-scale deflection for a given set of detector parameters. Under those conditions, sensitivity changes that might occur during the analysis can be more effectively monitored.

Note: *Different lots and types of PFK can contain varying levels of contamination, and excessive PFK (or other reference substance) may cause noise problems and contamination of the ion source necessitating increased frequency of source cleaning.*

10.2.2 The analysis time for the pesticides may exceed the long-term mass stability of the mass spectrometer. Because the instrument is operated in the high-resolution mode, mass drifts of a few ppm (e.g., 5 ppm in mass) can have serious adverse effects on instrument performance. Therefore, mass-drift correction is mandatory and a lock-mass m/z from perfluorokerosene (PFK) or other reference substance is used for drift correction. The lock-mass m/z is dependent on the exact m/z's monitored within each descriptor, as shown in Table 6. The deviation between each monitored exact m/z and the theoretical m/z (Table 6) must be less than 5 ppm.

10.2.3 Obtain a selected ion current profile (SICP) at the two exact m/z's specified in Table 6 and at ∃8,000 resolving power for each native and labeled pesticide. Because of the extensive mass range covered in each function, it may not be possible to maintain 8,000 resolution throughout the mass range during the function. Therefore, resolution must be ∃6,000 throughout the mass range and must be ∃8,000 in the center of the mass range for each function.

10.2.4 If the HRMS has the capability to monitor resolution during the analysis, it is acceptable to terminate the analysis when the resolution falls below the minimum (Section 10.2.1 and 10.2.3) to save re-analysis time.

10.3 Ion abundance ratios, minimum levels, and signal-to-noise ratios during calibration. Choose an injection volume of either 1 or 2 µL, consistent with the capability of the HRGC/HRMS instrument. Inject a 1 or 2 µL aliquot of the CS-1 calibration solution (Table 4) using the GC conditions in Section 10.1.1.

10.3.1 Measure the SICP areas for each pesticide, and compute the ion abundance ratios at the exact m/z's specified in Table 6. Compare the computed ratio to the theoretical ratio given in Table 6.

10.3.1.1 The exact m/z's to be monitored in each descriptor are shown in Table 6. Each group or descriptor must be monitored in succession as a function of GC retention time to ensure that the pesticides are detected. Additional m/z's may be monitored in each descriptor, and the m/z's may be divided among more than the descriptors listed in Table 6, provided that the laboratory is able to monitor the m/z's of all pesticides that may elute from the GC in a given RT window.

10.3.1.2 The mass spectrometer must be operated in a mass-drift correction mode, using PFK (or other reference substance) to provide lock m/z's. The lock mass for each group of m/z's is shown in Table 6. Each lock mass must be monitored and must not vary by more than ∀ 20% throughout its respective retention time window. Variations of lock mass by more than 20% indicate the presence of co-eluting interferences that raise the source pressure and may significantly reduce the sensitivity of the mass spectrometer. Re-injection of another aliquot of the sample extract may not resolve the problem and additional cleanup of the extract may be

required to remove the interference. A lock mass interference or suppression in a retention time region in which pesticides and labeled compounds do not elute may be ignored.

10.3.2 All pesticides and labeled compounds in the CS-1 standard must be within the QC limits in Table 6 for their respective ion abundance ratios; otherwise, the mass spectrometer must be adjusted and this test repeated until the m/z ratios fall within the limits specified. If the adjustment alters the resolution of the mass spectrometer, resolution must be verified (Section 10.2.1) prior to repeat of the test.

10.3.3 Verify that the HRGC/HRMS instrument meets the minimum levels (MLs) in Table 1. The peaks representing the pesticides and labeled compounds in the CS-1 calibration standard must have signal-to-noise ratios (S/N) ∃ 3; otherwise, the mass spectrometer must be adjusted and this test repeated until the minimum levels in Table 1 are met.

10.4 Calibration by isotope dilution – Isotope dilution is used for calibration of the native pesticides for which a labeled analog is available. The reference compound for each native compound is its labeled analog, as listed in Table 2. A 6-point calibration encompassing the concentration range is prepared for each native compound.

10.4.1 For the pesticides determined by isotope dilution, the relative response (RR) (labeled to native) vs. concentration in the calibration solutions (Table 4) is computed over the calibration range according to the procedures described below. Five calibration points are employed for less-sensitive HRMS instruments (e.g., VG 70); five or six points may be employed for more-sensitive instruments (e.g., Micromass Autospec Ultima).

10.4.2 Determine the response of each pesticide relative to its labeled analog using the area responses of both the primary and secondary exact m/z's specified in Table 6, for each calibration standard. Use the labeled compounds listed in Table 2 as the quantitation reference and the two exact m/z's listed in Table 6 for quantitation. The areas at the two exact m/z's for the compound is summed and divided by the summed area of the two exact m/z's for the quantitation reference.

Note: *Both exact m/z's are used as reference to reduce the effect of an interference at a single m/z. Other quantitation references and procedures may be used provided that the results produced are as accurate as results produced by the quantitation references and procedures described in this Section.*

10.4.3 Calibrate the native compounds with a labeled analog using the following equation:

$$RR = \frac{(A1_n + A2_n)\, C_l}{(A1_l + A2_l)\, C_n}$$

Where:

$A1_n$ and $A2_n$ = The areas of the primary and secondary m/z's for the pesticide

$A1_l$ and $A2_l$ = The areas of the primary and secondary m/z's for the labeled compound.

C_l = The concentration of the labeled compound in the calibration standard (Table 4).

C_n = The concentration of the native compound in the calibration standard (Table 4).

10.4.4 To calibrate the analytical system by isotope dilution, inject calibration standards CS-2 through CS-6 (Section 7.10 and Table 4) for a less sensitive instrument (e.g. VG 70) or CS-1 through CS-6 for a more sensitive instrument (e.g., Micromass Autospec Ultima). Use a volume identical to the volume chosen in Section 10.3, the procedure in Section 14, and the conditions in Section 10.1.1. Compute and store the relative response (RR) for each pesticide at each concentration. Compute the average (mean) RR and the RSD of the 6 RRs.

10.4.5 Linearity – If the RRs for any pesticide are constant (less than 20% RSD), the average RR may be used for that pesticide; otherwise, the complete calibration curve for that pesticide must be used over the calibration range.

10.5 Calibration by internal standard – Internal standard calibration is applied to determination of the native pesticides for which a labeled compound is not available, and to determination of the labeled compounds for performance tests and intra-laboratory statistics (Sections 9.4 and 15.6.4). The reference compound for each compound is listed in Table 2. For the labeled compounds, calibration is performed at a single concentration using data from the 6 points in the calibration (Section 10.4).

10.5.1 Response factors – Internal standard calibration requires the determination of response factors (RF) defined by the following equation:

$$RF = \frac{(A1_s + A2_s)\, C_{is}}{(A1_{is} + A2_{is})\, C_s}$$

Where:

$A1_s$ and $A2_s$ = The areas of the primary and secondary m/z's for the pesticide.

$A1_{is}$ and $A2_{is}$ = The areas of the primary and secondary m/z's for the internal standard.

C_{is} = The concentration of the internal standard (Table 4).

C_s = The concentration of the compound in the calibration standard (Table 4).

10.5.2 To calibrate the analytical system for pesticides that do not have a labeled analog, and for the labeled compounds, use the data from the 6-point calibration (Section 10.4.4 and Table 4).

10.5.3 Compute and store the response factor (RF) for all native pesticides that do not have a labeled analog and for the labeled compounds. Use the labeled compounds

listed in Table 2 as the quantitation reference and the two exact m/z's listed in Table 6 for quantitation. For example, the areas at the two exact m/z's for the compound is summed and divided by the summed area of the two exact m/z's for the quantitation reference.

10.5.4 Compute and store the response factor (RF) for the labeled compounds using the Labeled injection internal standard as the quantitation reference, as given in Table 2.

10.5.5 Linearity – If the RFs for any pesticide are constant (less than 35% RSD), the average RF may be used for that pesticide; otherwise, the complete calibration curve for that pesticide must be used over the calibration range.

10.6 Endrin/4,4'-DDT breakdown – This test is run after calibration (Section 10.4 and 10.5) or calibration verification (Section 15.3) to assure that the labile pesticides do not decompose in the GC.

10.6.1 Inject the endrin/4,4'-DDT breakdown solution (Section 7.13) using the same volume chosen in Section 10.3.

10.6.2 Measure and sum the peak areas for both exact m/z's separately for 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, endrin, endrin aldehyde, and endrin ketone using the calibration data from Section 10.4.

10.6.2.1 Add the summed peak areas for endrin aldehyde and endrin ketone and separately add the peak areas for 4,4'-DDD and 4,4'-DDE.

10.6.2.2 Calculate the endrin and 4,4'-DDT breakdown as follows:

$$\textit{Endrin breakdown (percent)} = \frac{\textit{(areas for endrin aldehyde + endrin ketone)}}{\textit{areas for endrin}} \times \textit{100}$$

$$\textit{4,4'-DDT breakdown (percent)} = \frac{\textit{(areas for 4,4'-DDD + 4,4'-DDE)}}{\textit{areas for 4,4'-DDT}} \times \textit{100}$$

10.6.2.3 If the breakdown of endrin or 4,4'-DDT exceeds 20 percent, endrin or 4,4'-DDT is decomposing. If decomposition greater than 20 percent of either endrin or 4,4'-DDT occurs, clean and recondition the injector, break off a short section of the inlet end of the column, or alter the GC conditions to reduce the decomposition to where the 20 percent criterion is met (see Section 6.9.1.2).

11.0 Sample preparation

11.1 Sample preparation involves modifying the physical form of the sample so that the pesticides can be extracted efficiently. In general, the samples must be in a liquid form or

in the form of finely divided solids in order for efficient extraction to take place. Table 7 lists the phases and suggested quantities for extraction of various sample matrices.

For samples known or expected to contain high levels of the pesticides, the smallest sample size representative of the entire sample should be used (see Section 18). For all samples, the blank and IPR/OPR aliquots must be processed through the same steps as the sample to check for contamination and losses in the preparation processes.

11.1.1 For samples that contain particles, percent solids and particle size are determined using the procedures in Sections 11.2 and 11.3, respectively.

11.1.2 Aqueous samples – Because the pesticides may be bound to suspended particles, the preparation of aqueous samples is dependent on the solids content of the sample.

11.1.2.1 Aqueous samples containing one percent solids or less are prepared per Section 11.4 and extracted directly using one of the extraction techniques in Section 12.2.

11.1.2.2 For aqueous samples containing greater than one percent solids, a sample aliquot sufficient to provide 10 g of dry solids is used, as described in Section 11.5.

11.1.3 Solid Samples - Solid samples are prepared using the procedure described in Section 11.5 followed by extraction using the SDS procedure in Section 12.3.

11.1.4 Multi-phase samples – The phase(s) containing the pesticides is separated from the non-pesticide phase using pressure filtration and centrifugation, as described in Section 11.6. The pesticides will be in the organic phase in a multi-phase sample in which an organic phase exists.

11.1.5 Procedures for grinding, homogenization, and blending of various sample phases are given in Section 11.7.

11.1.6 Tissue samples – Preparation procedures for fish and other tissues are given in Section 11.8.

11.2 Determination of percent suspended solids

Note: *This aliquot is used for determining the solids content of the sample, not for pesticide determination.*

11.2.1 Aqueous liquids and multi-phase samples consisting of mainly an aqueous phase.

11.2.1.1 Desiccate and weigh a GF/D filter (Section 6.5.3) to three significant figures.

11.2.1.2 Filter 10.0 ∀0.02 mL of well-mixed sample through the filter.

11.2.1.3 Dry the filter a minimum of 12 hours at 110 ± 5°C and cool in a desiccator.

11.2.1.4 Calculate percent solids as follows:

$$\% \text{ Solids} = \frac{\text{Weight of sample aliquot after drying (g)} - \text{weight of filter (g)}}{10 \text{ g}} \times 100$$

11.2.2 Non-aqueous liquids, solids, semi-solid samples, and multi-phase samples in which the main phase is not aqueous; but not tissues.

11.2.2.1 Weigh 5 to 10 g of sample to three significant figures in a tared beaker.

11.2.2.2 Dry a minimum of 12 hours at 110 ∀5°C, and cool in a desiccator.

11.2.2.3 Calculate percent solids as follows:

$$\% \text{ Solids} = \frac{\text{Weight of sample aliquot after drying}}{\text{Weight of sample aliquot before drying}} \times 100$$

11.3 Estimation of particle size

11.3.1 Spread the dried sample from Section 11.2.1.3 or 11.2.2.2 on a piece of filter paper or aluminum foil in a fume hood or glove box.

11.3.2 Estimate the size of the particles in the sample. If the size of the largest particles is greater than 1 mm, the particle size must be reduced to 1 mm or less prior to extraction using the procedures in Section 11.7.

11.4 Preparation of aqueous samples containing one percent suspended solids or less.

11.4.1 Aqueous samples containing one percent suspended solids or less are prepared using the procedure below and extracted using the one of the extraction techniques in Section 12.2.

11.4.2 Preparation of sample and QC aliquots

11.4.2.1 Mark the original level of the sample on the sample bottle for reference. Weigh the sample plus bottle to ∀ 1 g.

11.4.2.2 Spike 1.0 mL of the Labeled pesticide spiking solution (Section 7.12) into the sample bottle. Cap the bottle and mix the sample by shaking. Allow the sample to equilibrate for 1 to 2 hours, with occasional shaking.

11.4.2.3 For each sample or sample batch (to a maximum of 20 samples) to be extracted during the same 12-hour shift, place two 1.0-L aliquots of reagent water in clean sample bottles or flasks.

11.4.2.4 Spike 1.0 mL of the Labeled pesticide spiking solution (Section 7.12) into both reagent water aliquots. One of these aliquots will serve as the Method blank.

11.4.2.5 Spike 1.0 mL of the Native pesticide spiking solution (Section 7.11) into the remaining reagent water aliquot. This aliquot will serve as the OPR (Section 15.6).

11.4.2.6 For extraction using SPE, add 5 mL of methanol to the sample and QC aliquots. Cap and shake the sample and QC aliquots to mix thoroughly, and proceed to Section 12.2 for extraction.

11.5 Preparation of samples containing greater than one percent solids.

11.5.1 Weigh a well-mixed aliquot of each sample (of the same matrix type) sufficient to provide 10 g of dry solids (based on the solids determination in Section 11.2) into a clean beaker or glass jar, to a maximum of 1 L of sample.

11.5.2 Spike 1.0 mL of the Labeled pesticide spiking solution (Section 7.12) into the sample.

11.5.3 Prepare the blank and OPR aliquots per Sections 11.4.2.3 - 11.4.2.5.

11.5.4 Stir or tumble and equilibrate the aliquots for 1 to 2 hours.

11.5.5 Decant excess water. If necessary to remove water, filter the sample through a glass-fiber filter and discard the aqueous liquid.

11.5.6 If particles >1 mm are present in the sample (as determined in Section 11.3.2), spread the sample on clean aluminum foil in a hood. After the sample is dry, grind to reduce the particle size (Section 11.7).

11.5.7 Extract the sample and QC aliquots using the SDS procedure in Section 12.3.1.

11.6 Multi-phase samples, including high solids municipal sludge samples

11.6.1 Using the percent solids determined in Section 11.2.1.4 or 11.2.2.3, determine the volume of sample that will provide 10 g of solids, up to 1 L of sample.

11.6.2 Spike 1.0 mL of the Labeled pesticide spiking solution (Section 7.12) into the amount of sample determined in Section 11.6.1, and into the OPR and blank.

11.6.3 Prepare the blank and OPR aliquots per Sections 11.4.2.3 - 11.4.2.5.

11.6.4 Pressure filter the sample, blank, and OPR through Whatman GF/D glass-fiber filter paper (Section 6.5.3). If necessary to separate the phases and/or settle the solids, centrifuge these aliquots prior to filtration. Discard any aqueous phase (if present). Remove any non-aqueous liquid present and reserve the maximum amount filtered from the sample (Section 11.5.5) or 10 g, whichever is less, for combination with the solid phase (Section 12.3.1.5).

11.6.5 If particles >1 mm are present in the sample (as determined in Section 11.3.2) and the sample is capable of being dried, spread the sample and QC aliquots on clean aluminum foil in a hood. Observe the precaution in Section 5.3.1.

11.6.6 After the aliquots are dry or if the sample cannot be dried, reduce the particle size using the procedures in Section 11.7 and extract the reduced-size particles using the SDS procedure in Section 12.3. If particles >1 mm are not present, extract the particles and filter in the sample and QC aliquots directly using the SDS procedure in Section 12.3.

11.7 Sample grinding, homogenization, or blending – Samples with particle sizes greater than 1 mm (as determined in Section 11.3.2) are subjected to grinding, homogenization, or blending. The method of reducing particle size to less than 1 mm is matrix-dependent. In general, hard particles can be reduced by grinding with a mortar and pestle. Softer particles can be reduced by grinding in a Wiley mill or meat grinder, by homogenization, or in a blender.

11.7.1 Each size-reducing preparation procedure on each matrix must be verified by running the tests in Section 9.2 before the procedure is employed routinely.

11.7.2 The grinding, homogenization, or blending procedures must be carried out in a glove box or fume hood to prevent particles from contaminating the work environment.

11.7.3 Grinding – Certain papers and pulps, slurries, and amorphous solids can be ground in a Wiley mill or heavy duty meat grinder. In some cases, reducing the temperature of the sample to freezing or to dry ice or liquid nitrogen temperatures can aid in the grinding process. Grind the sample aliquots from Sections 11.5.7 or 11.6.6 in a clean grinder. Do not allow the sample temperature to exceed 50°C. Grind the blank and reference matrix aliquots using a clean grinder.

11.7.4 Homogenization or blending – Particles that are not ground effectively, or particles greater than 1 mm in size after grinding, can often be reduced in size by high speed homogenization or blending. Homogenize and/or blend the particles or filter from Sections 11.5.7 or 11.6.6 for the sample, blank, and OPR aliquots.

11.7.5 Extract the aliquots using the SDS procedure in Section 12.3.1.

11.8 Fish and other tissues – Prior to processing tissue samples, the laboratory must determine the exact tissue to be analyzed. Common requests for analysis of fish tissue include whole fish-skin on, whole fish-skin removed, edible fish fillets (filleted in the field or by the laboratory), specific organs, and other portions. Once the appropriate tissue has been determined, the sample must be homogenized.

11.8.1 Tissue homogenization

11.8.1.1 Samples are homogenized while still frozen, where practical. If the laboratory must dissect the whole fish to obtain the appropriate tissue for analysis, the unused tissues may be rapidly refrozen and stored in a clean glass jar for subsequent use.

11.8.1.2 Each analysis requires 10 g of tissue (wet weight). Therefore, the laboratory should homogenize at least 20 g of tissue to allow for re-extraction of a second aliquot of the same homogenized sample, if re-analysis is required. When whole fish analysis is necessary, the entire fish is homogenized.

11.8.1.3 Homogenize the sample in a tissue homogenizer (Section 6.3.3) or grind in a meat grinder (Section 6.3.4). Cut tissue too large to feed into the grinder into smaller pieces. To assure homogeneity, grind three times.

11.8.1.4 Transfer approximately 10 g (wet weight) of homogenized tissue to a clean, tared, 400- to 500-mL beaker.

11.8.1.5 Transfer the remaining homogenized tissue to a clean jar with a fluoropolymer-lined lid. Seal the jar and store the tissue at less than -10°C. Return any tissue that was not homogenized to its original container and store at less than -10°C.

11.8.2 Tissue QC aliquots

11.8.2.1 Prepare a Method blank by adding approximately 1-2 g of the oily liquid reference matrix (Section 7.6.4) to a 400- to 500-mL beaker. Record the weight to the nearest 10 mg.

11.8.2.2 Prepare an ongoing precision and recovery aliquot by adding 1-2 g of the oily liquid reference matrix (Section 7.6.4) to a separate 400- to 500-mL beaker. Record the weight to the nearest 10 mg.

11.8.3 Spiking

11.8.3.1 Spike 1.0 mL of the Labeled pesticide spiking solution (Section 7.12) into the sample, blank, and OPR aliquot.

11.8.3.2 Spike 1.0 mL of the Native spiking solution (Section 7.11) into the OPR aliquot.

11.8.4 Extract the aliquots using the Soxhlet procedure in Section 12.4.

12.0 Extraction and concentration

12.1 Extraction procedures include: solid phase (Section 12.2.1), separatory funnel (Section 12.2.2), or continuous liquid/liquid (Section 12.2.3) for aqueous liquids; Soxhlet/Dean-Stark (Section 12.3.1) for sludge, solids and filters; and Soxhlet extraction (Section 12.4) for tissues.

Macro-concentration procedures include: rotary evaporation (Section 12.6.1), heating mantle (Section 12.6.2), and Kuderna-Danish (K-D) evaporation (Section 12.6.3). Micro-concentration uses nitrogen evaporation (Section 12.7).

12.2 Extraction of aqueous liquids – separatory or continuous liquid/liquid extraction.

12.2.1 Solid-phase extraction of samples containing less than one percent solids

12.2.1.1 Disk preparation

12.2.1.1.1 Remove the test tube from the suction flask (Figure 4). Place an SPE disk on the base of the filter holder and wet with methylene chloride. While holding a GMF 150 filter above the SPE disk with tweezers, wet the filter with methylene chloride and lay the filter on the SPE disk, making sure that air is not trapped between the filter and disk. Clamp the filter and SPE disk between the 1-L glass reservoir and the vacuum filtration flask.

12.2.1.1.2 Rinse the sides of the reservoir with approx 15 mL of methylene chloride using a squeeze bottle or pipet. Apply vacuum momentarily until a few drops appear at the drip tip. Release the vacuum and allow the filter/disk to soak for approx one minute. Apply vacuum and draw all of the methylene chloride through the filter/disk. Repeat the wash step with approx 15 mL of acetone and allow the filter/disk to air dry.

12.2.1.2 Sample extraction

12.2.1.2.1 Pre-wet the disk by adding approx 20 mL of methanol to the reservoir. Pull most of the methanol through the filter/disk, retaining a layer of methanol approx 2 mm

thick on the filter. Do not allow the filter/disk to go dry from this point until the extraction is completed.

12.2.1.2.2 Add approx 20 mL of reagent water to the reservoir and pull most through, leaving a layer approx 2 mm thick on the filter/disk.

12.2.1.2.3 Allow the sample (Section 11.4.2.6) to stand for 1-2 hours, if necessary, to settle the suspended particles. Decant the clear layer of the sample, the blank (Section 11.4.2.4), or IPR/OPR aliquot (Section 11.4.2.5) into its respective reservoir and turn on the vacuum to begin the extraction. Adjust the vacuum to complete the extraction in no less than 10 minutes. For samples containing a high concentration of particles (suspended solids), the extraction time may be an hour or longer.

12.2.1.2.4 Before all of the sample has been pulled through the filter/disk, add approx 50 mL of reagent water to the sample bottle, swirl to suspend the solids (if present), and pour into the reservoir. Pull through the filter/disk. Use additional reagent water rinses until all solids are removed.

12.2.1.2.5 Before all of the sample and rinses have been pulled through the filter/disk, rinse the sides of the reservoir with small portions of reagent water.

12.2.1.2.6 Partially dry the filter/disk under vacuum for approx 3 minutes.

12.2.1.3 Elution of the filter/disk

12.2.1.3.1 Release the vacuum, remove the entire filter/disk/reservoir assembly from the vacuum flask, and empty the flask. Insert a test tube for eluant collection into the flask. The test tube should have sufficient capacity to contain the total volume of the elution solvent (approx 50 mL) and should fit around the drip tip. The drip tip should protrude into the test tube to preclude loss of sample from spattering when vacuum is applied (see Figure 4). Re-assemble the filter/disk/reservoir assembly on the vacuum flask.

12.2.1.3.2 Wet the filter/disk with 4-5 mL of acetone. Allow the acetone to spread evenly across the disk and soak for 15-20 seconds. Pull the acetone through the disk, releasing the vacuum when approx 1 mm thickness remains on the filter.

12.2.1.3.3 Rinse the sample bottle with approx 20 mL of methylene chloride and transfer to the reservoir. Pull approx half of the solvent through the filter/disk and release the vacuum. Allow the filter/disk to soak for approx 1 minute. Pull all of the solvent through the disk. Repeat the bottle rinsing and elution step with another 20 mL of methylene chloride. Pull all of the solvent through the disk.

12.2.1.3.4 Release the vacuum, remove the filter/disk/reservoir assembly, and remove the test tube containing the sample solution. Quantitatively transfer the solution to a 250-mL separatory funnel and proceed to Section 12.5 for back-extraction.

12.2.2 Separatory funnel extraction

12.2.2.1 Pour the spiked sample (Section 11.4.2.2) into a 2-L separatory funnel. Rinse the bottle or flask twice with 5 mL of reagent water and add these rinses to the separatory funnel.

12.2.2.2 Add 100 mL methylene chloride to the empty sample bottle. Cap the bottle and shake 60 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel, and extract the sample by shaking the funnel for 2 minutes with periodic venting. Allow the organic layer to separate from the aqueous phase for a minimum of 10 minutes. If an emulsion forms and is more than one-third the volume of the solvent layer, employ mechanical techniques to complete the phase separation

(see note below). Drain the methylene chloride extract through a solvent-rinsed glass funnel and dry over anhydrous sodium sulfate (Section 7.2.1) into an Erlenmeyer flask (1 L).

Note: *If an emulsion forms, the laboratory must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration through glass wool, use of phase separation paper, centrifugation, use of an ultrasonic bath with ice, addition of NaCl, or other physical methods. Alternatively, solid-phase (Section 12.2.1), CLLE (Section 12.2.3), or other extraction techniques may be used to prevent emulsion formation. Any alternative technique is acceptable so long as the requirements in Section 9.2 are met.*

12.2.2.3 Extract the water sample two more times with 100-mL portions of methylene chloride. Dry each portion over anhydrous sodium sulfate . After the third extraction, rinse the separatory funnel with at least 20 mL of methylene chloride, and add to the three 100-mL portions of methylene chloride. Repeat this rinse at least twice. Allow the methylene chloride extract to dry for 30 min. Transfer to a solvent-rinsed concentration device (Section 12.6).

12.2.2.4 Add 1 mL of a toluene "keeper" to the extract and concentrate using one of the macro-concentration procedures in Section 12.6, then proceed to back extraction in Section 12.5.

12.2.3 Continuous liquid/liquid extraction

12.2.3.1 Place 100-150 mL methylene chloride in each continuous extractor and 200-300 mL in each distilling flask.

12.2.3.2 Pour the sample(s), blank, and QC aliquots into the extractors. Rinse the sample containers with 50-100 mL methylene chloride and add to the respective extractors. Include all solids in the extraction process.

12.2.3.3 Begin the extraction by heating the flask until the methylene chloride is boiling. When properly adjusted, 1-2 drops of methylene chloride per second will fall from the condenser tip into the water. Extract for 16-24 hours.

12.2.3.4 Remove the distilling flask, estimate and record the volume of extract (to the nearest 100 mL), and pour the contents through a drying column containing 7 to 10 cm of granular anhydrous sodium sulfate into the concentration flask. Rinse the distilling flask with 30-50 mL of methylene chloride and pour through the drying column.

12.2.3.5 Add 1 mL of a toluene "keeper" to the extract and concentrate using one of the macro-concentration procedures in Section 12.6, then proceed to back extraction in Section 12.5.

12.3 Extraction of solids – Solid or sludge samples are extracted using a Soxhlet/Dean-Stark extractor (Section 12.3.1).

12.3.1 Soxhlet/Dean-Stark extraction

12.3.1.1 Charge a clean extraction thimble (Section 6.4.2.2) with 5.0 g of 100/200 mesh silica (Section 7.5.1.1) topped with 100 g of quartz sand (Section 7.3.2). Do not disturb the silica layer throughout the extraction process.

12.3.1.2 Place the thimble in a clean extractor. Place 30 to 40 mL of toluene in the receiver and 200 to 250 mL of toluene in the flask.

12.3.1.3 Pre-extract the glassware by heating the flask until the toluene is boiling. When properly adjusted, 1 to 2 drops of toluene will fall per second from the condenser tip into the receiver. Extract the apparatus for a minimum of 3 hours.

12.3.1.4 After pre-extraction, cool and disassemble the apparatus. Rinse the thimble with toluene and allow to air dry.

12.3.1.5 Load the wet sample and/or filter from Sections 11.5.7, 11.6.6, or 11.7.5 and any non-aqueous liquid from Section 11.6.4 into the thimble and manually mix into the sand layer with a clean metal spatula, carefully breaking up any large lumps of sample.

12.3.1.6 Reassemble the pre-extracted SDS apparatus, and add a fresh charge of 300 mL 80:20 toluene:acetone to the receiver and reflux flask. Apply power to the heating mantle to begin re-fluxing. Adjust the reflux rate to match the rate of percolation through the sand and silica beds until water removal lessens the restriction to toluene flow. Frequently check the apparatus for foaming during the first 2 hours of extraction. If foaming occurs, reduce the reflux rate until foaming subsides. Soxhlet extract for 12-24 hours.

12.3.1.7 Drain the water from the receiver at 1-2 hours and 8-9 hours, or sooner if the receiver fills with water. After 12-24 hours cool and disassemble the apparatus. Record the total volume of water collected.

12.3.1.8 Remove the distilling flask. Drain the water from the receiver and add any toluene in the receiver to the extract in the flask.

12.3.1.9 Concentrate the extracts from particles to approximately 10 mL using the rotary evaporator (Section 12.6.1) or heating mantle (Section 12.6.2), transfer to a 250-mL separatory funnel, and proceed with back-extraction (Section 12.5).

12.4 Soxhlet extraction of tissue

Note: *This procedure includes determination of the lipid content of the sample (Section 12.4.9), using the same sample extract that is analyzed by GC/HRMS. Alternatively, a separate sample aliquot may be used for the lipid determination. If a separate aliquot is used for GC/HRMS determination, use nitrogen to evaporate the main portion of the sample extract only to the extent necessary to effect the solvent exchange to n-hexane, so that loss of low molecular weight pesticides is avoided, i.e., it is not necessary to dry the main portion of the sample to constant weight (Section 12.4.8).*

12.4.1 Add 30 to 40 g of powdered anhydrous sodium sulfate (Section 7.2.2) to each of the beakers (Section 11.8.4) and mix thoroughly. Cover the beakers with aluminum foil and dry until the mixture becomes a free-flowing powder (30 minutes minimum). Remix prior to extraction to prevent clumping.

12.4.2 Assemble and pre-extract the Soxhlet apparatus per Sections 12.3.1-12.3.1.4, except use methylene chloride for the pre-extraction and rinsing and omit the quartz sand.

12.4.3 Re-assemble the pre-extracted Soxhlet apparatus and add a fresh charge of methylene chloride to the reflux flask.

12.4.4 Transfer the sample/sodium sulfate mixture (Section 12.4.1) to the Soxhlet thimble, and install the thimble in the Soxhlet apparatus.

12.4.5 Rinse the beaker with several portions of solvent and add to the thimble. Fill the thimble/receiver with solvent. Extract for 18-24 hours.

12.4.6 After extraction, cool and disassemble the apparatus.

12.4.7 Quantitatively transfer the extract to a macro-concentration device (Section 12.6) and concentrate to near dryness. Set aside the concentration apparatus for re-use.

12.4.8 Complete the removal of the solvent using the nitrogen blowdown procedure (Section 12.7) and a water bath temperature of 60°C. Weigh the receiver, record the weight, and return the receiver to the blowdown apparatus, concentrating the residue until a constant weight is obtained.

12.4.9 Percent lipid determination

12.4.9.1 Re-dissolve the residue in the receiver in hexane.

12.4.9.2 Transfer the residue/hexane to the anthropogenic isolation column (Section 13.6); retaining the boiling chips in the concentration apparatus. Use several rinses to assure that all material is transferred. If necessary, sonicate or heat the receiver slightly to assure that all

material is re-dissolved. Allow the receiver to dry. Weigh the receiver and boiling chips.

12.4.9.3 Calculate the lipid content to the nearest three significant figures as follows:

$$\textit{Percent lipid} = \frac{\textit{Weight of residue (g)}}{\textit{Weight of tissue (g)}} \times 100$$

12.4.9.4 The laboratory should determine the lipid content of the blank, IPR, and OPR to assure that the extraction system is working effectively.

12.5 Back-extraction with base and acid

Note: *Some pesticides may be decomposed by acid or base. If acid or base back-extraction is employed, the laboratory must evaluate the strengths of the acid and base solutions, and the exposure times, to preclude decomposition.*

12.5.1 Back-extraction may not be necessary for some samples, and back-extraction with strong acid and/or base with long contact times may destroy some pesticides. For some samples, the presence of color in the extract may indicate that back-extraction is necessary. If back-extraction is not necessary, concentrate the extract for cleanup or analysis (Section 12.6 and/or 12.7). If back-extraction is necessary, back-extract the extracts from Section 12.2.3.5 or 12.3.1.9 as follows:

12.5.2 Back-extract each extract three times sequentially with 500 mL of the aqueous sodium sulfate solution (Section 7.1.5), returning the bottom (organic) layer to the separatory funnel the first two times while discarding the top (aqueous) layer. On the final back-extraction, filter each pesticide extract through a prerinsed drying column containing 7 to 10 cm anhydrous sodium sulfate into a 500- to 1000-mL graduated cylinder. Record the final extract volume. Re-concentrate the sample and QC aliquots per Sections 12.6-12.7, and clean up the samples and QC aliquots per Section 13.

12.6 Macro-concentration – Extracts in toluene are concentrated using a rotary evaporator or a heating mantle; extracts in methylene chloride or hexane are concentrated using a rotary evaporator, heating mantle, or Kuderna-Danish apparatus.

Note: *In the concentration procedures below, the extract must not be allowed to concentrate to dryness because low molecular weight pesticides may be totally or partially lost. It may be advantageous to add 1 mL of toluene as a "keeper" to prevent loss of the low molecular weight pesticides.*

12.6.1 Rotary evaporation – Concentrate the extracts in separate round-bottom flasks.

12.6.1.1 Assemble the rotary evaporator according to manufacturer's instructions, and warm the water bath to 45°C. On a daily basis, pre-

clean the rotary evaporator by concentrating 100 mL of clean extraction solvent through the system. Archive both the concentrated solvent and the solvent in the catch flask for a contamination check if necessary. Between samples, three 2- to 3- mL aliquots of solvent should be rinsed down the feed tube into a waste beaker.

12.6.1.2 Attach the round-bottom flask containing the sample extract to the rotary evaporator. Slowly apply vacuum to the system, and begin rotating the sample flask.

12.6.1.3 Lower the flask into the water bath, and adjust the speed of rotation and the temperature as required to complete concentration in 15 to 20 minutes. At the proper rate of concentration, the flow of solvent into the receiving flask will be steady, but no bumping or visible boiling of the extract will occur.

Note: *If the rate of concentration is too fast, analyte loss may occur.*

12.6.1.4 When the liquid in the concentration flask has reached an apparent volume of approximately 2 mL, remove the flask from the water bath and stop the rotation. Slowly and carefully admit air into the system. Be sure not to open the valve so quickly that the sample is blown out of the flask. Rinse the feed tube with approximately 2 mL of solvent.

12.6.1.5 Proceed to Section 12.5 for back-extraction or Section 12.7 for micro-concentration and solvent exchange.

12.6.2 Heating mantle – Concentrate the extracts in separate round-bottom flasks.

12.6.2.1 Add one or two clean boiling chips to the round-bottom flask, and attach a three-ball macro Snyder column. Prewet the column by adding approximately 1 mL of solvent through the top. Place the round-bottom flask in a heating mantle, and apply heat as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood.

12.6.2.2 When the liquid has reached an apparent volume of approximately 10 mL, remove the round-bottom flask from the heating mantle and allow the solvent to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the glass joint into the receiver with small portions of solvent.

12.6.2.3 Proceed to Section 12.6 for preparation for back-extraction or Section 12.7 for micro-concentration and solvent exchange.

12.6.3 Kuderna-Danish (K-D) – Concentrate the extracts in separate 500-mL K-D flasks equipped with 10-mL concentrator tubes. The K-D technique is used for solvents such as methylene chloride and hexane. Toluene is difficult to concentrate using the K-D technique unless a water bath fed by a steam generator is used.

12.6.3.1 Add 1 to 2 clean boiling chips to the receiver. Attach a three-ball macro Snyder column. Prewet the column by adding approximately 1 mL of solvent through the top. Place the K-D apparatus in a hot water bath so that the entire lower rounded surface of the flask is bathed with steam.

12.6.3.2 Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

12.6.3.3 When the liquid has reached an apparent volume of 1 mL, remove the K-D apparatus from the bath and allow the solvent to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of solvent. A 5-mL syringe is recommended for this operation.

12.6.3.4 Remove the three-ball Snyder column, add a fresh boiling chip, and attach a two ball micro Snyder column to the concentrator tube. Prewet the column by adding approximately 0.5 mL of solvent through the top. Place the apparatus in the hot water bath.

12.6.3.5 Adjust the vertical position and the water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

12.6.3.6 When the liquid reaches an apparent volume of 0.5 mL, remove the apparatus from the water bath and allow to drain and cool for at least 10 minutes.

12.6.3.7 Proceed to 12.6 for preparation for back-extraction or Section 12.7 for micro-concentration and solvent exchange.

12.7 Micro-concentration and solvent exchange

12.7.1 Extracts to be subjected to GPC cleanup are exchanged into methylene chloride. Extracts to be cleaned up using silica gel, Florisil, the SPE cartridge, and/or HPLC are exchanged into hexane.

12.7.2 Transfer the vial containing the sample extract to a nitrogen evaporation device. Adjust the flow of nitrogen so that the surface of the solvent is just visibly disturbed.

Note: *A large vortex in the solvent may cause analyte loss.*

12.7.3 Lower the vial into a 30°C water bath and continue concentrating.

12.7.3.1 If the extract or an aliquot of the extract is to be concentrated to dryness for weight determination (Sections 12.4.8 and 13.6.4), blow dry until a constant weight is obtained.

12.7.3.2 If the extract is to be concentrated for injection into the GC/HRMS or the solvent is to be exchanged for extract cleanup, proceed as follows:

12.7.4 When the volume of the liquid is approximately 100 µL, add 2 to 3 mL of the desired solvent (methylene chloride for GPC and HPLC, or hexane for the other cleanups) and continue concentration to approximately 100 µL. Repeat the addition of solvent and concentrate once more.

12.7.5 If the extract is to be cleaned up by GPC, adjust the volume of the extract to 5.0 mL with methylene chloride. If the extract is to be cleaned up by HPLC, concentrate the extract to 1.0 mL. Proceed with GPC or HPLC cleanup (Section 13.2 or 13.5, respectively).

12.7.6 If the extract is to be cleaned up by column chromatography or the SPE cartridge, bring the final volume to 1.0 mL with hexane. Proceed with column cleanup (Sections 13.3, 13.4, 13.7, or 13.8).

12.7.7 If the extract is to be concentrated for injection into the GC/HRMS (Section 14), quantitatively transfer the extract to a 0.3-mL conical vial for final concentration, rinsing the larger vial with hexane and adding the rinse to the conical vial. Reduce the volume to approximately 100 µL. Add 20 µL of nonane to the vial, and evaporate the solvent to the level of the nonane. Seal the vial and label with the sample number. Store in the dark at room temperature until ready for GC/HRMS analysis. If GC/HRMS analysis will not be performed on the same day, store the vial at less than -10°C.

13.0 Extract cleanup

13.1 Cleanup may not be necessary for relatively clean samples (e.g., treated effluents, groundwater, drinking water). If particular circumstances require the use of a cleanup procedure, the laboratory may use any or all of the procedures below or any other appropriate procedure. Before using a cleanup procedure, the laboratory must demonstrate that the requirements of Section 9.2 can be met using the cleanup procedure. The following table suggests cleanups that may be used for the various analyte groups.

Analyte group	Suggested cleanups
All	GPC (13.2); SPE (13.3); Micro-silica (13.4)

Organo-chlorine	GPC, SPE, Micro-silica plus Florisil (13.7) or alumina (13.8)
Specific compounds	GPC, SPE, Micro-silica plus HPLC (13.5)

13.1.1 Gel permeation chromatography (Section 13.2) removes high molecular weight interferences that cause GC column performance to degrade. It should be used for all soil and sediment extracts. It may be used for water extracts that are expected to contain high molecular weight organic compounds (e.g., polymeric materials, humic acids). It should also be used for tissue extracts after initial cleanup on the anthropogenic isolation column (Section 13.6).

13.1.2 Micro-silica (Section 13.4), the SPE cartridge (Section 13.3), Florisil (Section 13.7), and alumina (Section 13.8) may be used to remove non-polar and polar interferences.

13.1.3 HPLC (Section 13.5) is used to provide specificity for certain pesticides.

13.1.4 The anthropogenic isolation column (Section 13.6) is used for removal of lipids from tissue samples.

13.2 Gel permeation chromatography (GPC)

13.2.1 Column packing

13.2.1.1 Place 70 to 75 g of SX-3 Bio-beads (Section 6.7.1.1) in a 400- to 500-mL beaker.

13.2.1.2 Cover the beads with methylene chloride and allow to swell overnight (a minimum of 12 hours).

13.2.1.3 Transfer the swelled beads to the column (Section 6.7.1.1) and pump solvent through the column, from bottom to top, at 4.5 to 5.5 mL/minute prior to connecting the column to the detector.

13.2.1.4 After purging the column with solvent for 1 to 2 hours, adjust the column head pressure to 7 to 10 psig and purge for 4 to 5 hours to remove air. Maintain a head pressure of 7 to 10 psig. Connect the column to the detector (Section 6.7.1.4).

13.2.2 Column calibration

13.2.2.1 Load 5 mL of the GPC calibration solution (Section 7.4) into the sample loop.

13.2.2.2 Inject the GPC calibration solution and record the signal from the detector. The elution pattern will be corn oil, bis(2-ethylhexyl) phthalate (BEHP), methoxychlor, perylene, and sulfur.

13.2.2.3 Set the "dump time" to allow >85% removal of BEHP and >85% collection of methoxychlor.

13.2.2.4 Set the "collect time" to the time of the sulfur peak maximum.

13.2.2.5 Verify calibration with the GPC calibration solution after every 20 extracts. Calibration is verified if the recovery of methoxychlor is greater than 85%. If calibration is not verified, the system must be recalibrated using the GPC calibration solution, and the previous sample batch must be re-extracted and cleaned up using a calibrated GPC system.

13.2.3 Extract cleanup – GPC requires that the column not be overloaded. The column specified in this Method is designed to handle a maximum of 0.5 g of material from an aqueous, soil, or mixed-phase sample in a 5-mL extract, and has been shown to handle 1.5 g of lipid from a tissue sample in a 5-mL extract. If the extract is known or expected to contain more than these amounts, the extract is split into aliquots for GPC, and the aliquots are combined after elution from the column. The residue content of the extract may be obtained gravimetrically by evaporating the solvent from a 50-µL aliquot.

13.2.3.1 Filter the extract or load through the filter holder (Section 6.7.1.3) to remove particles. Load the 5.0-mL extract onto the column.

13.2.3.2 Elute the extract using the calibration data determined in Section 13.2.2. Collect the eluate in a clean 400- to 500-mL beaker. Allow the system to rinse for additional 10 minutes before injecting the next sample.

13.2.3.3 Rinse the sample loading tube thoroughly with methylene chloride between extracts to prepare for the next sample.

13.2.3.4 If an extract is encountered that could overload the GPC column to the extent that carry-over could occur, a 5.0-mL methylene chloride blank must be run through the system to check for carry-over.

13.2.3.5 Concentrate the eluate per Sections 12.6 and 12.7 for further cleanup or injection into the GC/MS.

13.3 Solid-phase extraction (SPE) cartridge

13.3.1 Setup

13.3.1.1 Attach the Vac-elute manifold (6.7.6.1) to a water aspirator or vacuum pump with the trap and gauge installed between the manifold and vacuum source.

13.3.1.2 Place the SPE cartridge(s) in the manifold, turn on the vacuum source, and adjust the vacuum to 5 to 10 psig.

Note: *Do not allow the SPE cartridge to go dry during the following steps.*

13.3.2 Cartridge washing – Pre-elute the cartridge sequentially with two 6-mL volumes of 1:2:1 ethyl acetate:acetonitrile:toluene.

13.3.3 Using a pipette or a 1-mL syringe, transfer 1.0 mL of the extract in 1:2:1 ethyl acetate:acetonitrile:toluene (Section 12.2.3.5, 12.3.1.9, 12.4.8 or 12.5.2) onto the SPE cartridge followed by a rinse of 1 mL 1:2:1 ethyl acetate:acetonitrile:toluene.

13.3.4 As soon as the sample is loaded, begin to collect the eluate in a round bottom flask or centrifuge tube (if using a manifold). Elute the SPE cartridge with 11 mL of 1:2:1 ethyl acetate:acetonitrile: toluene.

13.3.5 Concentrate the eluted extract per Sections 12.6 and 12.7 and proceed to other cleanups or determination by HRGC/HRMS.

13.4 Micro-silica column

13.4.1 Place a small glass-wool plug in a clean Pasteur pipette. Rinse the pipette and glass wool twice with small (e.g., 2 - 5 mL) volumes of toluene, followed by two rinsings with small volumes of hexane. Allow the pipette to drain. Dry pack the column bottom to top with 0.75 gram of 10% deactivated silica (Section 7.5.1.1). Tap the column to settle the silica.

13.4.2 Rinse the column with hexane until the column is completely wetted (typically 5-10 mL). Allow the hexane to drain to the top of the silica.

13.4.3 Adjust the extract volume to 1.0 mL and apply to the column. Allow the extract to drain to the top of the silica. Rinse the extract onto the column with 500 µL of hexane.

13.4.4 Rinse the centrifuge tube that contained the extract with 300-µL of 10% methanol in dichloromethane and apply to the column. Collect the eluate in a round-bottom flask. Repeat this rinse and collect the eluate in the flask.

13.4.5 Elute the column with 5 mL of 10% methanol in dichloromethane. Collect the eluate in the round bottom flask.

13.4.6 Add 5 mL of acetone and 1 mL of iso-octane to the round bottom flask and concentrate the eluate per Section 12.6 and 12.7 for further cleanup or injection into the HPLC or GC/MS.

13.4.7 For extracts of samples known to contain large quantities of other organic compounds, it may be advisable to increase the capacity of the silica gel column. This may be accomplished by increasing the strength of the acid silica and including basic silica gel. The acid silica gel (Section 7.5.1.2) may be increased in strength to as much as 40% w/w (6.7 g sulfuric acid added to 10 g silica gel). The basic silica gel (Section 7.5.1.3) may be increased in strength to as much as 33% w/w (50 mL 1N NaOH added to 100 g silica gel), or the potassium silicate (Section 7.5.1.4) may be used. Larger columns may also be used if needed.

Note: *The use of stronger acid and basic silica gel (44% w/w) may lead to charring of organic compounds in some extracts. The charred material may retain some of the analytes and lead to lower recoveries of the pesticides. Increasing the strengths of the acid and basic silica gel may also require different volumes of eluants than those specified above to elute the analytes from the column. The performance of the Method after such modifications must be verified by the procedure in Section 9.2.*

13.5 HPLC (Reference 9)

13.5.1 Column calibration

13.5.1.1 Prepare a calibration standard containing the pesticides at the concentrations of the stock solution in Table 3, or at a concentration appropriate to the response of the detector.

13.5.1.2 Inject the calibration standard into the HPLC and record the signal from the detector. Collect the eluant for reuse.

13.5.1.3 Establish the collection time for the pesticides of interest. Following calibration, flush the injection system with solvent to ensure that residual pesticides are removed from the system.

13.5.1.4 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the pesticides is 75 to 125% compared to the calibration (Section 13.5.1.1). If calibration is not verified, the system must be recalibrated using the calibration solution, and the batch of samples run on the uncalibrated system must be re-extracted and cleaned up using a calibrated system.

13.5.2 Extract cleanup – HPLC requires that the column not be overloaded. The column specified in this Method is designed to handle a maximum of 50 Φg of a given pesticide, depending on the particular compound. If the amount of material in the extract will overload the column, split the extract into fractions and combine the fractions after elution from the column.

13.5.2.1 Rinse the sides of the vial containing the sample and adjust to the volume required for the sample loop for injection.

13.5.2.2 Inject the sample extract into the HPLC.

13.5.2.3 Elute the extract using the calibration data determined in Section 13.5.1. Collect the fraction(s) in clean 20-mL concentrator tubes.

13.5.2.4 If an extract containing greater than 500 µg of total material is encountered, a blank must be run through the system to check for carry-over.

13.5.2.5 Concentrate the eluate per Section 12.7 for injection into the GC/HRMS.

13.6 Anthropogenic isolation column (Reference 15) – Used for removal of lipids from tissue extracts

13.6.1 Prepare the column as given in Section 7.5.2.

13.6.2 Pre-elute the column with 100 mL of hexane. Drain the hexane layer to the top of the column, but do not expose the sodium sulfate.

13.6.3 Load the sample and rinses (Section 12.4.9.2) onto the column by draining each portion to the top of the bed. Elute the pesticides from the column into the apparatus used for concentration (Section 12.4.7) using 200 mL of hexane.

13.6.4 Remove a small portion (e.g., 50 µL) of the extract for determination of residue content. Estimate the percent of the total that this portion represents. Concentrate the small portion to constant weight per Section 12.7.3.1. Calculate the total amount of residue in the extract. If more than 500 mg of material remains, repeat the cleanup using a fresh anthropogenic isolation column.

13.6.5 If necessary, exchange the extract to a solvent suitable for the additional cleanups to be used (Section 13.2 - 13.8).

13.6.6 Clean up the extract using the procedures in Sections 13.2 - 13.8. GPC (Section 13.2) and Florisil (Section 13.7) are recommended as minimum additional cleanup steps.

13.6.7 Following cleanup, concentrate the extract to 20 ΦL per Section 12.7 and proceed with the analysis in Section 14.

13.7 Florisil

13.7.1 Begin to drain the n-hexane from the column (Section 7.5.4.3). Adjust the flow rate of eluant to 4.5 - 5.0 mL/min.

13.7.2 When the n-hexane is within 1 mm of the sodium sulfate, apply the sample extract (in hexane) to the column. Rinse the sample container twice with 1-mL portions of hexane and apply to the column, allowing the hexane to drain to the top of the sodium sulfate layer.

13.7.3 Elute Fraction 1 with 200 mL of 6% ethyl ether in n-hexane and collect the eluate. Elute Fraction 2 with 200 mL of 15% ethyl ether in hexane and collect the eluate. Elute Fraction 3 with 50% ethyl ether in hexane and collect the eluate. The exact volumes of solvents will need to be determined for each batch of Florisil. If the pesticides are not to be collected in separate fractions, elute all pesticides with 50% ethyl ether in hexane.

13.7.4 Concentrate the eluate(s) per Sections 12.6 - 12.7 for further cleanup or for injection into the HPLC or GC/HRMS.

13.8 Alumina

13.8.1 Begin to drain the hexane from the column (Section 7.5.5.2). Adjust the flow rate of eluant to 4.5 - 5.0 mL/min.

13.8.2 When the n-hexane is within 1 mm of the sodium sulfate, apply the sample extract (in hexane) to the column. Rinse the sample container twice with 1-mL portions of hexane and apply to the column, allowing the hexane to drain to the top of the sodium sulfate layer.

13.8.3 Elute the pesticides with 150 mL of n-hexane. If all pesticides are not eluted, elute the remaining pesticides with 50 mL of 15% methylene chloride in n-hexane.

13.8.4 Concentrate the eluate(s) per Sections 12.6 - 12.7 for further cleanup or for injection into the HPLC or GC/HRMS.

14.0 HRGC/HRMS analysis

14.1 Establish the operating conditions given in Section 10.1.

14.2 Add 2 µL of the labeled injection internal standard spiking solution (Section 7.14) to the 20 µL sample extract immediately prior to injection to minimize the possibility of loss by evaporation, adsorption, or reaction. If an extract is to be reanalyzed and evaporation has occurred, do not add more labeled injection internal standard spiking solution. Rather, bring the extract back to its previous volume (e.g., 19 µL) with pure nonane (18 µL if 2 µL injections are used).

14.3 Inject 1.0 or 2.0 µL of the concentrated extract containing the Labeled injection internal standards using on-column or splitless injection. The volume injected must be identical to the volume used for calibration (Section 10.3).

14.3.1 Start the GC column initial isothermal hold upon injection. Start MS data collection after the solvent peak elutes.

14.3.2 Monitor the exact m/z's for each pesticide throughout its retention time window. Where warranted, monitor m/z's associated with pesticides at higher levels of chlorination to assure that fragments are not interfering with the m/z's for pesticides at lower levels of chlorination. Also where warranted, monitor m/z's associated with interferents expected to be present.

14.3.3 Stop data collection after permethrin and cypermethrin have eluted. Return the column to the initial temperature for analysis of the next sample extract or standard.

15.0 System and laboratory performance

15.1 At the beginning of each 12-hour shift during which analyses are performed, GC/MS system performance and calibration are verified for all the pesticides and labeled compounds. For these tests, analysis of the CS-4 calibration verification (VER) standard (Section 7.10 and Table 4) must be used to verify all performance criteria. Adjustment and/or recalibration (Section 10) must be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, IPRs, and OPRs be analyzed.

15.2 MS resolution – Static resolving power checks must be performed at the beginning and at the end of each shift per Sections 10.2.1. If analyses are performed on successive shifts, only the beginning of shift static resolving power check is required. If the requirement in Section 10.2.1 cannot be met, the problem must be corrected before analyses can proceed. If any of the samples in the previous shift may be affected by poor resolution, those samples must be re-analyzed.

15.3 Calibration verification

15.3.1 Inject the VER (CS-4) calibration standard using the procedure in Section 14.

15.3.2 The m/z abundance ratios for all pesticides must be within the limits in Table 6; otherwise, the mass spectrometer must be adjusted until the m/z abundance ratios fall within the limits specified when the verification test is be repeated. If the adjustment alters the resolution of the mass spectrometer, resolution must be verified (Section 10.2.1) prior to repeat of the verification test.

15.3.3 The GC peak representing each native pesticide and labeled compound in the VER standard must be present with a S/N of at least 10; otherwise, the mass spectrometer must be adjusted and the verification test repeated.

15.3.4 Compute the concentration of the pesticides that have labeled analogs by isotope dilution and the concentration of the pesticides that do not have labeled analogs by the internal standard technique. These concentrations are computed based on the calibration data in Section 10.

15.3.5 For each compound, compare the concentration with the calibration verification limit in Table 5. If all compounds meet the acceptance criteria, calibration has been verified and analysis of standards and sample extracts may proceed. If, however, any compound fails its respective limit, the measurement system is not performing properly. In this event, prepare a fresh calibration standard or correct the problem and repeat the resolution (Section 15.2) and verification (Section 15.3) tests, or recalibrate (Section 10).

15.4 Retention times and GC resolution

15.4.1 Retention times.

15.4.1.1 Absolute – The absolute retention times of the Labeled compounds in the verification test (Section 15.3) must be within ∀ 15 seconds of the respective retention times in the calibration (Section 10.1)

15.4.1.2 Relative – The relative retention times of native pesticides and the labeled compounds in the verification test (Section 15.3) must be within their respective RRT limits in Table 2 or, if an alternate column or column system is employed, within their respective RRT limits for the alternate column or column system (Sections 9.1.2.3 and 6.9.1).

15.4.1.3 If the absolute or relative retention time of any compound is not within the limits specified, the GC is not performing properly. In this event, adjust the GC and repeat the verification test (Section 15.3) or recalibrate (Section 10), or replace the GC column and either verify calibration or recalibrate.

15.4.2 GC resolution and minimum analysis time

15.4.2.1 The resolution and minimum analysis time specifications in Sections 6.9.1.1.2 and 6.9.1.1.1, respectively, must be met for the DB-17 column or, if an alternate column or column system is employed, must be met as specified for the alternate column or column system (Sections 9.1.2.3 and 6.9.1). If these specifications are not met, the GC analysis conditions must be adjusted until the specifications are met, or the column must be replaced and the calibration verification tests repeated Sections 15.3 - 15.4), or the system must be recalibrated (Section 10).

15.4.2.2 After the resolution and minimum analysis time specifications are met, update the retention times and relative retention times, but not the relative responses and response factors. For the relative responses and response factors, the multi-point calibration data (Sections 10.4 and 10.5) must be used.

15.5 Endrin/4,4'-DDT breakdown – Perform the endrin/4,4'-DDT breakdown test (Section 10.6). The breakdown specification (Section 10.6.2.3) must be met before an OPR, sample, or blank may be analyzed.

15.6 Ongoing precision and recovery

15.6.1 Analyze the extract of the ongoing precision and recovery (OPR) aliquot (Section 11.4.2.5, 11.5.3, 11.6.3, or 11.8.3.2) prior to analysis of samples from the same batch.

15.6.2 Compute the percent recovery of the pesticides with labeled analogs by isotope dilution (Section 10.4). Compute the percent recovery of each labeled compound by the internal standard method (Section 10.5).

15.6.3 For the pesticides and labeled compounds, compare the recovery to the OPR limits given in Table 5. If all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may proceed. If, however, any individual concentration falls outside of the range given, the extraction/concentration processes are not being performed properly for that compound. In this event, correct the problem, re-prepare, extract, and clean up the sample batch and repeat the ongoing precision and recovery test (Section 15.6).

15.6.4 If desired, add results that pass the specifications in Section 15.6.3 to initial (Section 9.4) and previous ongoing data for each compound in each matrix. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory accuracy for each pesticide in each matrix type by calculating the average percent recovery (R) and the standard deviation of percent recovery (S_R). Express the accuracy as a recovery interval from R ! $2S_R$ to R + $2S_R$. For example, if R = 95% and S_R = 5%, the accuracy is 85 to 105%.

15.7 Blank – Analyze the Method blank extracted with each sample batch immediately following analysis of the OPR aliquot to demonstrate freedom from contamination and freedom from carryover from the OPR analysis. If pesticides will be carried from the OPR into the Method blank, analyze one or more aliquots of solvent between the OPR and the Method blank. The results of the analysis of the blank must meet the specifications in Section 9.5.2 before sample analyses may proceed.

16.0 Qualitative determination

A pesticide or labeled compound is identified in a standard, blank, or sample when all of the criteria in Sections 16.1 through 16.4 are met.

16.1 The signals for the two exact m/z's in Table 6 must be present and must maximize within the same two scans.

16.2 The signal-to-noise ratio (S/N) for the GC peak at each exact m/z must be greater than or equal to 2.5 for each pesticide detected in a sample extract, and greater than or equal to 10 for all pesticides in the calibration and verification standards (Sections 10.3.3 and 15.6.3).

16.3 The ratio of the integrated areas of the two exact m/z's specified in Table 6 must be within the limit in Table 6, or within ∀ 15 percent of the ratio in the midpoint (CS-4) calibration or calibration verification (VER), whichever is most recent.

16.4 The relative retention time of the peak for a pesticide must be within the RRT QC limits specified in Table 2 or within similar limits developed from calibration data (Section 10.1.2). If an alternate column (Section 9.1.2.3) is employed, the RRT for the pesticide must be within its respective RRT QC limits for the alternate column or column system (Section 6.9.1).

Note: *For native pesticides determined by internal standard quantitation, a pesticide with the same exact m/z's as other pesticides may fall within more than one RT window and be mis-identified unless the RRT windows are made very narrow, as in Table 2. Therefore, consistency of the RT and RRT with other pesticides and the labeled compounds may be required for rigorous pesticide identification. Retention time regression may aid in this identification.*

16.5 Because of pesticide RT overlap and the potential for interfering substances, it is possible that all of the identification criteria (Sections 16.1 - 16.4) may not be met. It is also possible that loss of one or more chlorines from a highly chlorinated pesticide or interferent may inflate or produce a false concentration for a less-chlorinated pesticide that elutes at the same retention time (see Section 18). If identification is ambiguous, an experienced spectrometrist (Section 1.5) must determine the presence or absence of the pesticide.

16.6 If the criteria for identification in Sections 16.1 - 16.5 are not met, the pesticide has not been identified and the result for that pesticide may not be reported or used for permitting or regulatory compliance purposes. If interferences preclude identification, a new aliquot of sample must be extracted, further cleaned up, and analyzed.

17.0 Quantitative determination

17.1 Isotope dilution quantitation

17.1.1 By adding a known amount of the labeled pesticides to every sample prior to extraction, correction for recovery of each pesticide can be made because the native

compound and its labeled analog exhibit similar effects upon extraction, concentration, and gas chromatography. Relative responses (RRs) are used in conjunction with the calibration data in Section 10.4 to determine concentrations in the final extract, so long as labeled compound spiking levels are constant.

17.1.2 Compute the concentrations of the pesticides in the extract using the RRs from the calibration data (Section 10.4) and following equation:

$$C_{ex}\ (ng/mL) = \frac{(A1_n + A2_n)\ C_l}{(A1_l + A2_l)\ RR}$$

Where:

C_{ex} = The concentration of the pesticide in the extract, and the other terms are as defined in Section 10.4.3

17.2 Internal standard quantitation and labeled compound recovery

17.2.1 Compute the concentrations in the extract of the native compounds that do not have labeled analogs using the response factors determined from the calibration data (Section 10.5) and the following equation:

$$C_{ex}\ (ng/mL) = \frac{(A1_s + A2_s)\ C_{is}}{(A1_{is} + A2_{is})\ RF}$$

Where:

C_{ex} = The concentration of the labeled compound in the extract, and the other terms are as defined in Section 10.5.1

17.2.2 Using the concentration in the extract determined above, compute the percent recovery of the labeled pesticides other labeled cleanup standard using the following equation:

$$Recovery\ (\%) = \frac{Concentration\ found\ (ng/mL)}{Concentration\ spiked\ (ng/mL)} \times 100$$

17.3 The concentration of a native compound in the solid phase of the sample is computed using the concentration of the compound in the extract and the weight of the solids (Section 11.2.2.3), as follows:

$$Concentration\ in\ solid\ (ng/kg) = \frac{(C_{ex} \times V_{ex})}{W_s}$$

Where:

C_{ex} = The concentration of the compound in the extract.
V_{ex} = The extract volume in mL.
W_s = The sample weight (dry weight) in kg.

17.4 The concentration of a native pesticide in the aqueous phase of the sample is computed using the concentration of the compound in the extract and the volume of water extracted (Section 11.4), as follows:

$$\text{Concentration in aqueous phase (pg/L)} = 1000 \times \frac{(C_{ex} \times V_{ex})}{V_s}$$

Where:

C_{ex} = The concentration of the compound in the extract.
V_{ex} = The extract volume in mL.
V_s = The sample volume in liters.

17.5 If the SICP area at either quantitation m/z for any pesticide exceeds the calibration range of the system, dilute the sample extract by the factor necessary to bring the concentration within the calibration range, adjust the concentration of the Labeled injection internal standard to 100 pg/µL in the extract, and analyze an aliquot of this diluted extract. If the pesticides cannot be measured reliably by isotope dilution, dilute and analyze an aqueous sample or analyze a smaller portion of a soil, tissue, or mixed-phase sample. Adjust the pesticide concentrations, detection limits, and minimum levels to account for the dilution.

17.6 Reporting of results

17.6.1 Reporting units and levels

17.6.1.1 Aqueous samples – Report results in pg/L (parts-per-quadrillion).

17.6.1.2 Samples containing greater than 1% solids (soils, sediments, filter cake, compost) – Report results in ng/kg based on the dry weight of the sample. Report the percent solids so that the result may be converted to aqueous units.

17.6.1.3 Tissues – Report results in ng/kg of wet tissue, not on the basis of the lipid content of the tissue. Report the percent lipid content, so that the data user can calculate the concentration on a lipid basis if desired.

17.6.2 Reporting level

17.6.2.1 Report the result for each pesticide in each sample, blank, or standard (VER, IPR, OPR) at or above the minimum level of quantitation (ML; Table 1) to 3 significant figures. Report the result below the ML in each sample as <ML (where ML is the concentration at the ML) or as required by the regulatory authority or permit.

17.6.2.2 Blanks – Report the result for each pesticide below the ML but above the MDL to 2 significant figures. Report results below the MDL as <MDL (where MDL is the concentration at the MDL) or as required by the regulatory authority or permit. In addition to reporting results for

the samples and blank(s) separately, the concentration of each pesticide in a method blank or field blank associated with the sample may be subtracted from the results for that sample, or must be subtracted if requested or required by a regulatory authority or in a permit.

17.6.2.3 Results for a pesticide in a sample that has been diluted are reported at the least dilute level at which the area at the quantitation m/z is within the calibration range (Section 17.5).

17.6.2.4 For a pesticide having a labeled analog, report results at the least dilute level at which the area at the quantitation m/z is within the calibration range (Section 17.5) and the labeled compound recovery is within the normal range for the Method (Section 9.3 and Table 5).

17.6.2.5 Results from tests performed with an analytical system that is not in control must not be reported or otherwise used for permitting or regulatory compliance purposes, but do not relieve a discharger or permittee of reporting timely results.

18.0 Analysis of complex samples

18.1 Some samples may contain high levels (>10 ng/L; >1000 ng/kg) of the compounds of interest, interfering compounds, and/or polymeric materials. Some extracts may not concentrate to 20 µL (Section 12.7); others may overload the GC column and/or mass spectrometer. A fragment ion from a pesticide at a higher level of chlorination may interfere with determination of a pesticide at a lower level of chlorination.

18.2 Analyze a smaller aliquot of the sample (Section 17.5) when the extract will not concentrate to 20 ΦL after all cleanup procedures have been exhausted. If a smaller aliquot of soils or mixed-phase samples is analyzed, attempt to assure that the sample is representative.

18.3 Perform integration of peak areas and calculate concentrations manually when interferences preclude computerized calculations.

18.4 Recovery of labeled compounds – In most samples, recoveries of the labeled compounds will be similar to those from reagent water or from the alternate matrix (Section 7.6).

18.4.1 If the recovery of any of the labeled compounds is outside of the normal range (Table 5), a diluted sample must be analyzed (Section 17.5).

18.4.2 If the recovery of any of the labeled compounds in the diluted sample is outside of normal range, the calibration verification standard (Section 7.10 and Table 5) must be analyzed and calibration verified (Section 15.3).

18.4.3 If the calibration cannot be verified, a new calibration must be performed and the original sample extract reanalyzed.

18.4.4 If calibration is verified and the diluted sample does not meet the limits for labeled compound recovery, the Method does not apply to the sample being analyzed and the result may not be reported or used for permitting or regulatory compliance purposes. In this case, alternate extraction and cleanup procedures in this Method or an alternate GC column must be employed to resolve the interference. If all cleanup procedures in this Method and an alternate GC column have been employed and labeled compound recovery remains outside of the normal range, extraction and/or cleanup procedures that are beyond this scope of this Method will be required to analyze the sample.

19.0 Pollution prevention

19.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. When wastes cannot be reduced at the source, the Agency recommends recycling as the next best option.

19.2 The pesticides in this Method are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

19.3 For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Governmental Relations and Science Policy, 1155 16th Street NW, Washington DC 20036, 202/872-4477 (http://membership.acs.org/c/ccs/pubs/less_is_better.pdf).

20.0 Waste management

20.1 The laboratory is responsible for complying with all Federal, State, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance is also required with any sewage discharge permits and regulations. An overview of requirements can be found in *Environmental Management Guide for Small Laboratories* (EPA 233-B-98-001).

20.2 Samples containing HCl or H_2SO_4 to pH <2, or KOH or NaOH to pH >12 must be handled as hazardous waste, or must be neutralized before being poured down a drain.

20.3 The pesticides decompose above 800°C. Low-level waste such as absorbent paper, tissues, animal remains, and plastic gloves may be burned in an appropriate incinerator. Gross

quantities (milligrams) should be packaged securely and disposed of through commercial or governmental channels that are capable of handling extremely toxic wastes.

20.4 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better-Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

21.0 Method performance

Method 1699 was validated and preliminary data were collected in a single laboratory (Reference 2). Single laboratory performance data are included in Table 8.

22.0 References

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23.0 Tables and Figures

Table 1. Names, CAS Registry numbers, and ambient water quality criteria for pesticides determined by isotope dilution and internal standard HRGC/HRMS.

Pesticide	CAS Number	Labeled analog	Lowest Ambient Criterion (pg/L) (1)	MDLs and MLs, matrix and concentration (2)				
				Water (pg/L)		Solid (ng/kg)		Extract (pg/µL)
Organochlorine				**MDL**	**ML**	**MDL**	**ML**	**ML**
Aldrin	309-00-2	$^{13}C_{12}$-Aldrin	49	6	90	0.6	10	3
BHC, alpha	319-84-6	$^{13}C_{6}$-BHC, alpha	2600	7	60	1.3	10	3
BHC, beta	319-85-7	$^{13}C_{6}$-BHC, beta	9100	6	60	0.6	10	3
BHC, delta	319-86-8	$^{13}C_{6}$-BHC, delta		5	60	2.0	10	3
BHC, gamma (lindane)	58-89-9	$^{13}C_{6}$-BHC, gamma	160000	9	60	0.7	10	3
Captan	133-06-2			182	500	35	100	25
Chlordane, alpha (cis)	5103-71-9		800	7	30	0.6	5	1.5
Chlordane, gamma (trans)	5103-74-2	$^{13}C_{10}$-Chlordane, gamma	800	6	50	0.8	5	2
Chlorothalonil	1897-45-6			35	100	1.9	10	5
Dacthal	1861-32-1			4	20	0.9	2	1
DDD, o,p-	53-19-0			3	30	0.8	5	1.5
DDD, p,p-	72-54-8		11	5	30	1.5	5	1.5
DDE, o,p-	3424-82-6			3	30	0.5	5	1.5
DDE, p,p-	72-55-9	$^{13}C_{12}$-p,p-DDE	11	6	30	0.7	5	1.5
DDT, o,p-	789-02-6	$^{13}C_{12}$-o,p-DDT		2	30	0.3	5	1.5
DDT, p,p-	50-29-3	$^{13}C_{12}$-p,p-DDT	11	1	30	0.3	5	1.5
Dieldrin	60-57-1	$^{13}C_{12}$-Dieldrin	52	5	30	0.5	5	1.5
Endosulfan-alpha	959-98-8	$^{13}C_{9}$-alpha-Endosulfan	8700	24	100	--	--	5
Endosulfan-beta	33213-65-9	$^{13}C_{9}$-beta-Endosulfan	8700	30	100	--	--	5
Endosulfan-sulfate	1031-07-8		62000000	13	40	11	50	2
Endrin	72-20-8	13C_{12}-Endrin	2300	3	30	0.4	5	1.5
Endrin-ketone	53494-70-5			12	40	1.6	5	2
Heptachlor	76-44-8	$^{13}C_{4}$-Heptachlor	79	7	30	--	--	1.5
Heptachlor-epoxide	1024-57-3	$^{13}C_{10}$-Heptachlor-epoxide	40	12	40	0.3	5	2
Hexachlorobenzene	118-74-1	$^{13}C_{6}$-Hexachlorobenzene		4	40	1.9	5	2
Methoxychlor	72-43-5	$^{13}C_{12}$-Methoxychlor	30000	7	30	0.3	5	1.5
Mirex	2385-85-5	$^{13}C_{8}$-Mirex	1000	35	100	--	--	5
Nonachlor, cis-	5103-73-1	$^{13}C_{10}$-Nonachlor, cis-		4	30	0.5	5	1.5
Nonachlor, trans-	39765-80-5	$^{13}C_{10}$-Nonachlor, trans-		11	40	0.8	5	2
Octachlorostyrene	29082-74-4			12	40	1.1	5	2
Oxychlordane	27304-13-8	$^{13}C_{10}$-Oxychlordane		7	60	0.5	10	3
Perthane	72-56-0			36	100	--	--	5
Quintozene	82-68-8			18	80	4.7	20	4
Tecnazene	117-18-0			22	80	3.2	10	4

Organophosphate								
Azinphos-methyl	86-50-0	Azinphos-methyl-d_6		57	200	1.4	20	10
Chlorpyriphos	2921-88-2			20	80	2.0	10	4
Chlorpyriphos-methyl	5598-13-0			19	100	3.0	10	5
Chlorpyriphos-oxon	5598-15-2			24	80	3.5	10	4
Diazinon	333-41-5	Diazinon-d_{10}	170000	27	80	24	100	4
Diazinon-oxon	962-58-3			22	80	--	--	4
Disulfoton	298-04-4			64	400	7.1	100	20
Disulfoton sulfone	2497 05 06			9	30	1.6	5	1.5
Fenitrothion	122-14-5			24	80	4.6	20	4
Fonofos	944-22-9	$^{13}C_6$-Fonofos		11	80	0.8	8	4
Malathion	121-75-5		100000	296	1000	41	200	50
Methamidophos	10265-92-6			269	1000	--	--	50
Parathion-ethyl	56-38-2		13000	15	80	3.5	10	4
Parathion-methyl	298-00-0			39	200	6.1	20	10
Phorate	298-02-2			49	200	3.5	20	10
Phosmet	732-11-6			63	200	12	50	10
Pirimiphos-methyl	29232-93-7			14	80	7.3	20	4
Triazine								
Ametryn	834-12-8			11	80	13	50	4
Atrazine	1912-24-9	$^{13}C_3$-Atrazine		14	80	--	--	4
Cyanazine	21725-46-2			38	80	--	--	4
Desethyl atrazine	6190-65-4			5	40	1.3	5	2
Hexazinone	51235-04-2			20	100	1.0	10	5
Metribuzin	21087-64-9			14	60	--	--	3
Simazine	122-34-9			12	80	1.4	10	4
Pyrethroid								
Cypermethrin	52315-07-8			66	200	2.4	20	10
Permethrins-peak 1	52645-53-1	$^{13}C_6$-cis/trans-Permethrin[3]		59	200	230	1000	10
Permethrins-peak 2	52645-53-1	$^{13}C_6$-cis/trans-Permethrin[3]		44	100	340	1000	5

1. *National Recommended Water Quality Criteria*, 2004, http://epa.gov/waterscience/criteria/wqcriteria.html, and Great Lakes Criteria (40 CFR 132.6), whichever is lower. A blank cell means there is no ambient criterion.
2. Method detection limits (MDLs) and minimum levels of quantitation (MLs) with no interferences present.
3. Elution order of cis/trans permethrin unknown

Table 2. Retention times (RTs); relative retention times (RRTs); and retention time and quantitation references for the pesticides

Pesticide	RT (1)	RRT (2)	RRT Limits (3)	Retention time and quantitation reference (4)	Quant Ref RT
Methamidophos	09:01	0.413	0.397 - 0.428	$^{13}C_{12}$-PCB-52	21:51
Tecnazene	14:44	0.927	0.906 - 0.948	13C_6-HCB	15:54
$^{13}C_6$-Hexachlorobenzene	15:54	0.728	0.712 - 0.743	$^{13}C_{12}$-PCB-52	21:51
Hexachlorobenzene	15:55	1.001	0.991 - 1.012	$^{13}C_6$-HCB	15:54
Phorate	16:11	0.741	0.725 - 0.756	$^{13}C_{12}$-PCB-52	21:51
BHC-alpha	16:35	0.909	0.890 - 0.927	$^{13}C_6$-gamma-BHC	18:15
Desethylatrazine	16:50	0.935	0.917 - 0.954	$^{13}C_3$-Atrazine	18:00
Diazinon-d10	17:32	0.802	0.787 - 0.818	$^{13}C_{12}$-PCB-52	21:51
Quintozene	17:39	1.110	1.089 - 1.131	$^{13}C_6$-HCB	15:54
Diazinon	17:44	1.011	1.002 - 1.021	Diazinon-d10	17:32
Diazinon-oxon	17:55	1.022	1.003 - 1.041	Diazinon-d10	17:32
$^{13}C_3$-Atrazine	18:00	0.824	0.809 - 0.839	$^{13}C_{12}$-PCB-52	21:51
Atrazine	18:01	1.001	0.992 - 1.010	$^{13}C_3$-Atrazine	18:00
$^{13}C_6$-gamma-BHC	18:15	0.835	0.820 - 0.850	$^{13}C_{12}$-PCB-52	21:51
gamma-BHC	18:16	1.001	0.992 - 1.010	$^{13}C_6$-gamma-BHC	18:15
Simazine	18:21	1.019	1.001 - 1.038	$^{13}C_3$-Atrazine	18:00
Fonofos	18:25	1.000	0.991 - 1.009	$^{13}C_6$-Fonofos	18:25
$^{13}C_6$-Fonofos	18:25	0.843	0.828 - 0.858	$^{13}C_{12}$-PCB-52	21:51
Disulfoton	18:34	0.850	0.834 - 0.865	$^{13}C_{12}$-PCB-52	21:51
$^{13}C_6$-beta-BHC	19:26	0.889	0.874 - 0.905	$^{13}C_{12}$-PCB-52	21:51
beta-BHC	19:27	1.001	0.992 - 1.009	$^{13}C_6$-beta-BHC	19:26
$^{13}C_4$-Heptachlor	19:36	0.897	0.882 - 0.912	$^{13}C_{12}$-PCB-52	21:51
Heptachlor	19:37	1.001	0.992 - 1.009	$^{13}C_4$-Heptachlor	19:36
$^{13}C_6$-delta-BHC	21:00	0.961	0.946 - 0.976	$^{13}C_{12}$-PCB-52	21:51
delta-BCH	21:01	1.001	0.993 - 1.009	$^{13}C_6$-delta-BHC	21:00
Chlorothalonil.	21:08	0.967	0.952 - 0.982	$^{13}C_{12}$-PCB-52	21:51
$^{13}C_{12}$-Aldrin	21:15	0.973	0.957 - 0.988	$^{13}C_{12}$-PCB-52	21:51
Aldrin	21:17	1.002	0.994 - 1.009	$^{13}C_{12}$-Aldrin	21:15
Chlorpyriphos-methyl	21:26	0.981	0.966 - 0.996	$^{13}C_{12}$-PCB-52	21:51
$^{13}C_{12}$-PCB-52	21:51	N/A	N/A	N/A	N/A
Parathion-methyl	22:28	1.028	1.013 - 1.043	$^{13}C_{12}$-PCB-52	21:51
Ametryn	22:41	1.038	1.023 - 1.053	$^{13}C_{12}$-PCB-52	21:51
Pirimiphos-methyl	22:42	1.039	1.024 - 1.054	$^{13}C_{12}$-PCB-52	21:51
Metribuzin	23:04	1.056	1.040 - 1.071	$^{13}C_{12}$-PCB-52	21:51
Octachlorostyrene	23:18	1.096	1.081 - 1.112	$^{13}C_{12}$-Aldrin	21:15
Dacthal	23:18	1.066	1.051 - 1.082	$^{13}C_{12}$-PCB-52	21:51
Chlorpyriphos	23:33	1.078	1.063 - 1.093	$^{13}C_{12}$-PCB-52	21:51
Fenitrothion	24:07	1.104	1.088 - 1.119	$^{13}C_{12}$-PCB-52	21:51
$^{13}C_{10}$-Oxychlordane	24:09	1.105	1.090 - 1.121	$^{13}C_{12}$-PCB-52	21:51
Oxychlordane	24:11	1.001	0.994 - 1.008	$^{13}C_{10}$-Oxychlordane	24:09
Malathion	24:12	1.108	1.092 - 1.123	$^{13}C_{12}$-PCB-52	21:51
Heptachlor-epoxide	25:14	0.962	0.956 - 0.969	$^{13}C_{12}$-Heptachlor-epoxide	25:11
^{13}C-Permethrins-Peak_2	42:21	1.114	1.099 - 1.130	$^{13}C_{12}$-PCB-52	21:51
Parathion-ethyl	24:26	1.118	1.103 - 1.133	$^{13}C_{12}$-PCB-52	21:51
Chlorpyriphos-oxon	24:30	1.121	1.106 - 1.137	$^{13}C_{12}$-PCB-52	21:51
$^{13}C_6$-Permethrins-Peak_1	42:04	1.124	1.108 - 1.139	$^{13}C_{12}$-PCB-52	21:51
Azinphos-ethyl-d6	24:33	1.124	1.108 - 1.139	$^{13}C_{12}$-PCB-52	21:51
$^{13}C_{12}$-Heptachlor-epoxide	25:11	1.153	1.137 - 1.168	$^{13}C_{12}$-PCB-52	21:51

$^{13}C_{10}$-t-Chlordane	26:39	1.220	1.204 - 1.235	$^{13}C_{12}$-PCB-52	21:51
t-Chlordane	26:41	1.001	0.995 - 1.008	$^{13}C_{10}$-t-Chlordane	26:39
$^{13}C_{10}$-t-Nonachlor	26:48	1.227	1.211 - 1.242	$^{13}C_{12}$-PCB-52	21:51
t-Nonachlor	26:50	1.001	0.995 - 1.007	$^{13}C_{10}$-t-Nonachlor	26:48
c-Chlordane	27:44	1.041	1.028 - 1.053	$^{13}C_{10}$-t-Chlordane	26:39
$^{13}C_{9}$-alpha-Endosulfan	27:51	1.275	1.259 - 1.290	$^{13}C_{12}$-PCB-52	21:51
Alpha-Endosulfan	27:53	1.001	0.995 - 1.007	$^{13}C_{9}$-alpha-Endosulfan	27:51
o,p-DDE	28:07	0.862	0.852 - 0.873	$^{13}C_{12}$-p,p-DDE	30:36
Cyanazine	28:13	1.291	1.276 - 1.307	$^{13}C_{12}$-PCB-52	21:51
$^{13}C_{12}$-Dieldrin	30:31	1.397	1.381 - 1.412	$^{13}C_{12}$-PCB-52	21:51
Dieldrin	30:34	1.002	0.996 - 1.007	$^{13}C_{12}$-Dieldrin	30:31
p,p-DDE	30:38	0.940	0.935 - 0.945	$^{13}C_{12}$-p,p-DDE	30:36
Captan	31:26	1.439	1.423 - 1.454	$^{13}C_{12}$-PCB-52	21:51
o,p-DDD	32:21	0.952	0.943 - 0.962	$^{13}C_{12}$-o,p-DDT	33:58
$^{13}C_{12}$-p,p-DDE	30:36	1.492	1.477 - 1.507	$^{13}C_{12}$-PCB-52	21:51
Disulfoton-Sulfone.	32:49	1.502	1.487 - 1.517	$^{13}C_{12}$-PCB-52	21:51
$^{13}C_{12}$-Endrin	32:53	1.505	1.490 - 1.520	$^{13}C_{12}$-PCB-52	21:51
Endrin	32:56	1.002	0.996 - 1.007	$^{13}C_{12}$-Endrin	32:53
Perthane	32:58	1.509	1.494 - 1.524	$^{13}C_{12}$-PCB-52	21:51
$^{13}C_{10}$-c-Nonachlor	33:17	1.523	1.508 - 1.539	$^{13}C_{12}$-PCB-52	21:51
c-Nonachlor	33:19	1.001	0.996 - 1.006	$^{13}C_{10}$-c-Nonachlor	33:17
$^{13}C_{12}$-o,p-DDT	33:58	1.555	1.539 - 1.570	$^{13}C_{12}$-PCB-52	21:51
o,p-DDT	33:59	1.000	0.996 - 1.005	^{13}C-o,p-DDT	33:58
^{13}C9-beta-Endosulfan	34:30	1.579	1.564 - 1.594	$^{13}C_{12}$-PCB-52	21:51
p,p-DDD	34:31	0.865	0.857 - 0.874	$^{13}C_{12}$-p,p-DDT	35:53
beta-Endosulfan	34:32	1.001	0.996 - 1.006	$^{13}C_{9}$-beta-Endosulfan	34:30
p,p-DDT	35:54	0.900	0.896 - 0.904	$^{13}C_{12}$-p,p-DDT	35:53
Endosulfan-sulfate	36:54	1.070	1.060 - 1.079	$^{13}C_{9}$-beta-Endosulfan	34:30
$^{13}C_{8}$-Mirex	39:29	1.807	1.792 - 1.822	$^{13}C_{12}$-PCB-52	21:51
Mirex	39:30	1.000	0.996 - 1.005	$^{13}C_{8}$-Mirex	39:29
Hexazinone	39:38	1.814	1.799 - 1.829	$^{13}C_{12}$-PCB-52	21:51
$^{13}C_{12}$-Methoxychlor	39:43	1.818	1.802 - 1.833	$^{13}C_{12}$-PCB-52	21:51
Methoxychlor	39:44	1.000	0.996 - 1.005	$^{13}C_{12}$-Methoxychlor	39:43
Endrin-Ketone	39:47	1.210	1.200 - 1.220	$^{13}C_{12}$-Endrin	32:53
$^{13}C_{12}$-p,p-DDT	35:53	1.825	1.810 - 1.841	$^{13}C_{12}$-PCB-52	21:51
Phosmet	40:55	1.873	1.857 - 1.888	$^{13}C_{12}$-PCB-52	21:51
Permethrins-Peak_1	42:04	1.714	1.707 - 1.72	$^{13}C_{6}$-Permethrins-Peak_1	42:04
Permethrins-Peak_2	42:21	1.739	1.732 - 1.746	$^{13}C_{6}$-Permethrins-Peak_2	42:21
Azinphos-methyl	42:39	1.737	1.730 - 1.744	Azinphos-methyl-d6	42:33
Cypermethrins-Peak_1	43:52	N/A	N/A	$^{13}C_{6}$-Permethrins-Peak_1+2	
Cypermethrins-Peak_2	44:03	N/A	N/A	$^{13}C_{6}$-Permethrins-Peak_1+2	
Cypermethrins-Peak_3	44:11	N/A	N/A	$^{13}C_{6}$-Permethrins-Peak_1+2	

1. Retention time of pesticide or labeled compound.
2. Relative retention time (RRT) between the target and reference compounds.
3. RRT limits based on estimated RRT variability.
4. Labeled compounds that form both the retention time and quantitation reference.
5. Method detection limits (MDLs) and minimum levels of quantitation (MLs) with no interferences present.

Table 3. Concentrations of native and labeled pesticides in stock solutions, spiking solutions, and final extracts

Pesticide	Stock (ng/mL)	Spiking solution (pg/mL)	In 20 µL extract (ng/mL; pg/µL)
Tecnazene	800	800	40
Hexachlorobenzene	800	800	40
Quintozene	1600	1600	80
Heptachlor	600	600	30
Alpha-BHC	1200	1200	60
gamma-BHC (Lindane)	1200	1200	60
beta-BHC	1200	1200	60
delta-BHC	1200	1200	60
Aldrin	1200	1200	60
Dacthal	400	400	20
Octachlorostyrene	600	600	30
Oxychlordane	1200	1200	60
Heptachlor epoxide B	600	600	30
Trans-Chlordane	600	600	30
cis-Chlordane	600	600	30
Trans-Nonachlor	800	800	40
cis-Nonachlor	600	600	30
Endosulfan I (alpha)	600	600	30
Endosulfan II (beta)	600	600	30
Dieldrin	600	600	30
2,4'-DDD	600	600	30
4,4'-DDD	600	600	30
2,4'-DDE	600	600	30
4,4'-DDE	600	600	30
2,4'-DDT	600	600	30
4,4'-DDT	600	600	30
Perthane	600	600	30
Endrin	600	600	30
Endosulfan sulfate	600	600	30
Mirex	600	600	30
Methoxychlor	600	600	30
Endrin ketone	600	600	30
Desethylatrazine	800	800	40
Simazine	1600	1600	80
Atrazine	1600	1600	80
Ametryn	1600	1600	80
Metribuzin	400	400	20
Cyanazine	1600	1600	80
Hexazinone	2000	2000	100

Permethrin	800	800	40
Cypermethrin	4000	4000	200
Chlorothalonil	800	800	40
Diazinon	1600	1600	80
Disulfoton	8000	8000	400
Phorate	1600	1600	80
Methamidophos	1600	1600	80
Diazinon-oxon	1600	1600	80
Fonofos	1600	1600	80
Chlorpyriphos-methyl	2000	2000	100
Parathion-methyl	4000	4000	200
Pirimphos-methyl	1600	1600	80
Chlorpyriphos	1600	1600	80
Fenitrothion	1600	1600	80
Malathion	20000	20000	1000
Parathion-ethyl	1600	1600	80
Chlorpyriphos-oxon	1600	1600	80
Disulfoton sulfone	400	400	20
Azinphos-methyl	2000	2000	100
Captan	4000	4000	200
Phosmet (Imidan)	4000	4000	200
13C6-HCB	1800	1800	90
13C6-gamma-BHC	2600	2600	130
13C4-Heptachlor	1400	1400	70
13C6-beta-BHC	1600	1600	80
13C6-delta-BHC	1600	1600	80
13C12-Aldrin	1600	1600	80
13C10-Oxychlordane	1600	1600	80
13C10-Heptachlor-epoxide	1600	1600	80
13C9-alpha-Endosulfan	1600	1600	80
13C12-Dieldrin	1600	1600	80
13C10-t-Chlordane	1600	1600	80
13C10-t-Nonachlor	1600	1600	80
13C12-p,p-DDE	1600	1600	80
13C12-Endrin	1600	1600	80
13C9-beta-Endosulfan	1600	1600	80
13C10-c-Nonachlor	1600	1600	80
13C12-o,p-DDT	1600	1600	80
13C12-p,p-DDT	1600	1600	80
13C8-Mirex	1600	1600	80
13C12-Methoxychlor	1600	1600	80
Azinphos-methyl-d6	1600	1600	80
Diazinon-d10	1600	1600	80
13C6-Fonofos	1600	1600	80

13C3-Atrazine	1600	1600	80
13C6-Permethrins	1600	1600	80
13C12 PCB 52	1600	1600	80

Table 4. Concentration of pesticides in calibration and calibration verification standards (ng/mL)

	Solution concentration (ng/mL)					
Pesticide	**CS-1 (Hi sens) (1)**	**CS-2**	**CS-3**	**CS-4 (VER)**	**CS-5**	**CS-6**
Tecnazene	2.0	8.0	16.0	40.0	100.0	200.0
Hexachlorobenzene	2.0	8.0	16.0	40.0	100.0	200.0
Quintozene	4.0	16.0	32.0	80.0	200.0	400.0
Heptachlor	1.5	6.0	12.0	30.0	75.0	150.0
alpha-BHC	3.0	12.0	24.0	60.0	150.0	300.0
gamma-BHC (Lindane)	3.0	12.0	24.0	60.0	150.0	300.0
beta-BHC	3.0	12.0	24.0	60.0	150.0	300.0
delta-BHC	3.0	12.0	24.0	60.0	150.0	300.0
Aldrin	3.0	12.0	24.0	60.0	150.0	300.0
Dacthal	1.0	4.0	8.0	20.0	50.0	100.0
Octachlorostyrene	1.5	6.0	12.0	30.0	75.0	150.0
Oxychlordane	3.0	12.0	24.0	60.0	150.0	300.0
Heptachlor epoxide	1.5	6.0	12.0	30.0	75.0	150.0
trans-Chlordane	1.5	6.0	12.0	30.0	75.0	150.0
cis-Chlordane	1.5	6.0	12.0	30.0	75.0	150.0
trans-Nonachlor	2.0	8.0	16.0	40.0	100.0	200.0
cis-Nonachlor	1.5	6.0	12.0	30.0	75.0	150.0
Endosulfan I (alpha)	1.5	6.0	12.0	30.0	75.0	150.0
Endosulfan II (beta)	1.5	6.0	12.0	30.0	75.0	150.0
Dieldrin	1.5	6.0	12.0	30.0	75.0	150.0
2,4'-DDD	1.5	6.0	12.0	30.0	75.0	150.0
4,4'-DDD	1.5	6.0	12.0	30.0	75.0	150.0
2,4'-DDE	1.5	6.0	12.0	30.0	75.0	150.0
4,4'-DDE	1.5	6.0	12.0	30.0	75.0	150.0
2,4'-DDT	1.5	6.0	12.0	30.0	75.0	150.0
4,4'-DDT	1.5	6.0	12.0	30.0	75.0	150.0
Perthane	1.5	6.0	12.0	30.0	75.0	150.0
Endrin	1.5	6.0	12.0	30.0	75.0	150.0
Endosulfan sulfate	1.5	6.0	12.0	30.0	75.0	150.0
Mirex	1.5	6.0	12.0	30.0	75.0	150.0
Methoxychlor	1.5	6.0	12.0	30.0	75.0	150.0
Endrin ketone	1.5	6.0	12.0	30.0	75.0	150.0
Desethylatrazine	2.0	8.0	16.0	40.0	100.0	200.0
Simazine	4.0	16.0	32.0	80.0	200.0	400.0
Atrazine	4.0	16.0	32.0	80.0	200.0	400.0
Ametryn	4.0	16.0	32.0	80.0	200.0	400.0
Metribuzin	1.0	4.0	8.0	20.0	50.0	100.0
Cyanazine	4.0	16.0	32.0	80.0	200.0	400.0
Hexazinone	5.0	20.0	40.0	100.0	250.0	500.0
Permethrin	2.0	8.0	16.0	40.0	100.0	200.0

Cypermethrin	10.0	40.0	80.0	200.0	500.0	1000.0
Chlorothalonil	2.0	8.0	16.0	40.0	100.0	200.0
Diazinon	4.0	16.0	32.0	80.0	200.0	400.0
Disulfoton	20.0	80.0	160.0	400.0	1000.0	2000.0
Phorate	4.0	16.0	32.0	80.0	200.0	400.0
Methamidophos (Monitor)	4.0	16.0	32.0	80.0	200.0	400.0
Diazinon-oxon	4.0	16.0	32.0	80.0	200.0	400.0
Fonofos (Dyfonate)	4.0	16.0	32.0	80.0	200.0	400.0
Chlorpyriphos-methyl	5.0	20.0	40.0	100.0	250.0	500.0
Parathion-methyl	10.0	40.0	80.0	200.0	500.0	1000.0
Pirimphos-methyl	4.0	16.0	32.0	80.0	200.0	400.0
Chlorpyriphos (Dursban)	4.0	16.0	32.0	80.0	200.0	400.0
Fenitrothion	4.0	16.0	32.0	80.0	200.0	400.0
Malathion	50.0	200.0	400.0	1000.0	2500.0	5000.0
Parathion-ethyl (Parathion)	4.0	16.0	32.0	80.0	200.0	400.0
Chlorpyriphos-oxon	4.0	16.0	32.0	80.0	200.0	400.0
Disulfoton sulfone	1.0	4.0	8.0	20.0	50.0	100.0
Azinphos-methyl	5.0	20.0	40.0	100.0	250.0	500.0
Captan	10.0	40.0	80.0	200.0	500.0	1000.0
Phosmet (Imidan)	10.0	40.0	80.0	200.0	500.0	1000.0
13C6-HCB	90.0	90.0	90.0	90.0	90.0	100.0
13C6-gamma-BHC	130.0	130.0	130.0	130.0	130.0	150.0
13C4-Heptachlor	70.0	70.0	70.0	70.0	70.0	100.0
13C6-beta-BHC	80.0	80.0	80.0	80.0	80.0	80.0
13C6-delta-BHC	80.0	80.0	80.0	80.0	80.0	80.0
13C12-Aldrin	80.0	80.0	80.0	80.0	80.0	80.0
13C10-Oxychlordane	80.0	80.0	80.0	80.0	80.0	80.0
13C10-Heptachlor-epoxide	80.0	80.0	80.0	80.0	80.0	80.0
13C9-alpha-Endosulfan	80.0	80.0	80.0	80.0	80.0	80.0
13C12-Dieldrin	80.0	80.0	80.0	80.0	80.0	80.0
13C10-t-Chlordane	80.0	80.0	80.0	80.0	80.0	80.0
13C10-t-Nonachlor	80.0	80.0	80.0	80.0	80.0	80.0
13C12-p,p-DDE	80.0	80.0	80.0	80.0	80.0	80.0
13C12-Endrin	80.0	80.0	80.0	80.0	80.0	80.0
13C9-beta-Endosulfan	80.0	80.0	80.0	80.0	80.0	80.0
13C10-c-Nonachlor	80.0	80.0	80.0	80.0	80.0	80.0
13C12-o,p-DDT	80.0	80.0	80.0	80.0	80.0	80.0
13C12-p,p-DDT	80.0	80.0	80.0	80.0	80.0	80.0
13C8-Mirex	80.0	80.0	80.0	80.0	80.0	80.0
13C12-Methoxychlor	80.0	80.0	80.0	80.0	80.0	80.0
Azinphos-methyl-d6	80.0	80.0	80.0	80.0	80.0	80.0
Diazinon-d10	80.0	80.0	80.0	80.0	80.0	80.0
13C6-Fonofos	80.0	80.0	80.0	80.0	80.0	80.0
13C3-Atrazine	80.0	80.0	80.0	80.0	80.0	80.0

13C6-Permethrins	80.0	80.0	80.0	80.0	80.0	80.0
13C12 PCB 52	80.0	80.0	80.0	80.0	80.0	80.0

1. Additional concentration used for calibration of high sensitivity HRGC/HRMS systems

Table 5. QC acceptance criteria for pesticides in VER, IPR, OPR, and samples[1]

Pesticide (1) (2)	VER (%) (3)	IPR Rec. Limits % (4)	IPR RSD Max	OPR Rec. limits (%) (5)	Recovery in samples (%) (6)
13C12-Aldrin	70-130	6 - 113	75	5 - 126	5 - 120
13C3-Atrazine	70-130	20 - 133	45	18 - 147	36 - 132
13C6-beta-BHC	70-130	19 - 127	46	17 - 141	32 - 130
13C10-c-Nonachlor	70-130	18 - 139	47	17 - 154	36 - 139
13C6-delta-BHC	70-130	18 - 135	47	16 - 150	36 - 137
13C12-Dieldrin	70-130	21 - 145	46	19 - 161	40 - 151
13C6-Fonofos	70-130	6 - 108	63	5 - 120	5 - 132
13C6-gamma-BHC	70-130	6 - 112	62	5 - 124	11 - 120
13C6-Hexachlorobenzene	70-130	6 - 108	70	5 - 120	5 - 120
13C4-Heptachlor	70-130	6 - 115	67	5 - 128	5 - 120
13C10-Heptachlor-epoxide	70-130	9 - 131	52	8 - 146	27 - 137
13C8-Mirex	70-130	6 - 125	56	5 - 138	5 - 120
13C12-o,p-DDT	70-130	16 - 180	51	14 - 200	5 - 199
13C10-Oxychlordane	70-130	6 - 129	54	5 - 144	23 - 135
13C12-p,p-DDE	70-130	29 - 152	43	26 - 169	47 - 160
13C12-p,p-DDT	70-130	15 - 180	52	13 - 200	5 - 120
13C6-Permethrin-Peak_1	70-130	35 - 180	43	32 - 200	35 - 189
13C6-Permethrin-Peak_2	70-130	35 - 180	43	31 - 200	31 - 192
13C10-T-Chlordane	70-130	17 - 130	47	15 - 144	21 - 132
13C10-T-Nonachlor	70-130	15 - 134	49	13 - 149	14 - 136
13C12-Endrin	70-130	22 - 141	45	20 - 157	35 - 155
13C12-Methoxychlor	70-130	8 - 180	54	8 - 200	5 - 120
13C9-alpha-Endosulfan	70-130	6 - 130	63	5 - 144	15 - 148
13C9-beta-Endosulfan	70-130	6 - 108	59	5 - 120	5 - 122
Diazinon-d10	75 -125	6 - 130	54	5 - 145	21 - 141
Azinphos-methyl-d6	70 -130	6 - 180	57	5 - 200	20 - 179
o,p'-DDD	75 - 125	55 - 108	30	50 - 120	
o,p'-DDE	75 - 125	26 - 111	30	24 - 123	
o,p'-DDT	75 - 125	55 - 108	30	50 - 120	
p,p'-DDD	75 - 125	47 - 108	30	42 - 120	
p,p'-DDE	75 - 125	55 - 108	30	50 - 120	
p,p'-DDT	75 - 125	55 - 108	30	50 - 120	
Aldrin	75 - 125	55 - 108	30	50 - 120	
Alpha-Endosulfan	75 - 125	55 - 108	30	50 - 120	
beta-Endosulfan	75 - 125	5-200	50	5-200	
Disulfoton	75 - 125	5-200	50	5-200	
alpha-BHC	75 - 125	55 - 108	30	50 - 120	
Ametryn	75 - 125	6 - 160	52	5 - 178	
Atrazine	75 - 125	55 - 108	30	50 - 120	
Azinphos-methyl	75 - 125	55 - 108	30	50 - 120	

beta-BHC	75 - 125	55 - 108	30	50 - 120	
c-Chlordane	75 - 125	55 - 108	30	50 - 120	
c-Nonachlor	75 - 125	55 - 108	30	50 - 120	
Captan	75 - 125	6 - 108	39	5 - 120	
Chlorothalonil	75 - 125	6 - 108	47	5 - 120	
Chlorpyrifos	75 - 125	21 - 147	46	19 - 163	
Chlorpyrifos-methyl	75 - 125	10 - 130	51	9 - 145	
Chlorpyrifos-oxon	75 - 125	6 - 143	43	5 - 158	
Octachlorostyrene	70 -130	55 - 158	30	50 - 175	
Cyanazine	75 - 125	10 - 176	53	9 - 195	
Dacthal	75 - 125	18 - 129	46	16 - 143	
delta-BHC	75 - 125	55 - 108	30	50 - 120	
Desethylatrazine	75 - 125	55 - 108	30	50 - 120	
Diazinon	75 - 125	55 - 108	30	50 - 120	
Diazinon-oxon	75 - 125	55 - 144	30	50 - 160	
Dieldrin	75 - 125	55 - 108	30	50 - 120	
Disulfoton sulfone	75 - 125	6 - 180	79	5 - 200	
Endosulfan-sulfate	75 - 125	55 - 180	30	50 - 200	
Endrin	75 - 125	55 - 108	30	50 - 120	
Endrin-ketone	75 - 125	55 - 120	30	50 - 134	
Fenitrothion	75 - 125	15 - 168	50	14 - 186	
Fonofos	75 - 125	55 - 108	30	50 - 120	
Gamma-BHC	75 - 125	55 - 108	30	50 - 120	
Hexachlorobenzene	75 - 125	55 - 108	30	50 - 120	
Heptachlor	75 - 125	55 - 108	30	50 - 120	
Heptachlor-epoxide	75 - 125	55 - 108	30	50 - 120	
Hexazinone	75 - 125	6 - 154	74	5 - 171	
Malathion	75 - 125	15 - 136	48	14 - 151	
Methamidophos	75 - 125	6 - 108	68	5 - 120	
Methoxychlor	75 - 125	55 - 108	30	50 - 120	
Metribuzin	75 - 125	6 - 134	58	5 - 149	
Mirex	75 - 125	55 - 108	30	50 - 120	
Oxychlordane	75 - 125	55 - 108	30	50 - 120	
Parathion-ethyl	75 - 125	13 - 147	50	12 - 164	
Parathion-methyl	75 - 125	7 - 136	53	7 - 151	
Perthane	75 - 125	26 - 180	46	24 - 200	
Phorate	75 - 125	6 - 108	291	5 - 120	
Phosmet	75 - 125	14 - 138	49	13 - 153	
Pirimiphos-methyl	75 - 125	6 - 151	64	5 - 168	
Quintozene	75 - 125	55 - 180	30	50 - 200	
Simazine	75 - 125	55 - 108	30	50 - 120	
t-Chlordane	75 - 125	55 - 108	30	50 - 120	
t-Nonachlor	75 - 125	55 - 108	30	50 - 120	
Technazene	75 - 125	55 - 154	30	50 - 171	

Total-Cypermethrins	75 - 125	55 - 108	30	50 - 120	
Total-Permethrins	75 - 125	55 - 180	30	50 - 200	

1. QC acceptance criteria for IPR, OPR, and samples based on a 20 µL extract final volume
2. For concentrations see Table 3 spike solutions.
3. Section 15.3.
4. Section 9.2.
5. Section 15.6.
6. Section 9.3: Recovery of labeled compounds from samples.

Table 6. Scan functions; exact m/z's (m1 and m2), ratios and tolerances; retention times (RTs); and quantitation references.

Func -tion	Pesticide	m1 (1)	m2 (1)	m1/m2		RT (min)	Quantified against labeled standard
				Ratio	Tolerance (+/-)		
1	Methamidophos	93.9642	94.9721		0.35	09:01	13C12-PCB-52
2	HCB	283.8102	285.8072	1.25	0.25	15:55	13C6-HCB
2	Tecnazene	258.8761	260.8732	0.78	0.35	14:44	13C6-HCB
2	13C6-HCB	289.8303	291.8273	1.25	0.25	15:54	13C12-PCB-52
2	Phorate	260.0128	262.0086	6.92	0.35	16:11	13C12-PCB-52
3	Desethylatrazine	172.0390	174.0360	3.11	0.35	16:50	13C3-Atrazine
3	Alpha-HCH	218.9116	220.9086	2.08	0.25	16:35	13C6-gamma-BHC
4	Atrazine	215.0938	217.0908	3.08	0.35	18:01	13C3-Atrazine
4	Simazine	201.0781	203.0752	3.1	0.35	18:21	13C3-Atrazine
4	Fonofos	246.0302	247.0336		0.35	18:25	13C6-Fonofos
4	gamma-HCH	218.9116	220.9086	2.08	0.25	18:16	13C6-gamma-BHC
4	Quintozene	236.8413	238.8384	1.56	0.35	17:39	13C6-HCB
4	13C3-Atrazine	218.1038	220.1009	3.08	0.35	18:00	13C12-PCB-52
4	13C6-Fonofos	252.0503	253.0537	1000	0.35	18:25	13C12-PCB-52
4	13C6-gamma-BHC	222.9346	224.9317	0.77	0.25	18:15	13C12-PCB-52
4	Diazinon-d10	282.1074	314.1638	1000	0.35	17:32	13C12-PCB-52
4	Disulfoton	274.0285	275.0318		0.35	18:34	13C12-PCB-52
4	Diazinon	276.0698	304.1011		0.35	17:44	Diazinon-d10
4	Diazinon-oxon	273.1004	288.1239		0.35	17:55	Diazinon-d10
5	Aldrin	262.8569	264.854	1.56	0.25	21:17	13C12-Aldrin
5	Beta-BHC	218.9116	220.9086	2.08	0.25	19:27	13C6-beta-BHC
5	Delta-BHC	218.9116	220.9086	2.08	0.25	21:01	13C6-delta-BHC
5	Heptachlor	271.8102	273.8072	1.25	0.25	19:37	13C4-Heptachlor
5	13C12-Aldrin	269.8804	271.8775	1.56	0.25	21:15	13C12-PCB-52
5	13C6-beta-BHC	222.9346	224.9317	0.77	0.25	19:26	13C12-PCB-52
5	13C6-delta-BHC	222.9346	224.9317	0.77	0.25	21:00	13C12-PCB-52
5	13C4-Heptachlor	276.8269	278.824	1.24	0.25	19:36	13C12-PCB-52
5	Chlorothalonil.	263.8816	265.8786	0.78	0.35	21:08	13C12-PCB-52
5	Chlorpyriphos-methyl	285.9261	287.9232	1.44	0.35	21:26	13C12-PCB-52
5	13C12-PCB-52	301.9626	303.9597	0.78	0.25	21:51	
6	Octachlorostyrene	270.8443	272.8413	0.63	0.25	23:18	13C12-Aldrin
6	Ametryn	227.1205	228.1238		0.35	22:41	13C12-PCB-52
6	Dacthal	298.8836	300.8807	0.78	0.35	23:18	13C12-PCB-52
6	Metribuzin	198.0701	199.0735		0.35	23:04	13C12-PCB-52
6	Parathion-methyl	263.0017	264.0051		0.35	22:28	13C12-PCB-52
6	Pirimiphos-methyl	276.0572	290.0728		0.35	22:42	13C12-PCB-52
7	Oxychlordane	262.8569	264.8540	1.56	0.25	24:11	13C10-Oxychlordane
7	13C10-Oxychlordane	269.8804	271.8775	1.56	0.25	24:09	13C12-PCB-52
7	Chlorpyriphos	313.9574	315.9545	1.44	0.35	23:33	13C12-PCB-52
7	Chlorpyriphos-oxon	269.9490	271.9462	1.54	0.35	24:30	13C12-PCB-52

7	Fenitrothion	260.0146	277.0174		0.35	24:07	13C12-PCB-52
7	Malathion	283.9942	285.0020		0.35	24:12	13C12-PCB-52
7	Parathion-ethyl	291.0330	292.0364		0.35	24:26	13C12-PCB-52
8	Heptachlor-epoxide	262.8569	264.8540	1.56	0.25	24:14	13C12-Heptachlor-epoxide
8	alpha-Endosulfan	262.8569	264.8540	1.56	0.25	27:53	13C9-alpha-Endosulfan
8	Dieldrin	262.8569	264.8540	1.56	0.25	30:34	13C12-Dieldrin
8	o,p-DDE	246.0003	247.9974	1.56	0.25	28:07	13C12-p,p-DDE
8	p,p-DDE	246.0003	247.9974	1.56	0.25	30:38	13C12-p,p-DDE
8	13C12-Heptachlor-epoxide	269.8804	271.8775	1.56	0.25	25:11	13C12-PCB-52
8	13C9-alpha-Endosulfan	269.8804	271.8775	1.56	0.25	27:51	13C12-PCB-52
8	13C12-Dieldrin	269.8804	271.8775	1.56	0.25	30:31	13C12-PCB-52
8	13C12-p,p-DDE	258.0406	260.0376	1.56	0.25	32:36	13C12-PCB-52
8	13C10-t-Chlordane	269.8804	271.8775	1.56	0.25	26:39	13C12-PCB-52
8	13C10-t-Nonachlor	269.8804	271.8775	1.56	0.25	26:48	13C12-PCB-52
8	Cyanazine	240.0890	242.0861	3.06	0.35	28:13	13C12-PCB-52
8	c-Chlordane	262.8569	264.854	1.56	0.25	27:44	13C10-t-Chlordane
8	t-Chlordane	262.8569	264.854	1.56	0.25	26:41	13C10-t-Chlordane
8	t-Nonachlor	262.8569	264.854	1.56	0.25	26:50	13C10-t-Nonachlor
9	Endrin	262.8569	264.854	1.56	0.25	32:56	13C12-Endrin
9	c-Nonachlor	262.8569	264.854	1.56	0.25	33:19	13C10-c-Nonachlor
9	o,p-DDD	235.0081	237.0052	1.56	0.25	32:21	13C12-o,p-DDT
9	13C12-Endrin	269.8804	271.8775	1.56	0.25	32:53	13C12-PCB-52
9	Captan	263.9653	265.9623	1.44	0.35	31:26	13C12-PCB-52
9	Disulfoton-Sulfone.	213.0173	214.0251		0.35	32:49	13C12-PCB-52
9	Perthane	224.1520	223.1487		0.35	32:58	13C12-PCB-52
10	beta-Endosulfan	264.8540	262.8569	0.64	0.25	34:32	13C9-beta-Endosulfan
10	Endosulfan-sulfate	264.8540	262.8569	0.64	0.25	36:54	13C9-beta-Endosulfan
10	o,p-DDT	235.0081	237.0052	1.56	0.25	33:59	13C12-o,p-DDT
10	p,p-DDD	235.0081	237.0052	1.56	0.25	34:31	13C12-p,p-DDT
10	p,p-DDT	235.0081	237.0052	1.56	0.25	35:54	13C12-p,p-DDT
10	13C9-beta-Endosulfan	269.8804	271.8775	1.56	0.25	34:30	13C12-PCB-52
10	13C10-c-Nonachlor	269.8804	271.8775	1.56	0.25	33:17	13C12-PCB-52
10	13C12-o,p-DDT	247.0484	249.0454	1.56	0.25	33:58	13C12-PCB-52
10	13C12-p,p-DDT	247.0484	249.0454	1.56	0.25	39:53	13C12-PCB-52
11	Endrin-ketone	247.8521	249.8491	0.63	0.25	39:47	13C12-Endrin
11	Methoxychlor	227.1072	228.1106		0.35	39:44	13C12-Methoxychlor
11	Mirex	236.8413	238.8384	1.56	0.25	39:30	13C8-Mirex
11	13C12-Methoxychlor	239.1475	240.1508		0.35	39:43	13C12-PCB-52
11	13C8-Mirex	241.8581	243.8551	1.56	0.25	39:29	13C12-PCB-52
11	13C6-Permethrins-Peak_1	189.1011	190.1045		0.35	24:33	13C12-PCB-52
11	13C6-Permethrins-Peak_2	189.1011	190.1045		0.35	24:21	13C12-PCB-52
11	Azinphos-methyl-d6	160.0511	161.0544		0.35	24:33	13C12-PCB-52
11	Hexazinone	171.0882	172.0916		0.35	39:38	13C12-PCB-52
11	Phosmet	160.0399	161.0432		0.35	40:55	13C12-PCB-52

11	Permethrins-Peak_1	183.0081	184.0843		0.35	42:04	13C6-Permethrins-Peak_1
11	Cypermethrins-Peak_1	163.0081	165.0052	1.56	0.35	43:52	13C6-Permethrins-Peak_1+2
11	Cypermethrins-Peak_2	163.0081	165.0052	1.56	0.35	44:03	13C6-Permethrins-Peak_1+2
11	Cypermethrins-Peak_3	163.0081	165.0052	1.56	0.35	44:11	13C6-Permethrins-Peak_1+2
11	Permethrins-Peak_2	183.0081	184.0843		0.35	42:21	13C6-Permethrins-Peak_2
11	Azinphos-methyl	160.0511	161.0544		0.35	42:39	Azinphos-methyl-d6
11	Total-Cypermethrins	163.0081	165.0052	1.56	0.35		
11	Total-Permethrins	183.0081	184.0843		0.35		

1. Isotopic masses used for accurate mass calculation

^{1}H	1.0078
^{12}C	12.0000
^{13}C	13.0034
^{35}Cl	34.9689
^{37}Cl	36.9659
^{19}F	18.9984
^{14}N	14.0031
^{16}O	15.9949

Table 7. Suggested sample quantities to be extracted for various matrices [1]

Sample matrix[2]	Example	Percent solids	Phase	Quantity extracted
Single-phase				
Aqueous	Drinking water	<1	–[3]	1000 mL
	Groundwater			
	Treated wastewater			
Solid	Dry soil	>20	Solid	10 g
	Compost			
	Ash			
Organic	Waste solvent	<1	Organic	10 g
	Waste oil			
	Organic polymer			
Tissue	Fish	–	Organic	10 g
	Human adipose			
Multi-phase				
Liquid/Solid				
Aqueous/Solid	Wet soil	1-30	Solid	10 g
	Untreated effluent			
	Digested municipal sludge			
	Filter cake			
	Paper pulp			
Organic/solid	Industrial sludge	1-100	Both	10 g
	Oily waste			
Liquid/Liquid				
Aqueous/organic	In-process effluent	<1	Organic	10 g
	Untreated effluent			
	Drum waste			
Aqueous/organic/solid	Untreated effluent	>1	Organic & solid	10 g
	Drum waste			

1. The quantity of sample to be extracted is adjusted to provide 10 g of solids (dry weight). One liter of aqueous samples containing one percent solids will contain 10 grams of solids. For aqueous samples containing greater than one percent solids, a lesser volume is used so that 10 grams of solids (dry weight) will be extracted.

2. The sample matrix may be amorphous for some samples. In general, when the pesticides are in contact with a multi-phase system in which one of the phases is water, they will be preferentially dispersed in or adsorbed on the alternate phase because of their low water solubility.

3. Aqueous samples are filtered after spiking with the labeled compounds. The filtrate and the materials trapped on the filter are extracted separately, and the extracts are combined for cleanup and analysis.

Table 8. Performance data from single laboratory validation.

	Solid-Based			Reagent Water-Based			Biosolids-Based on 8 (native)		
	on 5 samples			on 4 samples			or 6 (label) samples		
	Solids Average Recovery	Solids Standard Deviation	Solids Relative Standard Deviation	Water Average Recovery	Water Standard Deviation	Water Relative Standard Deviation	Biosolids Average Recovery	Biosolids Standard Deviation	Biosolids Relative Standard Deviation
13C12-ENDRIN	90.43	31.77	28.73	86.81	8.60	7.46	104.57	13.16	13.76
13C12-METHOXYCHLOR	128.41	32.12	41.25	100.11	7.61	7.62	20.64	53.78	11.10
13C9-ALPHA-ENDOSULPHAN	78.70	35.89	28.25	58.13	8.21	4.77	99.81	7.05	7.04
13C9-BETA-ENDOSULPHAN	41.74	33.54	14.00	29.95	7.71	2.31	84.12	10.42	8.76
13C-ALDRIN	63.24	40.59	25.67	44.20	23.24	10.27	55.15	13.54	7.47
13C-ATRAZINE	79.29	33.93	26.90	87.33	6.03	5.27	85.34	7.72	6.59
13C-BETA-HCH	82.51	31.58	26.05	74.98	6.06	4.55	84.55	11.92	10.08
13C-C-NONACHLOR	90.04	31.72	28.56	79.79	5.75	4.59	89.72	8.54	7.66
13C-DELTA-HCH	88.30	31.10	27.46	76.82	6.14	4.72	91.15	6.01	5.48
13C-DIELDRIN	93.89	31.51	29.59	85.15	5.83	4.96	103.06	5.37	5.53
13C-FONOFOS	50.73	40.18	20.38	49.40	27.30	13.49	90.65	8.48	7.69
13C-GAMMA-HCH	68.29	32.52	22.21	48.74	23.03	11.22	71.55	17.26	12.35
13C-HCB	52.33	31.93	16.71	29.07	37.79	10.98	54.51	17.21	9.38
13C-HEPTACHLOR	68.85	33.42	23.01	42.58	27.19	11.58	55.32	34.65	19.17
13C-HEPTACHLOR-EPOXIDE	83.80	31.90	26.73	68.38	10.13	6.93	90.61	7.70	6.98
13C-MIREX	79.07	31.94	25.25	59.67	9.65	5.76	46.84	24.47	11.46
13C-O,P-DDT	123.18	30.97	38.16	99.18	7.07	7.02	48.25	43.86	21.16
13C-OXYCHLORDANE	83.09	30.78	25.58	62.67	10.04	6.29	87.54	11.61	10.16
13C-P,P-DDE	99.83	30.31	30.25	94.99	6.26	5.95	111.80	5.68	6.34
13C-P,P-DDT	140.36	30.94	43.42	110.00	4.43	4.87	33.09	50.74	16.79
13C-PERMETHRINS-PEAK_1	116.88	32.00	37.40	128.45	6.05	7.77	97.01	11.02	10.69
13C-PERMETHRINS-PEAK_2	118.48	32.32	38.30	130.18	6.11	7.95	94.06	11.15	10.49
13C-T-CHLORDANE	86.23	29.38	25.34	71.73	7.67	5.50	72.27	21.41	15.47
13C-T-NONACHLOR	87.23	31.29	27.30	74.09	7.72	5.72	66.33	26.20	17.38
2,4'-DDD	81.14	1.09	0.89	102.00	1.33	1.36	270.95	81.98	222.13
2,4'-DDE	84.86	15.50	13.15	58.65	7.59	4.45	101.19	2.88	2.91
2,4'-DDT	97.46	0.50	0.48	98.77	0.77	0.76	98.26	9.75	9.58
4,4'-DDD	45.89	3.04	1.40	46.43	1.70	0.79	430.55	97.71	420.69
4,4'-DDE	99.45	0.67	0.66	92.40	1.23	1.14	102.43	7.38	7.56
4,4'-DDT	95.81	0.94	0.90	97.66	0.66	0.64	97.19	7.90	7.68
ALDRIN	97.30	1.88	1.83	99.82	5.14	5.13	97.94	15.24	14.93
ALPHA-ENDOSULPHAN	94.02	4.50	4.23	91.74	4.03	3.70	90.06	13.49	12.15

ALPHA-HCH	86.44	2.34	2.03	80.00	12.67	10.14	94.43	11.18	10.56
AMETRYN	38.46	57.63	22.17	105.31	6.94	7.31	124.18	15.18	18.85
ATRAZINE	99.47	1.51	1.50	98.95	0.76	0.75	108.25	25.55	27.66
AZINPHOS-METHYL	95.50	1.32	1.27	91.43	2.10	1.92	92.45	12.98	12.00
BETA-ENDOSULPHAN	*	*	*	*	*	*	97.41	12.31	11.99
BETA-HCH	101.26	1.23	1.24	103.95	0.62	0.65	96.72	10.14	9.81
CAPTAN	2.03			39.76	15.77	6.27	*	*	*
C-CHLORDANE	97.89	1.77	1.73	95.29	2.47	2.36	109.10	98.97	107.98
CHLOROTHALONIL	18.32	43.37	7.94	46.46	12.52	5.82	5.43	124.49	6.76
CHLORPYRIPHOS	95.97	30.61	29.38	85.71	9.45	8.10	112.15	9.12	10.23
CHLORPYRIPHOS-METHYL	82.40	31.23	25.73	70.07	17.14	12.01	95.67	16.32	15.61
CHLORPYRIPHOS-OXON	0.64	42.62	0.27	84.10	14.59	12.27	59.89	47.09	28.20
CL8-STYRENE	122.49	16.49	20.20	109.15	12.37	13.50	132.75	7.10	9.42
C-NONACHLOR	99.49	1.65	1.64	99.95	0.99	0.99	99.96	7.50	7.50
CYANAZINE	104.03	38.26	39.80	99.88	5.26	5.25	121.57	31.95	38.84
D10-DIAZINON	77.75	36.37	28.28	71.13	15.58	11.08	88.11	16.37	14.43
D6-AZINPHOS-METHYL	93.60	42.99	40.24	118.19	4.83	5.71	90.12	18.63	16.79
DACTHAL	81.66	31.90	26.05	77.16	12.06	9.31	100.50	25.52	25.65
DELTA-HCH	102.16	1.56	1.59	99.31	3.08	3.06	97.90	9.92	9.71
DESETHYLATRAZINE	99.84	2.63	2.63	86.50	4.41	3.81	111.16	20.68	22.99
DIAZINON	98.58	1.85	1.83	98.08	1.36	1.33	93.14	7.25	6.75
DIAZINON-OXON	*	*	*	131.28	5.15	6.76	82.64	36.03	29.78
DIELDRIN	101.70	0.95	0.97	103.87	0.71	0.74	95.23	16.77	15.97
DIMETHOATE	75.95	38.51	29.25	85.52	10.34	8.84	114.22	20.38	23.28
DISULFOTON	*	*	*	*	*	*	60.23	46.33	27.91
DISULFOTON SULFONE	477.45	41.55	198.40	651.23	39.89	259.79	139.98	57.09	79.91
ENDOSULPHAN-SULPHATE	231.97	1.94	4.50	271.05	3.40	9.22	88.54	20.18	17.87
ENDRIN	101.22	3.00	3.04	103.49	1.60	1.66	98.44	9.29	9.15
ENDRIN-KETONE	104.25	10.11	10.54	95.59	8.87	8.48	71.24	41.83	29.80
ETHION	90.12	61.34	55.28	49.68	4.65	2.31	167.53	32.83	54.99
FENITROTHION	101.29	35.89	36.36	98.41	9.50	9.35	151.55	17.43	26.42
FONOFOS	106.68	2.93	3.13	98.46	2.43	2.40	98.06	7.60	7.45
GAMMA-HCH	95.92	1.44	1.39	94.86	0.82	0.78	95.09	10.82	10.28
HCB	102.21	0.10	0.10	102.89	2.58	2.65	100.09	7.31	7.32
HEPTACHLOR	100.14	1.30	1.30	99.58	3.27	3.26	91.71	9.12	8.37
HEPTACHLOR-EPOXIDE	101.40	1.84	1.87	101.50	2.31	2.35	102.08	7.48	7.64
HEXAZINONE	27.26	90.24	24.60	89.16	19.32	17.23	130.31	21.84	28.45
MALATHION	83.84	34.77	29.15	81.02	7.61	6.17	95.88	37.53	35.98
METHAMIDOPHOS	29.71	37.83	11.24	*	*	*	32.62	40.38	13.17
METHOXYCHLOR	101.14	2.51	2.54	98.17	1.44	1.41	105.54	9.42	9.95
METRIBUZIN	80.54	37.63	30.31	67.41	5.70	3.84	130.08	13.38	17.41
MIREX	103.40	2.45	2.53	94.01	3.69	3.46	103.95	10.84	11.27
OXYCHLORDANE	97.11	0.88	0.86	103.17	2.14	2.21	93.78	12.65	11.86
PARATHION-ETHYL	91.86	34.54	31.72	82.74	7.33	6.06	146.11	22.28	32.55

PARATHION-METHYL	82.54	35.71	29.48	74.72	13.29	9.93	139.95	16.25	22.74
PERTHANE	120.82	31.57	38.14	108.82	6.85	7.45	144.00	23.38	33.67
PHORATE	5.72	167.39	9.57	14.53	137.43	19.96	81.78	21.37	17.48
PHOSMET	80.19	36.61	29.36	86.54	8.34	7.22	86.09	75.25	64.78
PIRIMIPHOS-METHYL	73.75	48.99	36.13	87.90	8.09	7.11	112.14	33.53	37.60
QUINTOZENE	129.60	3.06	3.96	163.36	15.06	24.60	142.76	25.37	36.22
SIMAZINE	106.25	2.05	2.18	104.43	3.02	3.15	110.07	11.58	12.75
T-CHLORDANE	101.65	5.24	5.33	104.93	1.01	1.06	104.36	26.02	27.16
TECNAZENE	120.17	3.76	4.52	107.67	23.63	25.44	132.60	36.97	49.02
TERBUFOS	7.50	167.78	12.58	20.86	127.25	26.54	84.92	26.80	22.75
T-NONACHLOR	100.91	1.81	1.83	102.74	4.02	4.13	94.64	35.56	33.65
TOTAL-CYPERMETHRINS	93.50	3.29	3.07	93.57	4.36	4.08	71.64	7.06	5.06
TOTAL-PERMETHRINS	145.58	16.20	23.58	144.77	2.53	3.66	655.40	124.74	817.53

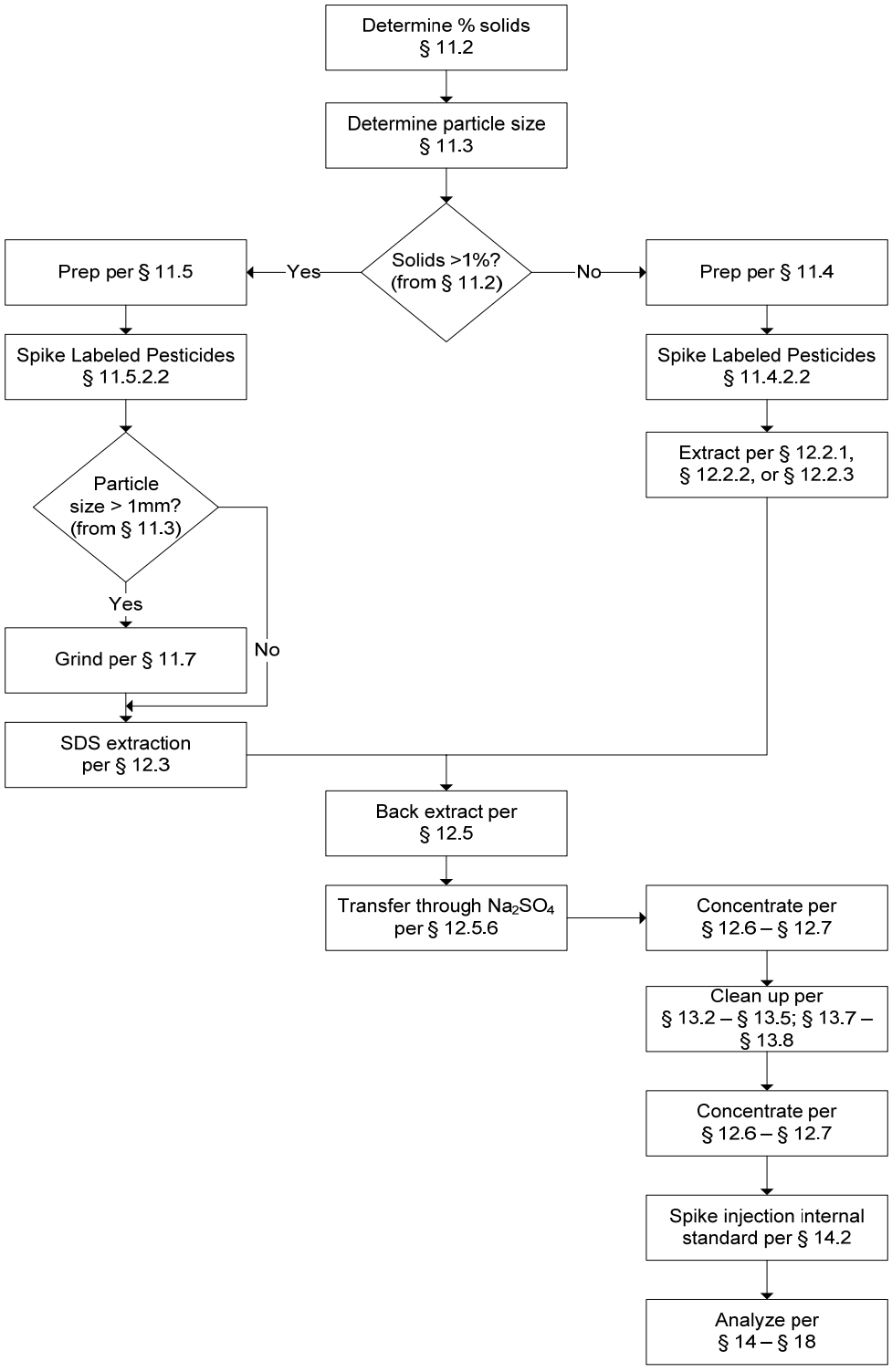


Figure 1 Flow Chart for Analysis of Aqueous and Solid Samples

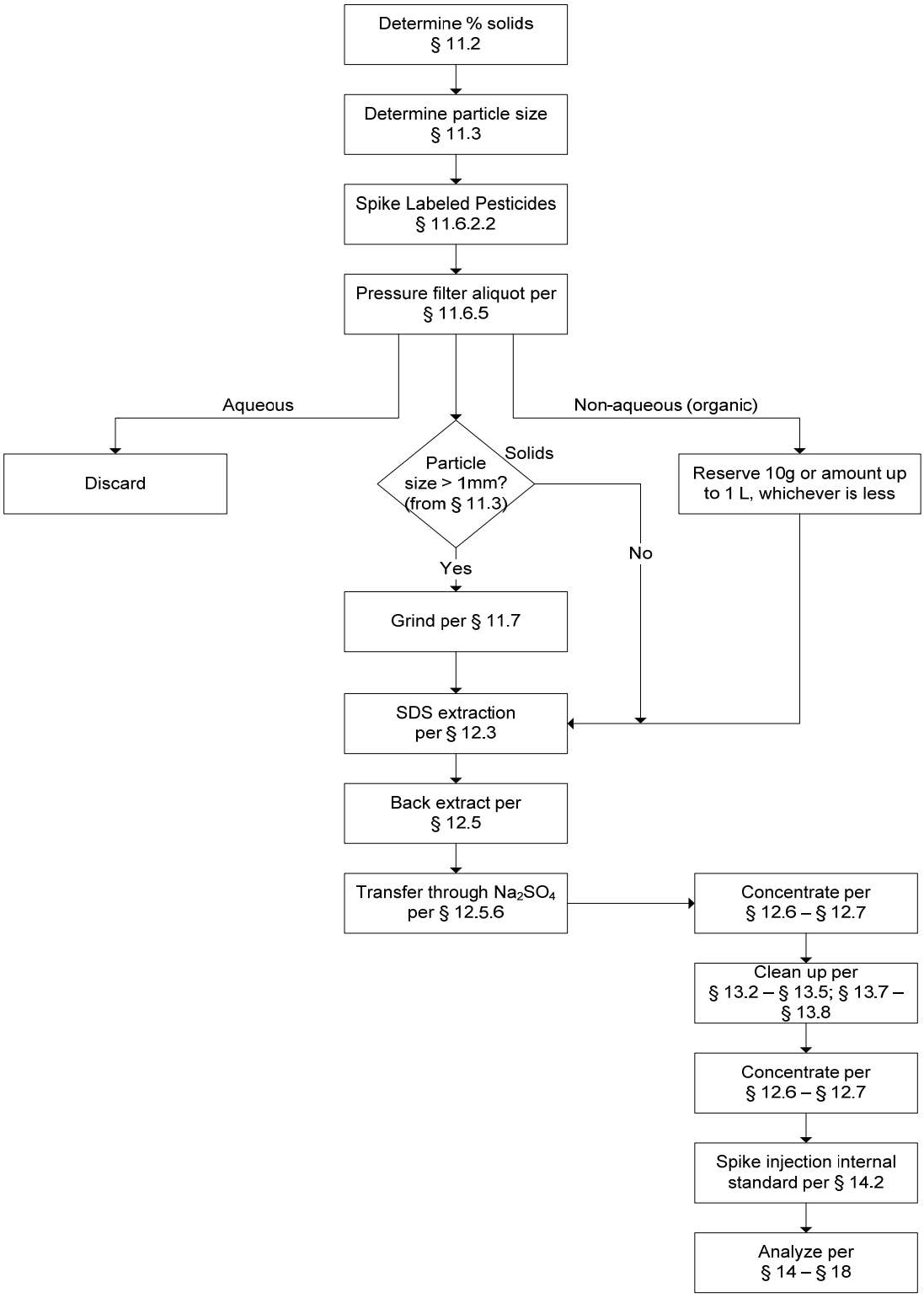


Figure 2 Flow Chart for Analysis of Multi-Phase Samples

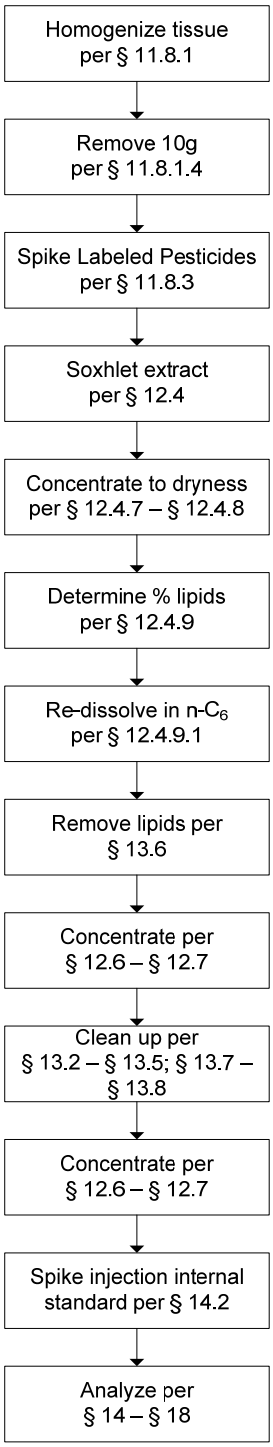


Figure 3 Flow Chart for Analysis of Tissue Samples

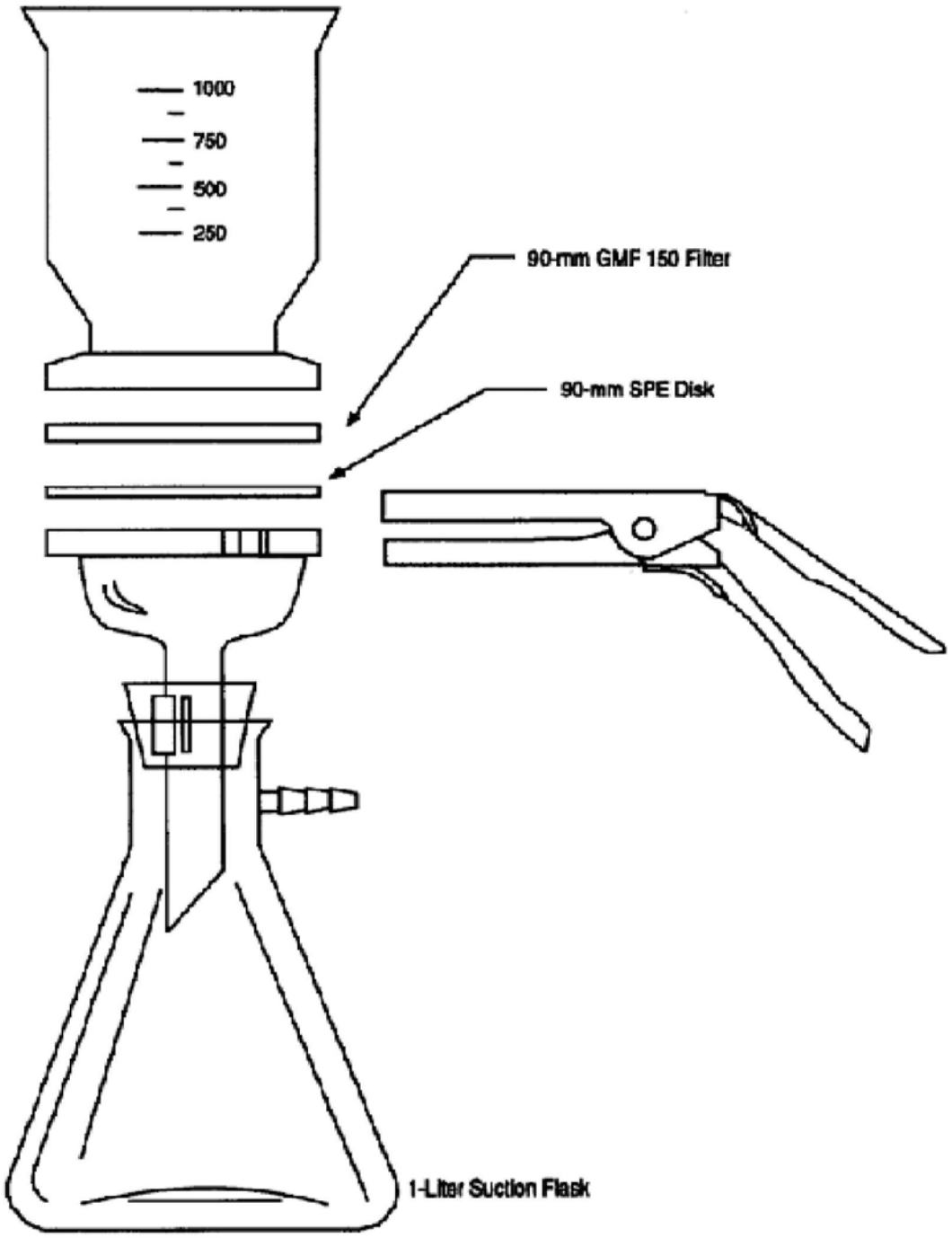


Figure 4 Solid-phase Extraction Apparatus

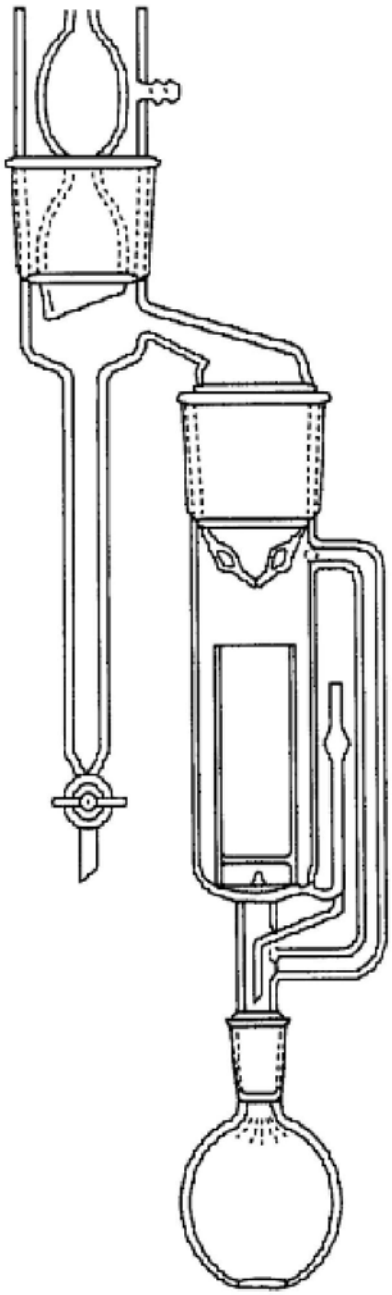


Figure 5 Soxhlet/Dean-Stark Extractor

24.0 Glossary

These definitions and purposes are specific to this Method but have been conformed to common usage to the extent possible.

24.1 Units of weight and measure

24.1.1 Symbols

EC	degrees Celsius
ΦL	microliter
Φm	micrometer
<	less than
>	greater than
%	percent

24.1.2 Alphabetical abbreviations

cm	centimeter
g	gram
h	hour
ID	inside diameter
in.	inch
L	liter
M	Molecular ion
m	mass or meter
mg	milligram
min	minute
mL	milliliter
mm	millimeter
m/z	mass-to-charge ratio
N	normal; gram molecular weight of solute divided by hydrogen equivalent of solute, per liter of solution
OD	outside diameter
pg	picogram
ppb	part-per-billion
ppm	part-per-million
ppq	part-per-quadrillion
ppt	part-per-trillion
psig	pounds-per-square inch gauge
v/v	volume per unit volume
w/v	weight per unit volume

25.0 Definitions and acronyms (in alphabetical order)

Analyte – A pesticide tested for by this Method. The analytes are listed in Table 1.

Calibration standard (CAL) – A solution prepared from a secondary standard and/or stock solution and used to calibrate the response of the HRGC/HRMS instrument.

Calibration verification standard (VER) – The mid-point calibration standard (CS-4) that is used to verify calibration. See Table 4.

CS-1, CS-2, CS-3, CS-4, CS-5, CS-6 – See Calibration standards and Table 4.

Field blank – An aliquot of reagent water or other reference matrix that is placed in a sample container in the field, and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the field blank is to determine if the field or sample transporting procedures and environments have contaminated the sample.

GC – Gas chromatograph or gas chromatography

GPC – Gel permeation chromatograph or gel permeation chromatography

HPLC – High performance liquid chromatograph or high performance liquid chromatography

HRGC – High resolution GC

HRMS – High resolution MS

Labeled injection internal standard – Labeled PCB52 is spiked into the concentrated extract immediately prior to injection of an aliquot of the extract into the HRGC/HRMS.

Internal standard – a labeled compound used as a reference for quantitation of other labeled compounds and for quantitation of native pesticides other than the pesticide of which it is a labeled analog. See Internal standard quantitation.

Internal standard quantitation – A means of determining the concentration of (1) a naturally occurring (native) compound by reference to a compound other than its labeled analog and (2) a labeled compound by reference to another labeled compound.

IPR – Initial precision and recovery; four aliquots of a reference matrix spiked with the analytes of interest and labeled compounds and analyzed to establish the ability of the laboratory to generate acceptable precision and recovery. An IPR is performed prior to the first time this Method is used and any time the Method or instrumentation is modified.

Isotope dilution quantitation – A means of determining a naturally occurring (native) compound by reference to the same compound in which one or more atoms has been isotopically enriched. In this Method, labeled are enriched with carbon-13 to produce ^{13}C-labeled analogs. The ^{13}C-labeled pesticides are spiked into each sample to allow identification and correction of the concentration of the native compounds in the analytical process.

K-D – Kuderna-Danish concentrator; a device used to concentrate the analytes in a solvent

Laboratory blank – See Method blank

Laboratory control sample (LCS) – See Ongoing precision and recovery standard (OPR)

Laboratory reagent blank – See Method blank

May – This action, activity, or procedural step is neither required nor prohibited.

May not – This action, activity, or procedural step is prohibited.

Method blank – An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with samples. The Method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

Method detection limit (MDL) – The lowest concentration at which a pesticide can be detected under routine operating conditions (see 40 CFR 136, appendix B). MDLs are listed in Table 1.

Minimum level (ML) – The greater of a multiple of the MDL or the lowest calibration point (see 68 FR 11790, March 12, 2003.)

MS – Mass spectrometer or mass spectrometry

Must – This action, activity, or procedural step is required.

OPR – Ongoing precision and recovery standard (OPR); a method blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in this Method for precision and recovery.

Perfluorokerosene (PFK) – A mixture of compounds used to calibrate the exact m/z scale in the HRMS.

Preparation blank – See Method blank

Quality control check sample (QCS) – A sample containing all or a subset of the analytes at known concentrations. The QCS is obtained from a source external to the laboratory or is prepared from a source of standards different from the source of calibration standards. It is used to check laboratory performance with test materials prepared external to the normal preparation process.

Reagent water – water demonstrated to be free from the analytes of interest and potentially interfering substances at the method detection limit for the analyte.

Relative standard deviation (RSD) – The standard deviation times 100 divided by the mean. Also termed "coefficient of variation."

RF – Response factor. See Section 10.5

RR – Relative response. See Section 10.4

RSD – See relative standard deviation

SDS – Soxhlet/Dean-Stark extractor; an extraction device applied to the extraction of solid and semi-solid materials (Reference 3 and Figure 5).

Signal-to-noise ratio (S/N) – The height of the signal as measured from the mean (average) of the noise to the peak maximum divided by the width of the noise.

Should – Although this action, activity, or procedural step is suggested and not required, you may be asked why you changed or omitted this action, activity, or procedural step.

SICP – Selected ion current profile; the line described by the signal at an exact m/z.

SPE – Solid-phase extraction; an extraction technique in which an analyte is extracted from an aqueous sample by passage over or through a material capable of reversibly adsorbing the analyte. Also termed liquid-solid extraction.

Stock solution – A solution containing an analyte that is prepared using a reference material traceable to EPA, the National Institute of Science and Technology (NIST), or a source that will attest to the purity and authenticity of the reference material.

Unique GC resolution or uniquely resolved – Two adjacent chromatographic peaks in which the height of the valley is less than 10 percent of the height of the shorter peak (see Section 6.9.1.1.2).

VER – See Calibration verification.

ANNEXURE-II

10.7.5 Organochlorine Pesticides

A. Liquid-liquid extraction by gas chromatography with electron capture detector

This method covers the determination of certain organochlorine pesticides in water and wastewater samples. The following parameters can be determined by this method:

aldrin, a-BHC, b-BHC, d-BHC, g-BHC, chlordane, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, dieldrin, endosulphan-I, endosulphan-II, endrin, endosulphan sulphate, heptachlor, heptachlor epoxide and toxaphene. This method is applicable for the determination of the above listed compounds in water. The method detection limit for each parameter is listed in Table 10.7.1 at the end of this chapter. A single sample may be extracted to measure the parameters included in this method. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, to apply appropriate cleanup procedures. The analyst is allowed to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters. This method is restricted to be used by or under the supervision of analysts experienced in the use of a gas chromatograph and in the interpretation of gas chromatograms.

10.7.5.1 Principle

A measured volume of sample, approximately 1 L, is extracted with 15% diethyl ether in n-hexane using a separatory funnel. The extract is concentrated to a volume of 0.5 mL or less. The extract is separated by gas chromatography and the parameters are then measured with an electron capture detector. The method provides a Florisil column cleanup procedure and an elemental sulphur removal procedure to aid in the elimination of interferences that may be encountered.

10.7.5.2 Apparatus and equipment

Sampling equipment, for discrete or composite sampling :

Grab sample bottle: 1 L, amber glass, fitted with a screw cap lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber colour bottles are not available, protect samples from light. The bottle and cap liner must be washed, rinsed with acetone or n-hexane and dried before use to minimise contamination. Automatic sampler (optional)-the sampler must incorporate glass sample containers for the collection of a minimum of 250 mL of sample. Sample containers must be kept refrigerated at 4°C and protected from light.

Glassware :

a. Separatory funnel: 2 L, with Teflon stopcock

b. Drying column: Chromatographic column, approximately 400 mm long x 19 mm ID, with coarse frit filter disc

c. Chromatographic column: 400 mm long x 22 mm ID, with Teflon stopcock and coarse frit filter disc (Kontes K-42054 or equivalent)

d. Concentrator tube, Kuderna-Danish-10 mL, graduated (Kontes K 570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

e. Evaporative flask, Kuderna-Danish-500 mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs

f. Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000 0121 or equivalent)

g. Vials: 10 to 15 mL, amber colour glass, with Teflon-lined screw cap

h. Boiling chips: approximately 10/40 mesh. Heat to 400°C for 30 minutes or soxhlet extract with methylene chloride

i. Water bath: heated, with concentric ring cover, capable of temperature control (±2°C).The bath should be used in a hood.

j. Balance: Analytical, capable of accurately weighing 0.0001 g

Gas Chromatograph: An analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector and strip-chart recorder. A data system is recommended for measuring peak areas.

1. Column 1: DB-1, 10 mm film, 30 m x 0.32 mm ID, fused silica capillary with methyl polysiloxane phase, oven temperature from 180-260°C at 4°C/min (hold at 260°C till all compounds elute).

2. Column 2: Durawax-Dx3, 0.25 mm film, 30 m x 0.32 mm ID, fused silica capillary with 1:1 dimethyl silicone and polyethylene glycol, oven temperature from 100-210°C at 8°C/min (hold at 210°C till all compounds elute).

3. Column 3: OV-17, 1.5 mm, 25m x 0.32 mm ID, fused silica capillary with 50:50 methyl-phenyl silicone, oven temperature from 100-260°C at 4°C/min (hold at 260°C till all compounds elute).

4. Column 4: Supelco Inc., fused silica open tubular column pair DB-5 (0.83 mm film thickness) 30 m x 0.25 nm ID, column temperature 100°C (2 min hold) to 270°C (1 min hold) at 2.8°C/min.

10.7.5.3 Reagents and standards

a. Reagent water: reagent water is defined as water in which an interferent is not observed at the method detection limit of the parameters of interest.

b. Sodium hydroxide solution (10N): dissolve 40 g of NaOH in reagent water and dilute to 100 mL.

c. Sodium thiosulphate: granular.

d. Sulphuric acid (1+1): slowly, add 50 mL of H_2SO_4 to 50 mL of reagent water.

e. Acetone, hexane, isooctane: pesticide quality or equivalent.

f. Ethyl-ether: nano-grade, redistilled in glass if necessary.

g. Sodium sulphate: granular, anhydrous. Purify by heating at 400°C for four hours in a shallow tray

h. Florisil: PR grade (60/100 mesh): activated at 1250°F and store in the dark in glass containers with ground glass stoppers or foil-lined screw caps. Before use, activate each batch at least 16 hours at 130°C in a foil-covered glass container and allow cooling

i. Mercury: triple distilled

j. Copper powder: activated

k. Stock standard solutions (1 µg/µL): stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

Prepare stock standard solutions by accurately weighing about 0.01 g of pure material. Dissolve the material in n-hexane and dilute to volume in a 10 mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration, if they are certified by the manufacturer or by an independent source.

10.7.5.4 Sample collection, preservation and storage

Grab samples must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle must not be prerinsed with sample before collection. Composite samples should be collected and refrigerated in glass containers in accordance with the requirements of the programme. All samples must be iced or refrigerated at 4°C from the time of collection until extraction. If the samples will not be extracted within 72 hours of collection, the sample should be adjusted to a pH range of 5 - 9 with sodium hydroxide solution or sulphuric acid. Record the volume of acid or base used. If aldrin is to be determined, add sodium thiosulphate when residual chlorine is present. All samples must be extracted within seven days of collection and completely analysed within 40 days of extraction.

10.7.5.5 Calibration and standardisation

Establish GC operating parameters :

Warning: Endrin is easily degraded in the injection port if the injection port or front of the column is dirty. This is the result of buildup of high boiling residue from sample injection. Check for degradation problems daily by injecting a midlevel standard containing only endrin. Look for the degradation products of endrin (endrin ketone and endrin aldehyde). If degradation of endrin exceeds 20%, take corrective action before proceeding with calibration. Calculate percent breakdown as follows:

$$\frac{\text{Total endrin degradation peak area (endrin aldehyde + endrin ketone)}}{\text{Total endrin peak area (endrin + endrin aldehyde + endrin ketone)}} \times 100\%$$

..... [Equation 10.7.1]

At least three calibration standards are needed; five are recommended. Guidance on the number of standards is as follows: A minimum of three calibration standards are required to calibrate a range of a factor of 20 in concentration. For a factor of 50 use at least four standards, and for a factor of 100 at least five standards. The lowest standard should represent analyte concentrations near, but above, their respective MDLs. The remaining standards should bracket the analyte concentrations expected in the sample extracts, or should define the working range of the detector.

To prepare a calibration standard (CAL), add an appropriate volume of a secondary dilution standard to a 35 mL aliquot of reagent water in a 40 mL bottle. Do not add less than 20 µL of an alcoholic standard to the reagent water. Use a 25 µL micro syringe and rapidly inject the methanolic standard into the middle point of the water volume. Remove the needle as quickly as possible after injection. Mix by inverting and shaking the capped bottle several times. Aqueous standards must be prepared fresh daily.

Starting with the standard of lowest concentration, prepare, extract, and analyse each calibration standard and tabulate peak height or area response versus the concentration in the standard. The results are to be used to prepare a calibration curve for each compound by plotting the peak height or area response versus the concentration. Alternatively,

if the ratio of concentration to response (calibration factor) is a constant over the working range (20% RSD or less), linearity to the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

Note: Toxaphene, chlordane, and Aroclor standards must be injected separately.

The working calibration curve or calibration factor must be verified on each working day by the measurement of a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (greater than eight hours), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analyses. If the response for any analyte varies from the predicted response by more than the criteria, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve. For those analytes that failed the calibration verification, results from field samples analysed since the last passing calibration should be considered suspect. Reanalyse sample extracts for these analytes after acceptable calibration is restored.

Note: It is suggested that a calibration verification standard of one multicomponent analyte, either chlordane, toxaphene or an Aroclor also be analysed each work shift. By selecting a different multi-component analyte for this calibration verification each work shift, continuing calibration data can be obtained for all of these analytes over the course of several days.

Instrument Performance: Check the performance of the entire analytical system daily using data gathered from analyses of laboratory reagent blanks (LRB), CAL, and laboratory duplicate samples (LD1 and LD2). Significant peak tailing in excess of that shown for the target compounds in the method chromatograms must be corrected. Tailing problems are generally traceable to active sites on the GC column, improper column installation, or operation of the detector. Check the precision between replicate analyses. Poor precision is generally traceable to pneumatic leaks, especially at the injection port. If the GC system is apparently performing acceptably but with decreased sensitivity, it may be necessary to generate a new curve or set of calibration factors to verify the decreased responses before searching for the source of the problem.

10.7.5.6 Procedure

Sample extraction :

Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2 L separatory funnel. Add 60 mL of 15% diethyl ether in n-hexane to the sample bottle, seal, and shake 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the extract in a 250 mL Erlenmeyer flask.

Add a second 60 mL volume of 15% diethyl ether to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner with 60 mL of n-hexane and 36 mL saturated sodium sulphate solution. Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10 mL concentrator tube to a 500 mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D concentrator. Pour the combined extract through a solvent-rinsed drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20-30 mL of n-hexane to complete the quantitative transfer. Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Pre-wet the Snyder column by adding about 1 mL of n hexane to the top. Place the K-D apparatus on a hot water bath (60-65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapour. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15-20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

Clean-up and Separation :

Clean-up procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a cleanup procedure, the analyst may use either procedure below or any other appropriate procedure. The florisil column allows for a select fractionation of the compounds and will eliminate polar interferences. Elemental sulphur, which interferes with the electron capture gas chromatography of certain pesticides, can be removed by the technique described in the following section.

Florisil column cleanup: Place florisil (20 g), into a chromatographic column. Tap the column to settle the florisil and add 1-2 cm of anhydrous sodium sulphate to the top.

Add 60 mL of hexane to wet and rinse the sodium sulphate and florisil. Just prior to exposure of the sodium sulphate layer to the air, stop the elution of the hexane by closing the stopcock on the chromatographic column. Discard the eluate.

Adjust the sample extract volume to 10 mL with n-hexane and transfer it from the K-D concentrator tube onto the column. Rinse the tube twice with 1-2 mL of n-hexane, adding each rinse to the column.

Place a 500 mL K-D flask and clean concentrator tube under the chromatographic column. Drain the column into the flask until the sodium sulphate layer is nearly exposed. Elute the column with 200 mL of 6% diethyl-ether in n-hexane (v/v) (Fraction 1) at a rate of about 5 mL/min. Remove the K-D flask and set it aside for later concentration. Elute the column again, using 200 mL of 15% diethyl - ether in n-hexane (v/v) (Fraction 2), into a second K-D flask. Perform the third elution using 200 mL of 50% diethyl-ether in n-Hexane (v/v) (Fraction 3).

Concentrate the fractions as described in the 'sample extraction' section, except use n-hexane to prewet the column and set the water bath at about 85°C. When the apparatus is cool, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with n-hexane. Adjust the volume of each fraction to 10 mL with n-hexane and analyse by gas chromatography as per the details given below.

Confirmation test for DDT: It can be carried by treating the sample extract with 0.1N potassium hydroxide with gentle heating. If DDT is present, the same will be converted to DDE.

Gas Chromatography: Table 10.7.1 summarises the recommended operating conditions for the gas chromatograph. Included in this table are retention times and MDL that can be achieved under these conditions. Examples of the separations achieved by Column 1 are shown in Figures 10.7.1- 10.7.3 Other packed or capillary (open tubular) columns, chromatographic conditions, or detectors may be used. If the internal standard calibration procedure is being used, the internal standard must be added to the sample extract and mixed thoroughly immediately before injection into the gas chromatograph. Inject 1 µL of the sample extract or standard into the gas chromatograph using the solvent-flush technique. Record the volume injected to the nearest 0.05 µL, the total extract volume, and the resulting peak size in area or peak height units. Identify the parameters in the sample by comparing the retention times of the peaks in the sample chromatogram with those of the peaks in standard chromatograms. The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms. If the response for a peak exceeds the working range of the system, dilute the extract and reanalyse. If the measurement of the peak response is prevented by the presence of interferences, further clean-up is required.

10.7.5.7 Data analysis and calculations

If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor determined in the section 10.7.5.6. The concentration in the sample can be calculated from equation 10.7.2.

$$\text{Concentration (mg/L)} = \frac{(A)\ (V_t)}{(V_i)\ (V_s)} \quad \ldots\ldots \text{[Equation 10.7.2]}$$

where:

A = Amount of material injected (ng).

V_i = Volume of extract injected (µL).

V_t = Volume of total extract (µL).

V_s = Volume of water extracted (mL).

If the internal standard calibration procedure is used, calculate the concentration in the sample using the response factor (RF) determined in Equation 10.7.3.

$$\text{Concentration (mg/L)} = \frac{(A_s)\ (I_s)}{(V_{is})\ (RF)\ (v_O)} \quad \ldots\ldots \text{[Equation 10.7.3]}$$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

I_s = Amount of internal standard added to each extract (µg) . sV

V_o = Volume of water extracted (L).

For multi component mixtures (chlordane, toxaphene) match retention times of peaks in the standards with peaks in the sample. Quantitate every identifiable peak unless interference with individual peaks persists after cleanup. Add peak height or peak area of each identified peak in the chromatogram. Calculate as total response in the sample versus total response in the standard.

Report results in µg/L without correction for recovery data. All QC data obtained should be reported with the sample results.

10.7.5.8 Method performance and quality control

The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL concentrations listed in Table 10.7.1 were obtained using reagent water. The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and matrix effects.

Minimum quality control (QC) requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks (LRB), laboratory fortified blanks (LFB), laboratory fortified sample matrix (LFM), and quality control samples (QCS). A MDL for each analyte must also be determined.

Laboratory reagent blanks (LRB); before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, an LRB must be analysed. If within the retention time window of any analyte the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples/ Initial Demonstration of capability:

Select a representative fortified concentration (about 10 times MDL) or at a concentration in the middle of the calibration range for each analyte. Prepare a standard concentrate containing each analyte at 1000 times the selected concentration. With a syringe, add 35 µL of the concentrate to each of four to seven 35 mL aliquots of reagent water, and analyse each aliquot according to procedures. For each analyte the mean recovery value for these samples must fall in the range of ±30% of the fortified amount. The RSD for these measurements must be 20% or less. For those compounds that meet the acceptance criteria, performance is considered acceptable. For those compounds that fail these criteria, this procedure must be repeated using fresh replicate samples until satisfactory performance has been demonstrated. For each analyte, determine the MDL. Prepare a minimum of seven LFBs at a low concentration. Use calibration data obtained in Section 10.7.5.5 to estimate a concentration for each analyte that will produce a peak with a three to five times signal to noise response. Extract and analyse each replicate according to Sections 10.7.5.6. It is recommended that these LFBs be prepared and analysed over a period of several days, so that day to day precision is reflected in the precision measurements. Calculate mean recovery and standard deviation for each analyte.

The initial demonstration of capability is used primarily to preclude a laboratory from analysing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. It is expected that as laboratory personnel gain experience with this method the quality of data will improve beyond those required here. The analyst is permitted to modify GC columns, GC conditions, concentration techniques (i.e., evaporation techniques), internal standards or surrogate compounds. Each time such method modifications are made, the analyst must repeat the procedures.

Assessing laboratory performance - laboratory fortified blank (LFB): The laboratory must analyse at least one LFB per sample set (all samples extracted within a 24 hour period). If the sample set contains more than 20 samples, analyse one LFB for every 20 samples. Ideally the fortifying concentration of each analyte in the LFB sample should be the same as that selected. Calculate accuracy as percent recovery (Xi). If the recovery of any analyte falls outside the control limits, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses. Because this LFB is prepared and analysed in the same way as a calibration verification standard, it can be used to satisfy the calibration requirement.

Note: It is suggested that one multi-component analyte (toxaphene, chlordane or an Aroclor) LFB also be analysed with each sample set. By selecting a different multi-component analyte for this LFB each work shift, LFB data can be obtained for all of these analytes over the course of several days. Until sufficient data become available from within their own laboratory, usually a minimum of results from 20-30 analyses, the laboratory may assess laboratory performance against the control limits that are derived from the data. When sufficient internal performance data becomes available, develop control limits from the mean percent recovery (x) and standard deviation (s) of the percent recovery. These data are used to establish upper and lower control limits as follows:

Upper Control Limit = $\underline{x} + 3s$ ..... [Equation 10.7.4]

Lower Control Limit = $\underline{x} - 3s$ ..... [Equation 10.7.5]

After each five to 10 new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points. These calculated control limits should not exceed the fixed limits. It is recommended that the laboratory periodically determine and document its detection limit capabilities for analytes of interest.

Caution: No attempts to establish low detection limits should be made before instrument optimisation and adequate conditioning of both the column and the GC system. Conditioning includes the processing of LFB and LFM samples containing moderate concentration levels of these analytes. At least quarterly the laboratory should analyse quality control samples (QCS). If acceptance criteria are not met, corrective action should be taken and documented.

Assessing analyte recovery-Laboratory fortified sample matrix (LFM): The laboratory must add a known concentration to a minimum of 10% of the routine samples or one LFM per set, whichever is greater. The fortified concentration should not be less than the background concentration of the sample selected for fortification. Ideally the LFM concentration should be the same as that used for the LFB. Periodically, samples from all routine sample sources should be fortified. Calculate the percent recovery for each analyte, corrected for background concentrations measured in the unfortified sample. If the recovery of any such analyte falls outside the range of ±35% of the fortified amount, and the laboratory performance for that analyte is shown to be in control, the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects. The laboratory may adopt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analysed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage. It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depends upon the need of laboratory and the nature of the samples. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used.

10.7.5.9 Interferences

Interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks. Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Solvent rinsing should be followed by detergent washing with hot water and rinses with tap water and distilled water. The glassware should then be drained dry, and heated in a muffle furnace at 400°C for 15-30 minutes. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminium foil.

The use of high purity reagents and solvents helps to minimise interference problems. Purification of solvents by distillation in all-glass systems may be required.

Interferences by phthalate esters can pose a major problem in pesticide analysis when using the electron capture detector. These compounds generally appear in the chromatogram as large late eluting peaks, especially in the 15 and 50% fractions from Florisil. Common flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination. The interferences from phthalate esters can be avoided by using a micro-colorimetric or electrolytic conductivity detector. Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature of sample. The clean-up can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the method detection limit listed in Table 10.7.1.

10.7.5.10 Safety

The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified for the information of the analyst. The following parameters covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogens: 4,4'-DDT, 4,4' -DDD and the BHCs. Primary standards of these toxic compounds should be prepared in a hood.

10.7.5.11 Bibliography

1. Standard Methods for the Examination of Water and Wastewater; APHA, AWWA and WEF, 21st Edition, 2005.

Table 10.7.1: Chromatographic conditions and method detection limits

Parameter	Retention time (min)				Method detection limit (µg/L)
	Column 1	Column 2	Column 3	Column 4	
a-BHC				12.35	0.003
g-BHC	12.3	18.4	18.7	14.14	0.004
b-BHC				13.80	0.006
Heptachlor	15.9	17.5	20.0	18.34	0.003
d-BHC				15.49	0.009
Aldrin	17.6	18.4	21.4	20.37	0.004
Heptachlor	19.0	24.6	24.6	22.83	0.083
epoxide					
Endosulfan I				25.00	0.014
4,4'-DDE				26.80	0.004
Dieldrin	23.2	45.1	27.8	26.60	0.002
Endrin	23.2	33.3	29.2	27.86	0.006
4,4'-DDD				29.32	0.011
Endosulfan II				28.45	0.004
4,4'-DDT				31.62	0.012
Endrin aldehyde				29.63	0.023
Endosulfan sulfate				31.62	0.066
Chlordane	mr	mr		mr	0.014
Toxaphene	mr	mr		mr	0.240

Column 1 conditions: DB-1, 10 mm film, 30 m x 0.32 mm ID, fused silica capillary with methyl polysiloxane phase, oven temperature from 180-260°C at 4°C/min (hold at 260°C till all compounds elute).

Column 2 conditions: Durawax-Dx3, 0.25 mm film, 30 m x 0.32 mm ID, fused silica capillary with 1:1 dimethyl silicone and polyethylene glycol, oven temperature from 100-210°C at 8°C/min (hold at 210°C till all compounds elute).

Column 3 conditions: OV-17, 1.5 mm, 25 m x 0.32 mm ID, fused silica capillary with 50:50 methyl-phenyl silicone, oven temperature from 100-260°C at 4°C/min (hold at 260°C till all compounds elute).

Column 4 conditions: Supelco Inc, fused silica open tubular column pair. DB-5 (0.83 mm film thickness) 30 m x 0.25 nm ID, column temperature 100°C (2 min hold) to 270°C (1 min hold) at 2.8°C/min.

mr = multiple peak response

Table 10.7.2: Distribution of chlorinated pesticides into florisil column fractions

Parameter	Percent recovery by fraction[a]		
	1	2	3
Aldrin	100	....	....
a -BHC	100	....	....
b -BHC	97	....	....
d-BHC	98	....	....
g-BHC	100	....	....
Chlordane	100	....	....
4,4'-DDD	99	....	....
4,4'-DDE	98	....	....
4,4'-DDT	100	....	....
Dieldrin	0	100	....
Endosulfan I	37	64	....
Endosulfan II	0	7	91
Endosulfan sulfate	0	0	106
Endrin	4	96	....
Endrin aldehyde	0	68	26
Heptachlor	100	....	....
Heptachlor epoxide	100	....	....
Toxaphene	96	....	....

Fraction 1 - 6% diethyl ether in n-hexane
Fraction 2 - 15% diethyl ether in n-hexane
Fraction 3 - 50% diethyl ether in n-hexane
[a] Eluent composition

Table 10.7.3: QC acceptance criteria

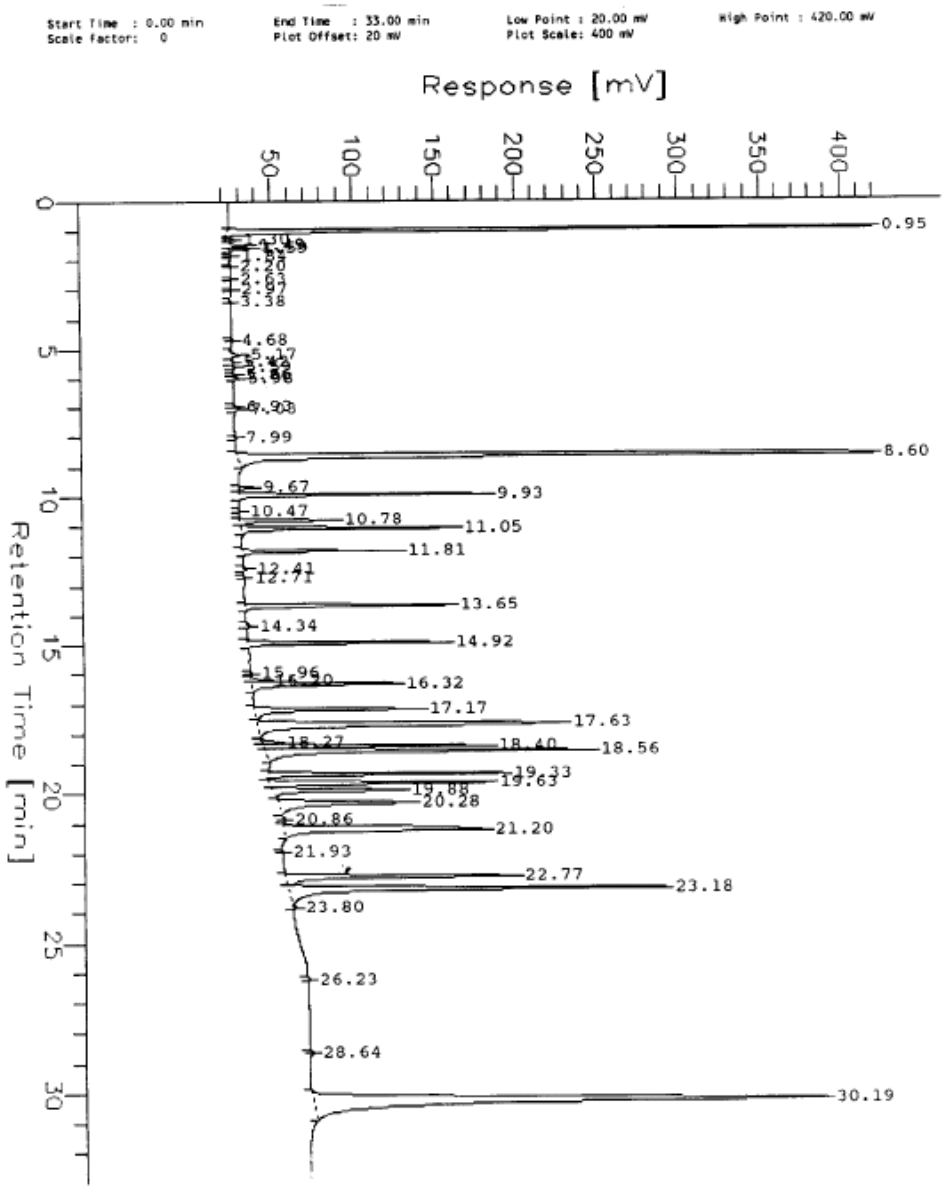
Parameter	Test conc. (mg/L)	Limit for s (mg/L)	Range for X (mg/L)	Range for P, Ps (%)
Aldrin	2.0	0.42	1.0-2.24	42-122
a-BHC	2.0	0.48	0.98-2.44	37-134
b-BHC	2.0	0.64	0.78-2.60	17-147
d-BHC	2.0	0.72	1.01-2.37	19-140
g-BHC	2.0	0.46	0.86-2.32	32-127
Chlordane	50	10.0	27.6-54.3	45-119
4,4'-DDD	10	2.8	4.8-12.6	31-141
4,4'-DDE	2.0	0.55	1.08-2.60	30-145
4,4'-DDT	10	3.6	4.6-13.7	25-160
Dieldrin	2.0	0.76	1.15-2.49	36-146
Endosulphan I	2.0	0.49	1.14-2.82	45-153
Endosulphan II	10	6.1	2.2-17.1	D-202
Endosulphan sulphate	10	2.7	2.8-13.2	26-144
Endrin	10	3.7	5.1-12.6	30-147
Heptachlor	2.0	0.40	0.86-2.00	34-111
Heptachlor epoxide	2.0	0.41	1.13-2.63	37-142
Toxaphene	50	12.7	27.8-55.6	41-126

s = Standard deviation of four recovery measurements, in µg/L (Section 10.7.5.8)

X= Average recovery for four recovery measurements, in µg/L (Section 10.7.5.8)

P, Ps = Percent recovery measured

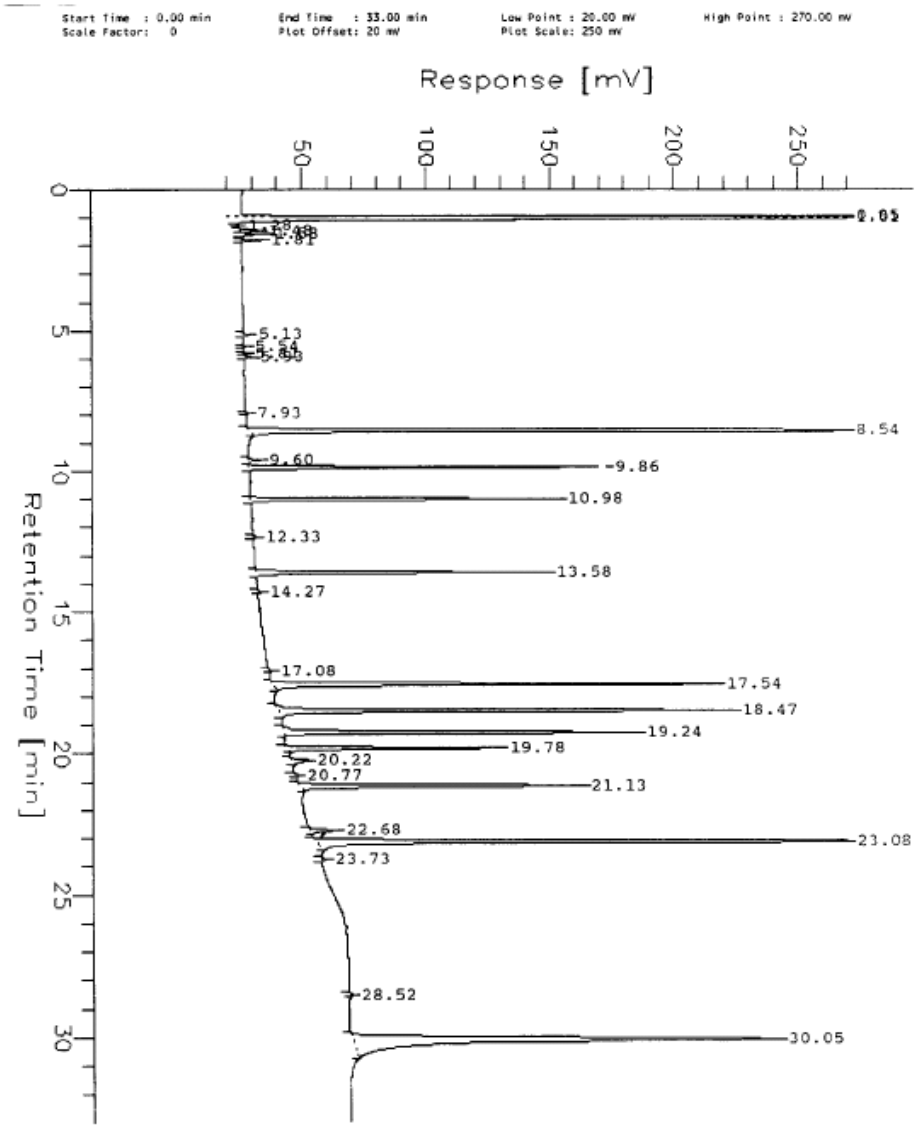
D = Detected; result must be greater than zero



Column: 30-m x 0.25-mm ID, DB-5

Temperature program: 100°C (hold 2 minutes) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier He at 16 psi

Figure 10.7.1 Gas chromatogram of the mixed organochlorine pesticide standard



Column: 30-m x 0.25-mm ID, DB-5
Temperature program: 100°C (hold 2 minutes) to 160°C at 15°C/min, then at 5°C/min to 270°C; carrier He at 16 psi.

Figure 10.7.2 Gas chromatogram of the mixed organochlorine pesticide standard Mix A

10.7.6 Organophosphorus Pesticides

A. Liquid-liquid extraction by gas chromatography with nitrogen phosphorus/flame photometric detector

This is a gas chromatographic (GC) method used to determine the concentration of organophosphorus (OP) compounds. The compounds listed in the Table 10.7.1 below can be determined by GC using capillary column or packed column with a flame photometric detector (FPD) or a nitrogen phosphorus detector (NPD). The analyst must select columns, detectors and calibration procedures for the specific analytes of interest in a study. The method can also be used to analyse some other organophosphorus pesticides mentioned in EPA method 8141.

Table 10.7.1: Organophosphorus compounds

Sr. No.	Compound Name	CAS Registry No.
1	Chlorpyriphos	2921-88-2
2	Diazinon	333-41-5
3	Dichlorvos (DDVP)	62-73-7
4	Dimethoate	60-51-5
5	Ethion	563-12-2
6	Fenitrothion	122-14-5
7	Fenthion	55-38-9
8	Malathion	121-75-5
9	Monocrotophos	6923-22-4
10	Parathion, ethyl	56-38-2
11	Parathion, methyl	298-02-2
12	Phosphamidon	13171-21-6

Two detectors can be used for the listed OP chemicals. The FPD works by measuring the emission of phosphorus or sulphur-containing species. Detector performance is optimised by selecting the proper optical filter and adjusting the hydrogen and air flows to the flame. The NPD is a flame ionisation detector with a rubidium ceramic flame tip which enhances the response of phosphorus- and nitrogen-containing analytes. The FPD is more sensitive and more selective.

10.7.6.1 Principle

This method provides gas chromatographic conditions for the detection of ppb concentrations of organophosphorus compounds. Prior to the gas chromatography, appropriate sample preparation techniques must be used. Water samples are extracted at a neutral pH with methylene chloride by using a separatory funnel. Spiked samples are used to verify the applicability of the chosen extraction technique to each new sample type. A gas chromatograph with a flame photometric or nitrogen-phosphorus detector is used for this multi-residue procedure.

10.7.6.2 Apparatus and equipment

Gas chromatograph: An analytical system complete with a gas chromatograph suitable for on-column or split/splitless injection, and all required accessories, including syringes, analytical columns, gases, suitable detector(s), and a recording device. The analyst should select the detector for the specific measurement application, either the flame photometric detector or the nitrogen-phosphorus detector.

Capillary columns (0.53 mm, 0.32 mm or 0.25 mm ID x 15 m or 30 m length, depending on the resolution required). Columns of 0.53-mm ID are recommended for the analysis.

Column 1: 15 or 30 m x 0.53 mm wide-bore capillary column, 1.0 µm film thickness, chemically bonded with 50% trifluoropropyl polysiloxane, 50% methyl polysiloxane (DB-210) or equivalent.

Column 2: 15 or 30 m x 0.53 mm wide-bore capillary column, 0.83 µm film thickness, chemically bonded with 35% phenyl methyl polysiloxane (DB-608, SPB-608, RTx-35), or equivalent.

Column 3: 15- or 30 m x 0.53 mm wide-bore capillary column, 1.0 µm film thickness, chemically bonded with 5% phenyl polysiloxane, 95% methyl polysiloxane (DB-5, SPB-5, RTx-5), or equivalent.

Column 4: 15 or 30 m x 0.53 mm ID fused-silica open-tubular column, chemically bonded with methyl polysiloxane (DB-1, SPB-1, or equivalent), 1.0 µm or 1.5 µm film thickness.

(Optional) Column rinsing kit: Bonded-phase column rinse kit (JandW Scientific, Catalog no. 430-3000 or equivalent).

Detectors:

Flame photometric detector (FPD) operated in the phosphorus-specific mode is recommended. Nitrogen-phosphorus detector (NPD) operated in the phosphorus-specific mode is less selective but can detect triazine herbicides.

10.7.6.3 Reagents and standards

Solvents:

a. n-Hexane: Pesticide quality or equivalent.

b. Acetone: Pesticide quality or equivalent.

c. Methylene chloride: Pesticide quality or equivalent

Stock standard solutions (1000 mg/L): Can be prepared from pure standard materials or can be purchased as certified solutions. Prepare stock standard solutions by accurately weighing about 0.0100 g of pure compounds. Dissolve the compounds in hexane and dilute to volume in a 10 mL volumetric flask. If compound purity is 96 percent or greater, the weight can be used without correction to calculate the concentration of the stock standard solution. Commercially prepared stock standard solutions can be used at any concentration, if they are certified by the manufacturer or by an independent source.

Composite stock standard: This standard can be prepared from individual stock solutions. The analyst must demonstrate that the individual analytes and common oxidation products are resolved by the chromatographic system. For composite stock standards containing less than 25 components, take exactly 1 mL of each individual stock solution at 1000 mg/L, add solvent and mix the solutions in a 25 mL volumetric flask. Store the standard solutions at 4°C in Teflon-sealed containers in the dark. All standard solutions should be replaced after two months or sooner if routine QC (Section 10.7.6.8) indicates a problem. It is recommended that lots of standards be subdivided and stored in small vials. Individual vials should be used as working standards to minimise the potential for contamination or hydrolysis of the entire lot. Calibration standards should be prepared at a minimum of five concentrations by dilution of the composite stock standard with isooctane or hexane. The concentrations should correspond to the expected range of concentrations found in real samples and should bracket the linear range of the detector. Organophosphorus calibration standards should be replaced after one or two months.

10.7.6.4 Sample collection, preservation and handling

Grab water samples must be collected in glass containers. Conventional sampling practices should be followed except that the bottle must not be prerinsed with sample before collection. Composite sample should be collected and refrigerated in glass containers. Extracts are refrigerated at 4°C and analysed within 40 days of extraction.

10.7.6.5 Calibration and standardisation

Establish gas chromatographic operating conditions equivalent to those given in Table 10.7.6 and 10.7.7. The gas chromatographic system can be calibrated using the external standard technique or the internal standard technique as described below.

External standard calibration procedure: Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with n-hexane. One of the external standards should be at a concentration near, but above, the MDL (Table 10.7.2) and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector. Using injections of 1-2 µL, analyse each calibration standard according to Section 10.7.5.7 and tabulate peak height or area responses against the mass is injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards that are similar in analytical behaviour to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples. Using injections of 1-2 µL, analyse each calibration standard and tabulate peak height or area responses against concentration for each compound and internal standard. Calculate response factors (RF) for each compound using Equation 10.7.1.

$$RF = \frac{(A_s)\ (C_{is})}{(A_{is})\ (C_s)} \qquad \text{..... [Equation 10.7.1]}$$

where:

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard (µg/L)

C_s = Concentration of the parameter to be measured (µg/L)

If the RF value over the working range is a constant (<10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. concentration ratios C_s/C_{is}.

10.7.6.6 Procedure

Extraction and clean-up:

a. In general, water samples are extracted at a neutral pH with 15 % diethyl-ether in n-hexane.

b. Extraction and clean-up procedures that use solutions below pH 4 or above pH 8 are not appropriate for this method.

c. If required, the samples may be cleaned up. Florisil column clean-up and sulphur clean-up may have particular application for OPPs. Gel permeation clean-up should not generally be used for OP pesticides.

d. Prior to gas chromatographic analysis, the extraction solvent may be exchanged to n-hexane. The analyst must ensure quantitative transfer of the extract concentrate. Single-laboratory data indicate that samples should not be transferred with 100 percent hexane during sample workup, as more polar organophosphorus compounds may be lost. Transfer of organophosphorus esters is best accomplished using methylene chloride.

e. Methylene chloride may be used as an injection solvent with both the FPD and the NPD.

Gas chromatographic conditions:

a. Four 0.53 mm ID capillary columns are suggested for the determination of organophosphates by this method. Column 1 (DB-210 or equivalent) and Column 2 (SPB-608 or equivalent) of 30 m length are recommended, if a large number of organophosphorus analytes are to be determined. If superior chromatographic resolution is not required, 15 m lengths columns may be appropriate. Operating conditions for 15-m columns are listed in Table 10.7.6. Operating conditions for 30 m columns are listed in Table 10.7.7.

b. Retention times and chromatograms for analytes on each set of columns are presented in Table 10.7.3, Table 10.7.4 and Figures 10.7.1 to 10.7.6.

c. Record the sample volume injected to the nearest 0.05 µL and the resulting peak sizes (in area units or peak heights). Using either the internal or external calibration procedure, determine the identity and quantity of each component peak in the sample chromatogram, which corresponds to the compounds used for calibration purposes.

d. If peak detection and identification is prevented by the presence of interferences, the use of an FPD or further sample cleanup is required. Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to establish elution patterns and to determine recovery of target compounds. The absence of interference from reagents must be demonstrated by routine processing of reagent blanks through the chosen cleanup procedure.

Detector maintenance:

a. Older FPDs may be susceptible to stray light in the photomultiplier tube compartment. This stray light will decrease the sensitivity and the linearity of the detector. Analysts can check for leaks by initiating an analysis in a dark room and turning on the lights. A shift in the baseline indicates that light may be leaking into the photomultiplier tube compartment. Additional shielding should be applied to eliminate light leaks and minimise stray light interference.

b. The bead of the NPD will become exhausted with time, which will decrease the sensitivity and the selectivity of the detector. The collector may become contaminated which decreased detector sensitivity.

c. Both types of detectors use a flame to generate a response. Flow rates of air and hydrogen should be optimised to give the most sensitive, linear detector response for target analytes.

10.7.6.7 Data analysis and calculations

Refer section 10.7.5.7

10.7.6.8 Method performance and quality control

Estimated MDLs and associated chromatographic conditions for water are listed in Table 10.7.2. As detection limits will vary with the particular matrix to be analysed, recoveries for several method analytes are provided in Table 10.7.5. To confirm an identification of a compound, the background corrected mass spectrum of the compound must be obtained from the sample extract and must be compared with a mass spectrum from a stock or calibration standard analysed under the same chromatographic conditions. At least 25 ng of material should be injected into the GC/MS. The following criteria must be met for qualitative confirmation. The qualitative identification of compounds determined by this method is based on retention time, and on comparison of the sample mass spectrum, after background correction, with characteristic ions in a reference mass spectrum. The reference mass spectrum must be generated by the laboratory using the conditions of this method. The characteristic ions from the reference mass spectrum are defined to be the three ions of greatest relative intensity, or any ions over 30% relative intensity, if less than three such ions occur in the reference spectrum. Compounds should be identified as present when the criteria below are met. The intensities of the characteristic ions of a compound maximise in the same scan or within one scan of each other. Selection of a peak by a data system target compound search routine where the search is based on the presence of a target chromatographic peak containing ions specific for the target compound at a compound-specific retention

time will be accepted as meeting this criterion. Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte. When gas chromatographic peaks obviously represent more than one sample component (i.e., a broadened peak with shoulder(s) or a valley between two or more maxima), appropriate selection of analyte spectra and background spectra is important. Examination of extracted ion current profiles of appropriate ions can aid in the selection of spectra, and in qualitative identification of compounds. When analytes coelute (i.e., only one chromatographic peak is apparent), the identification criteria can be met, but each analyte spectrum will contain extraneous ions contributed by the coeluting compound. Where available, chemical ionisation mass spectra may be employed to aid in the qualitative identification process because of the extensive fragmentation of organophosphorus pesticides during electron impact MS processes. Should the MS procedure fail to provide satisfactory results, additional steps may be taken before reanalysis. These steps may include the use of alternate packed or capillary GC columns or additional sample cleanup. For quality control procedure refer to section 10.7.5.8 given in the method of organochlorine pesticides.

10.7.6.9 Interferences

Refer to Section 10.7.5.9 given in the method of organochlorine pesticides. Use of an FPD often eliminates the need for sample cleanup. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each analyte is not less than 85 percent. The use of Gel permeation cleanup (GPC) for sample cleanup has been demonstrated to yield recoveries of less than 85 percent for many method analytes because they elute before bis-(2-ethylhexyl) phthalate. Use of FPD in the phosphorus mode will minimise interferences from materials that do not contain phosphorus or sulphur. Elemental sulphur will interfere with the determination of certain organophosphorus compounds by flame photometric gas chromatography. Certain analytes will coelute, particularly on 15 m columns (Table 10.7.3). If coelution is observed, analysts should (1) select a second column of different polarity for confirmation, (2) use 30 m x 0.53 mm columns or (3) use 0.25 or 0.32 mm ID columns. Analytical difficulties encountered for target analytes include:

a. The water solubility of Dichlorvos (DDVP) is 10 g/L at 20°C, and recovery is poor from aqueous solution.

b. Retention times of some analytes, particularly Monocrotophos, may increase with increasing concentrations in the injector. Analysts should check for retention time shifts in highly contaminated samples.

c. Many analytes will degrade on reactive sites in the chromatographic system. Analysts must ensure that injectors and splitters are free from contamination and are silanised. Columns should be installed and maintained properly.

d. Performance of chromatographic systems will degrade with time. Column resolution, analyte breakdown and baselines may be improved by column washing (Section 10.7.5.6). Oxidation of columns is not reversible.

Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts or elevated baselines in gas chromatograms. All these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by analysing reagent blanks. NP Detector interferences: Triazine herbicides, such as Atrazine and Simazine, and other nitrogen-containing compounds may interfere.

10.7.6.10 Safety

Refer to Section 10.7.5.10 described in the method of organochlorine pesticides.

10.7.6.11 Bibliography

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4. Manual of Analytical Methods for the Analysis of Pesticides in Human and Environmental Samples, EPA-600/8-80-038, U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, North Carolina.

5. Method Detection Limit and Analytical Curve Studies, EPA Methods 606, 607, and 608, Special letter report for EPA Contract 68-03-2606, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, June 1980.

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7. Standard Methods for the Examination of Water and Wastewater; APHA, AWWA and WEF, 21st Edition, 2005.

8. Hatcher,M.D., D.M.Hickey, P.J.Marsden, and L.D. Betowski, (1988). Development of a GC/MS Module for RCRA Method 8141; final report to the U.S., EPA Environmental Protection Agency on Contract 68-03-1958; S-Cubed, San Diego, CA.

9. Method 622, Organophosphorus Pesticides; U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268.

Table 10.7.2: Method detection limits in water matrix using 15 m columns and a flame photometric detector

Compound	Regent Water (mg/L)
Chlorpyrifos	0.07
Diazinon	0.20
Dichlorvos (DDVP)	0.80
Dimethoate	0.26
Fenthion	0.08
Malathion	0.11
Parathion, ethyl	0.06
Parathion, methyl	0.12

Table 10.7.3: Retention times for analytes employing 15 m columns

	Capillary Column		
	Compound	DB-5	SPB-608
Dichlorvos (DDVP)	9.63	7.91	12.79
Monochrotophos	20.11	20.11	31.42
Dimethoate	23.71	20.18	27.96
Diazinon	26.82	20.02	19.68
Chlorpyrifos	26.82	26.88	25.18
Malathion	31.17	28.78	32.58
Parathion, methyl	31.72	23.71	32.17
Parathion, ethyl	31.84	27.62	33.39
Fenthion	38.83	29.45	28.86

Table 10.7.4: Retention times for analytes employing 30 m Columns

compound	RT (min)			
	DB-5	DB-210	DB-608	DB-1
Dichlorvos (DDVP)	7.45	6.99	6.56	10.73
Diazinon	13.23	18.60	24.03	21.87
Chlorpyrifos	17.06	22.22	29.48	24.85
Fenthion	17.87	22.11	29.14	24.63
Monocrotophos	19.08	15.98	19.62	19.3
Dimethioate	18.11	17.21	20.59	19.87
Malathion	19.83	21.75	28.87	24.57
Parathion, methyl	20.15	20.45	25.98	22.97
Fenithrothion	20.63	21.42		
Parathion, ethyl	21.38	22.22	29.29	24.82
Ethion	22.55	27.12	37.61	

DB-5 and DB-210: 30 m x 0.53 mm ID column, DB-5 (1.50 Fm film thickness) and DB-210 (1.0 Fm film thickness). Both connected to a press-fit Y-shaped inlet splitter. Temperature programme: 120°C (3 min hold) to 270°C (10 min hold) at 5°C/min; injector temperature 250°C; detector temperature 300°C; bead temperature 400°C; bias voltage 4.0; hydrogen gas pressure 20 psi; helium carrier gas 6 mL/min; helium makeup gas 20 mL/min

DB-608: 30 m x 0.53 mm ID column, DB-608 (1.50 Fm film thickness) installed in packed column inlet. Temperature programme: 110°C (0.5 min hold) to 250°C (4 min hold) at 3°C/min; injector temperature 250°C; helium carrier gas 5 mL/min; flame photometric detector.

DB-1: 30 m x 0.32 mm ID column, DB-1 (0.25 Fm film thickness) split/splitless with head pressure of 10 psi, split valve closure at 45 sec, injector temp. 250°C, 50°C (1 min hold) to 280°C (2 min hold) at 6°C/min, mass spectrometer full scan 35-550 amu.

Table 10.7.5: Percent recovery of organophosphates by separatory funnel extraction

Compound	Percent recovery		
	Low	Medium	High
Chlorpyriphos	7	89+6	86
Diazinon	136	121+905	82
Dichlorvos	80	79+11	72
Dimethoate	NR	47+3	101
Fenthion	NR	48+10	89
Malathion	127	92+6	86
Monocrotophos	NR	18+4	NR
Parathion, ethyl	101	94+5	86
Parathion, methyl	NR	46+4	44

NR = Not recovered.

Table 10.7.6: Suggested operating conditions for 15-M Columns

Columns 1 and 2, DB-210 and SPB-608 or their equivalent	
Carrier gas (He) flow rate	5 mL/min
Initial temperature	50°C, hold for 1 minute
Temperature programme	50°C to 140 °C at 5°C/min, hold for 10 minutes, followed by 140°C to 240°C at 10°C/min, hold for 10 minutes (or a sufficient amount of time for last compound to elute)
Column 3 (DB-5 or equivalent)	5 mL/min
Carrier gas (He) flow rate	130°C, hold for 3 minutes
Initial temperature	130°C, to 180°C at 5°C/min, hold for 10 minutes, followed by 180°C to 250°C at 2°C/min, hold for 15 minutes (or a sufficient amount of time for last compound to elute).
Temperature programme	

Table 10.7.7: Suggested operating conditions for 30 m Columns

Column 1:	
Type:	DB-210
Dimensions:	30 m x 0.53 mm ID
Film Thickness (µm):	1.0
Column 2:	
Type:	DB-5
Dimensions:	30 m x 0.53 mm ID
Film Thickness (µm):	1.5
Carrier gas flowrate (mL/min):	6 (Helium)
Makeup gas flowrate (mL/min):	20 (Helium)
Temperature programme:	120°C (3 min hold) to 270°C (10 min hold) at 5°C/min
Injector temperature:	250°C
Detector temperature:	300°C
Injection volume:	2 µL
Solvent:	Hexane
Type of injector:	Flash vaporisation
Detector type:	Dual NPD
Range:	1
Attenuation:	64
Type of splitter:	Y-shaped or Tee
Data system:	Integrator
Hydrogen gas pressure:	20 psi
Bead temperature:	400°C
Bias voltage:	4

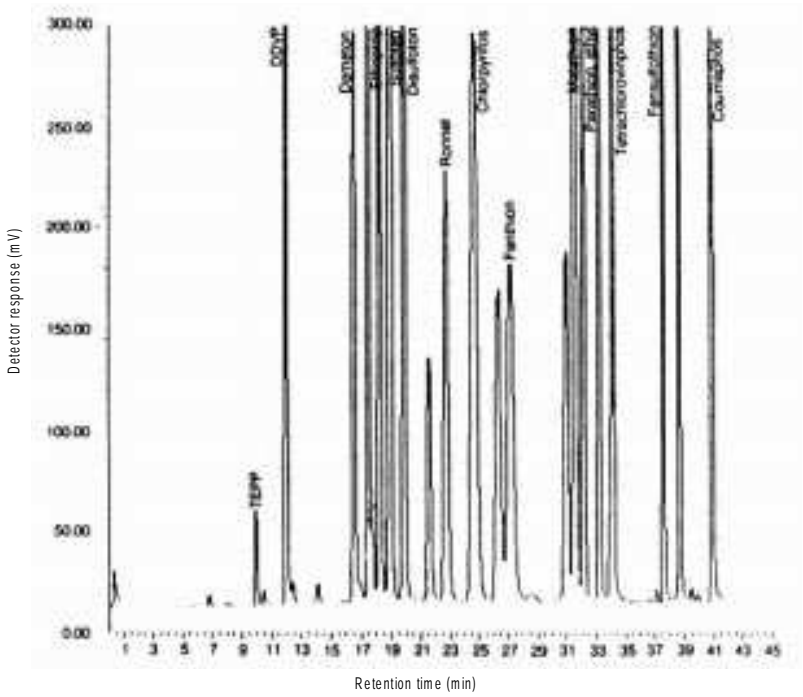


Figure 10.7.1: Chromatogram of target organophosphorus compounds from a 15 m DB-210 column with FPD detector

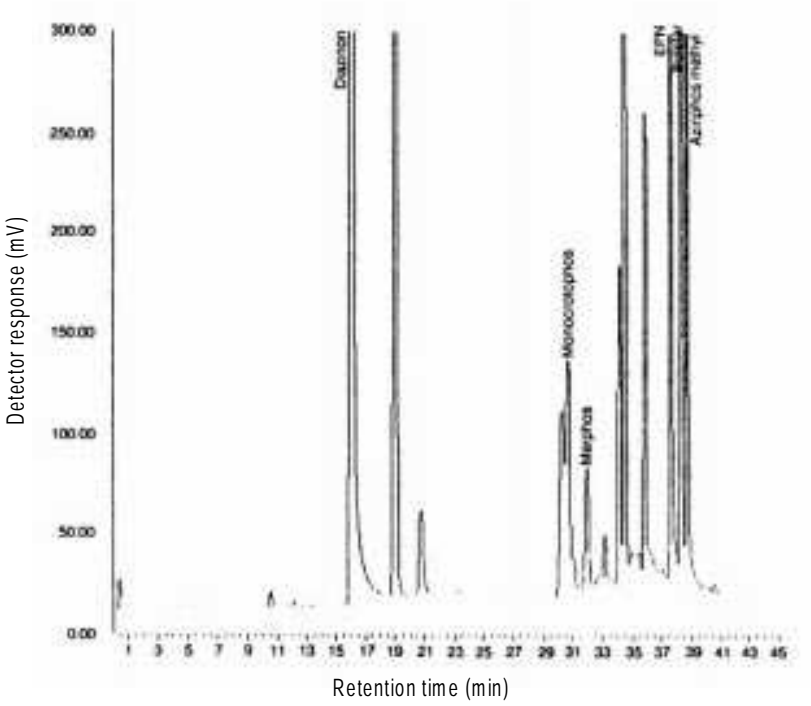


Figure 10.7.2: Chromatogram of target organophosphorus compounds from a 15 m DB-210 column with FPD detector

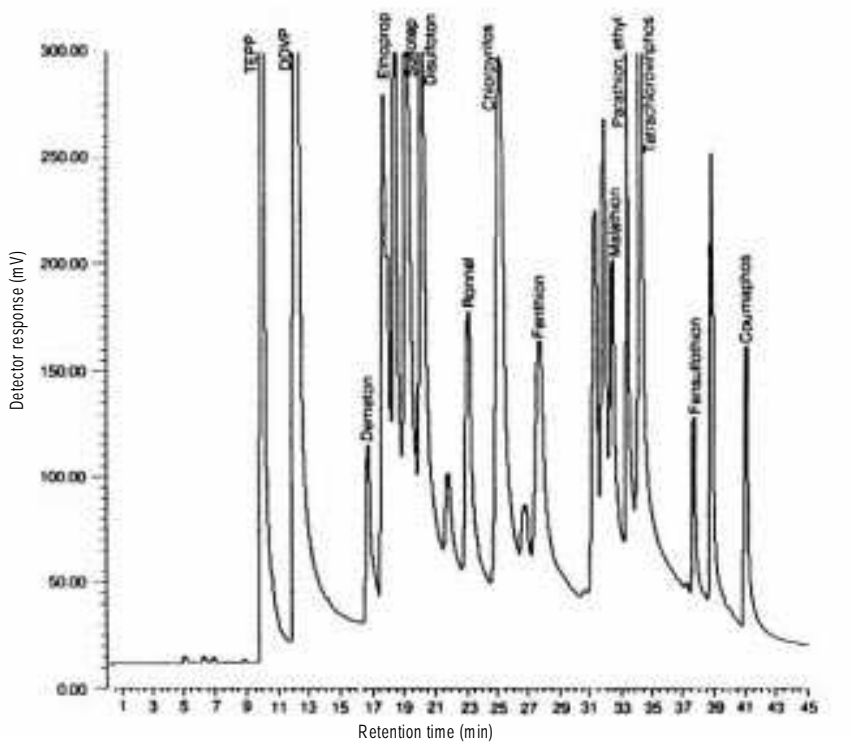


Figure 10.7.3: Chromatogram of target organophosphorus compounds from a 15 m DB-210 column with NPD detector

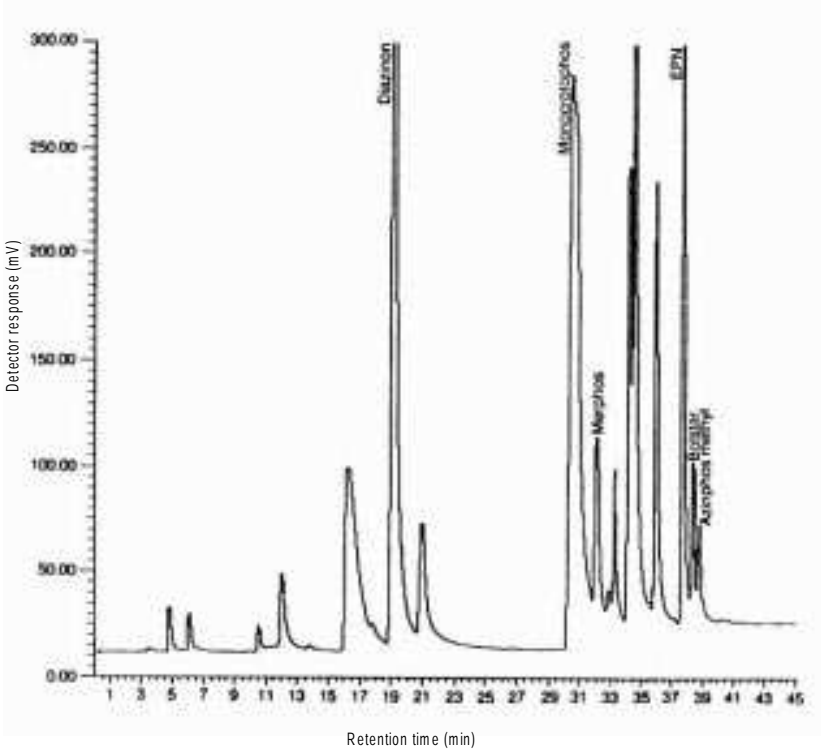


Figure 10.7.4: Chromatogram of target organophosphorus compounds from a 15 m DB-210 column with NPD detector

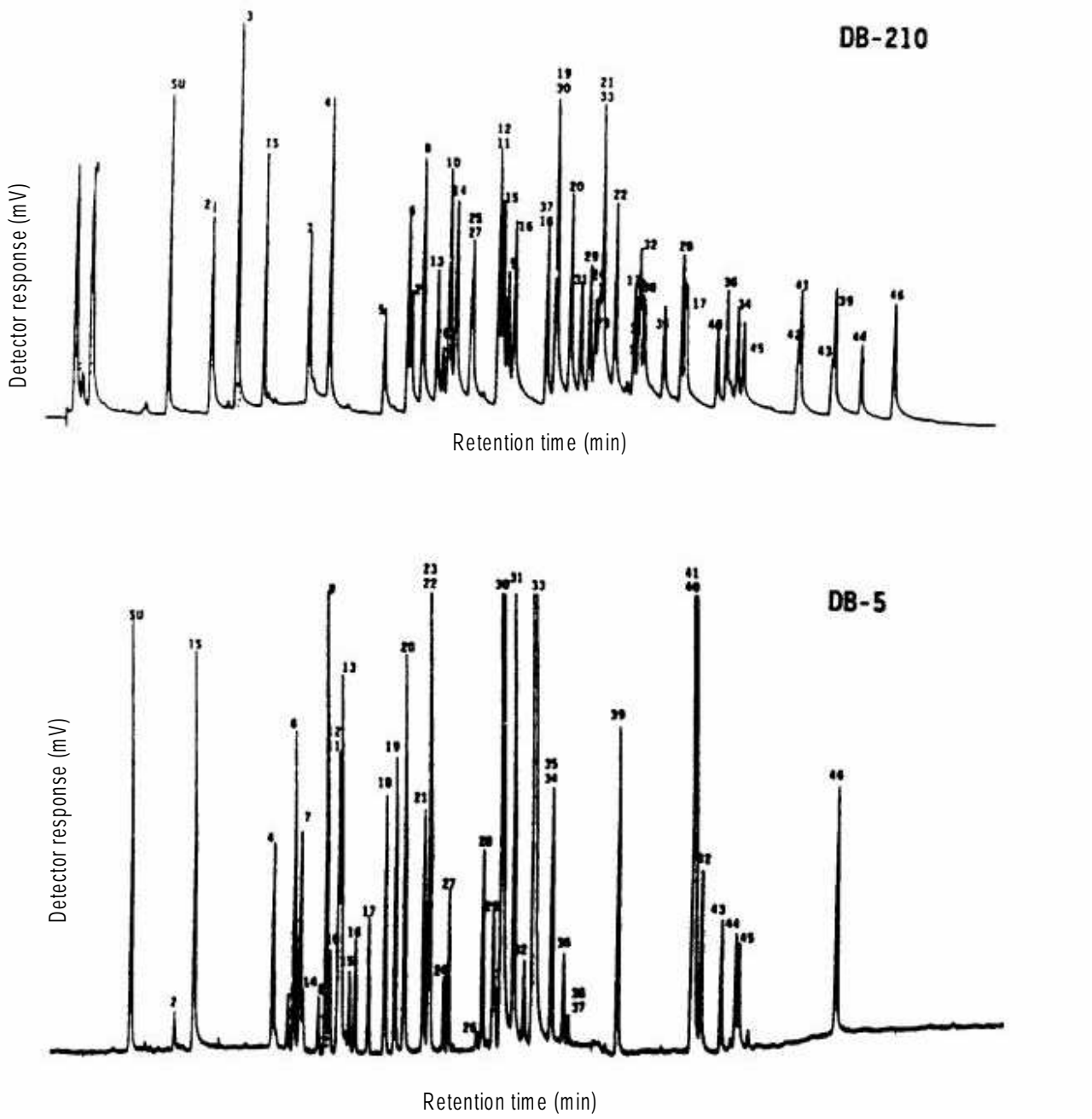


Figure 10.7.5: Chromatogram of target organophosphorus compounds on a 30 m DB-5/DB-210 column pair with NPD detector, without Simazine, Atrazine and Carbophenothion

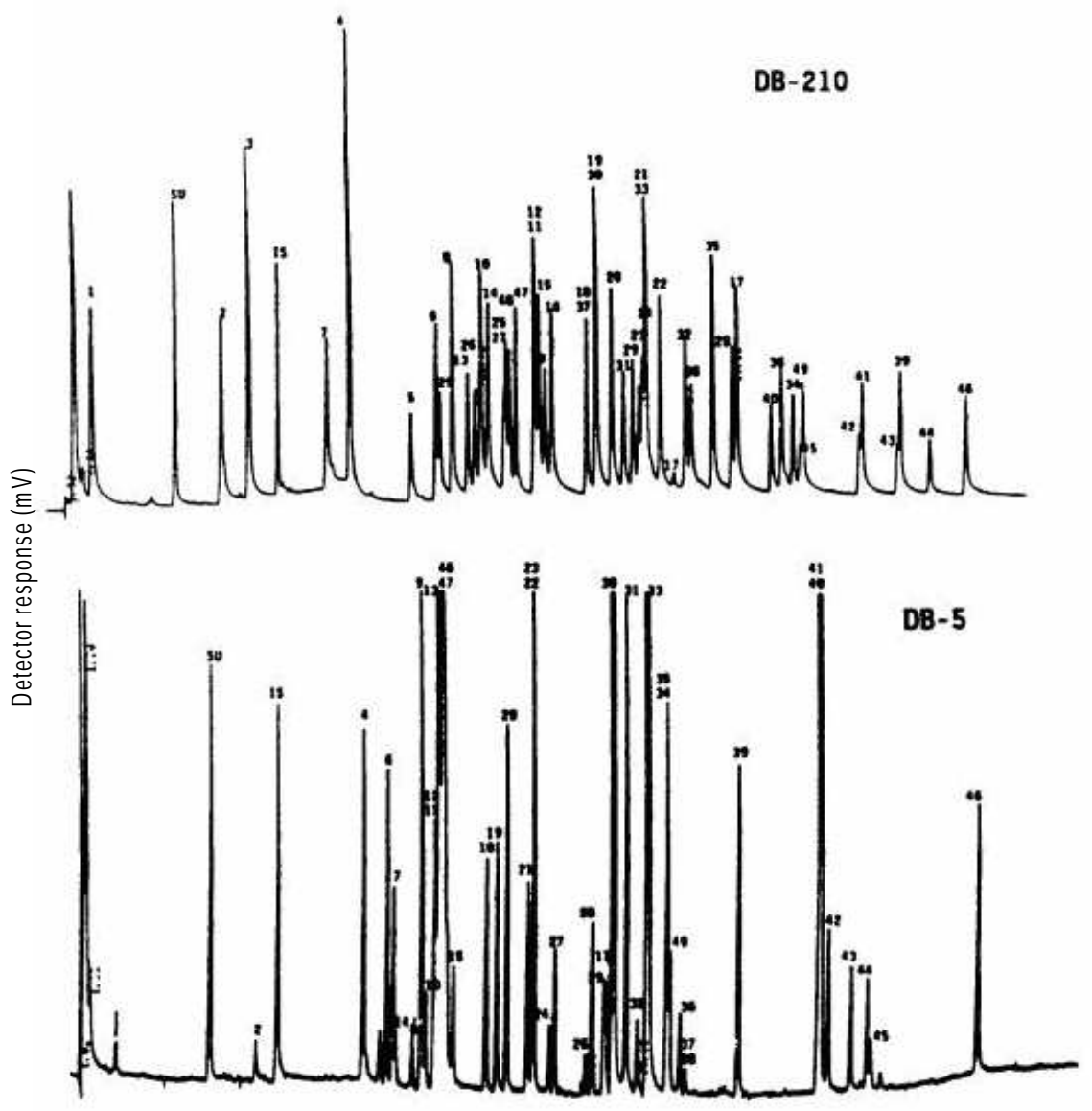


Figure 10.7.6: Chromatogram of target organophosphorus compounds on a 30 m DB-5/DB-210 column pair with NPD detector, with Simazine, Atrazine and Carbophenothion.

10.7.7 Carbamate Pesticides

A. Liquid-liquid extraction by high performance liquid chromatography

10.7.7.1 Principle

This method is used to determine the concentration of n-methyl carbamates in water. The method detection limits (MDLs) for determining the target analytes in organic-free reagent water are listed in Table 10.7.1. This method is restricted to use by, or under the supervision of, analysts experienced in the use of high performance liquid chromatography (HPLC) and skilled in the interpretation of chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method.

N-methylcarbamates are extracted from aqueous samples with methylene chloride. The extract solvent is exchanged to methanol/ethylene glycol, and then the extract is cleaned upon a C-18 cartridge, filtered and eluted on a C-18 analytical column. After separation, the target analytes are hydrolysed, followed by post-column derivatisation then quantitated fluorometrically. Due to the specific nature of this analysis, confirmation by a secondary method is not essential. However, fluorescence due to post-column derivatisation may be confirmed by substituting the NaOH and o-phthalaldehyde solutions with organic-free reagent water and reanalysing the sample. If fluorescence is still detected, then a positive interference is present and care should be taken in the interpretation of the results. The sensitivity of the method usually depends on the level of interferences present, rather than on the instrumental conditions. Waste samples with a high level of extractable fluorescent compounds are expected to yield significantly higher detection limits.

10.7.7.2 Apparatus and equipment

a. HPLC system

b. An HPLC system capable of injecting 20 µL aliquots and performing multilinear gradients at a constant flow. The system must also be equipped with a data system to measure the peak areas

c. C-18 reverse phase HPLC column, 25 cm x 4.6 mm (5 µm)

d. Post column reactor with two solvent delivery systems (Kratos PCRS 520 with two Kratos Spectro flow 400 Solvent Delivery Systems, or equivalent)

e. Fluorescence detector (Kratos Spectroflow 980, or equivalent)

f. Centrifuge

g. Analytical balance $\pm$ 0.0001 g

h. Top loading balance $\pm$ 0.01 g

i. Platform shaker

j. Heating block, or equivalent apparatus, that can accommodate 10 mL graduated vials

k. HPLC injection syringe, 50 µL

l. Filter paper, (Whatman #113 or #114, or equivalent)

m. Volumetric pipettes, Class A, glass, assorted sizes

n. Reverse phase cartridges, (C-18 Sep-PakR [Waters Associates], or equivalent)

o. Glass syringes, 5 mL

p. Volumetric flasks, Class A: sizes as appropriate

q. Erlenmeyer flasks with teflon-lined screw caps, 250 mL

r. Assorted glass funnels

s. Separatory funnels, with ground glass stoppers and teflon stopcocks: 250 mL

t. Graduated cylinders, 100 mL

u. Graduated glass vials, 10 mL, 20 mL

v. Centrifuge tubes, 250 mL.

w. Vials, 25 mL, glass with Teflon lined screw caps or crimp top

x. Positive displacement micro-pipettor, 3 to 25 µL displacement, (Gilson Microman [Rainin #M-25] with tips, [Rainin #CP-25], or equivalent)

y. Nylon filter unit, 25 mm diameter, 0.45 µm pore size, disposable (Alltech Associates, #2047, or equivalent)

10.7.7.3 Reagents and standards

a. Acetonitrile (CH_3CN) : HPLC grade, minimum UV cut-off at 203 nm (EM Omnisolv #AX0142-1 or equivalent).

b. Methanol (CH_3OH) : HPLC grade, minimum UV cut-off at 230 nm (EM Omnisolv #MX0488-1 or equivalent).

c. Methylene chloride (CH_2Cl_2) : HPLC grade, minimum UV cut-off at 230 nm (EM Omnisolv #DX0831-1 or equivalent).

d. Hexane (C_6H_{14}) : pesticide grade, (EM Omnisolv #HX0298-1 or equivalent).

e. Ethylene glycol ($HOCH_2CH_2OH$) : Reagent grade (EM Science or equivalent).

f. Organic-free reagent water: all references to water in this method refer to organic-free reagent water.

g. Sodium hydroxide, (NaOH) : reagent grade, 0.05N NaOH solution.

h. Phosphoric acid, (H_3PO_4) : reagent grade.

i. pH 10 borate buffer (J.T. Baker #5609-1or equivalent).

j. o-Phthalaldehyde (OPA), (o-$C_6H_4(CHO)_2$) : reagent grade (Fisher #0-4241 or equivalent).

k. 2-Mercaptoethanol, ($HSCH_2CH_2OH$) : reagent grade (Fisher #0-3446 or equivalent).

l. N-methylcarbamate neat standards (equivalence to EPA standards must be demonstrated for purchased solutions)

m. Chloroacetic acid ($ClCH_2COOH$) : 0.1N

n. Standard solutions : Stock standard solutions- prepare individual 1000 mg/L solutions by adding 0.025 g of carbamate to a 25 mL volumetric flask, and diluting to the mark with methanol. Store solutions, under refrigeration, in glass vials with Teflon lined screw caps or crimp tops. Replace every six months. Intermediate standard solution prepare a mixed 50 mg/L solution by adding 2.5 mL of each stock solution to a 50 mL volumetric flask, and diluting to the mark with methanol. Store solutions, under refrigeration, in glass vials with teflon lined screw caps or crimp tops. Replace every three months. Working standard solutions - prepare 0.5, 1, 2, 3 and 5 mg/L solutions by adding 0.25, 0.5, 1, 1.5 and 2.5 mL of the intermediate mixed standard to respective 25 mL volumetric flasks, and diluting each to the mark with methanol. Store solutions, under refrigeration, in glass vials with Teflon lined screw caps or crimp tops. Replace every two months or sooner if necessary. Mixed QC standard solution - prepare a 40 mg/L solution from another set of stock standard solutions. Add 2 mL of each stock solution to a 50 mL volumetric flask and dilute to the mark with methanol. Store the solution, under refrigeration, in a glass vial with a Teflon lined screw cap or crimp top. Replace every three months.

10.7.7.4 Sample collection, preservation and storage

Due to the extreme instability of N-methylcarbamates in water it should be preserved immediately after collection by acidifying to pH 4-5 with 0.1N chloroacetic acid. Store samples at 4°C and out of direct sunlight, from the time of collection through analysis. N-methylcarbamates are sensitive to alkaline hydrolysis and heat. All samples must be extracted within seven days of collection, and analysed within 40 days of extraction.

10.7.7.5 Calibration and standardisation

Establish the liquid chromatographic operating conditions. The liquid chromatographic system can be calibrated using the external standard technique or the internal standard technique as described below. External standard calibration procedure: Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with appropriate solvents. One of the external standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

10.7.7.6 Procedure

Sample extraction: Measure 100 mL of sample into a 250 mL separatory funnel and extract by shaking vigorously for about 2 minutes with 30 mL of methylene chlop ride. Repeat the extraction two more times. Combine all three extracts in a 100 mL volumetric flask and dilute to volume with methylene chloride. If cleanup is required, refer section. 10.7.6.6.

Cleanup: Pipette 20 mL of the extract into a 20 mL glass vial containing 100 µL of ethylene glycol. Place the vial in a heating block set at 50°C, and gently evaporate the extract under a stream of nitrogen (in a fume hood) until only the ethylene glycol keeper remains. Dissolve the ethylene glycol residue in 2 mL of methanol, pass the extract through a pre-washed C-18 reverse phase cartridge, and collect the eluate in a 5 mL volumetric flask. Elute the cartridge with methanol, and collect the eluate until the final volume of 5 mL is obtained (dilution factor = 0.25). Using a disposable 0.45 µm filter, filter an aliquot of the clean extract directly into a properly labelled auto sampler vial. The extract is now ready for analysis.

Solvent exchange: Pipette 10 mL of the extract into a 10 mL graduated glass vial containing 100 µl of ethylene glycol. Place the vial in a heating block set at 50 °C, and gently evaporate the extract under a stream of nitrogen (in a fume hood) until only the ethylene glycol keeper remains. Add methanol to the ethylene glycol residue, drop wise, until the total

volume is 1 mL (dilution factor = 0.1). Using a disposable 0.45 µm filter, filter this extract directly into a properly labelled auto sampler vial. The extract is now ready for analysis.

Sample analysis: Analyse the samples using the chromatographic conditions. Table 10.7.1 provides the retention times that were obtained under these conditions during method development. A chromatogram of the separation is shown in Figure 10.7.1.

Chromatographic conditions (recommended):

Solvent A: Organic-free reagent water, acidified with 0.4 mL of phosphoric acid per Litre of water

Solvent B: Methanol/acetonitrile (1:1, v/v)

Flow rate: 1 mL/min

Injection Volume: 20 µL

Solvent delivery system programme:

Time min.	Function	Value	Duration min	File
0.00	FR	1.0		0
0.00	B%	10		0
0.02	B%	80	20	0
20.02	B%	100	5	0
25.02	B%	100	5	0
30.02	B%	10	3	0
33.02	B%	10	7	0
36.02	ALARM		0.01	0

Post-column Hydrolysis Parameters (Recommended)	
Solution:	0.05N aqueous sodium hydroxide
Flow Rate:	0.7 mL/min
Temperature:	95°C
Residence Time:	35 seconds (1 mL reaction coil)
Post-column derivatisation parameters (recommended)	
Solution:	o-phthalaldehyde/2-mercaptoethanol (Sec. 10.7.7.3)
Flow Rate:	0.7 mL/min
Temperature	40°C
Residence time:	25 seconds (1 mL reaction coil)
Fluorometer parameters (recommended)	
Cell:	10 µL
Excitation wavelength:	340 nm
Emission wavelength:	418 nm cut-off filter
Sensitivity wavelength:	0.5 µA
PMT voltage:	-800 V
Time constant:	2 sec

If the peak areas of the sample signals exceed the calibration range of the system, dilute the extract as necessary and reanalyse the diluted extract.

10.7.7.7 Data analysis and calculations

Calculate each response factor as follows (mean value based on 5 points):

$$RF = \frac{\text{Concentration of standards}}{\text{Area of the signal}} \quad \text{..... [Equation 10.7.1]}$$

$$\text{mean RF} = \overline{RF} = \frac{(\sum_{i}^{5} RFi)}{5} \quad \text{..... [Equation 10.7.2]}$$

$$\%\ \overline{RSD}\ \text{of RF} = \frac{[(\sum_{i}^{5} RFi - \overline{RF})^2]^{1/2}/4}{\overline{RF}} \times 100 \text{.....[Equation 10.7.3]}$$

Calculate the concentration of each N-methyl carbamate as follows:

$$\mu g/g \text{ or } mg/l = (\overline{RF}) \text{ (area of signal) (dilution factor)}$$

..... [Equation 10.7.4]

10.7.7.8 Method performance and quality control

Table 10.7.1 lists the single operator method detection limit (MDL) for each compound in organic-free reagent water and soil. Seven/ten replicate samples were analysed, as indicated in the table. Table 10.7.2 lists the single operator average recoveries and standard deviations for organic-free reagent water. Ten replicate samples were analysed at each indicated spike concentration for each matrix type. The method detection limit, accuracy and precision obtained will be determined by the sample matrix.

Before processing any sample, the analyst must demonstrate, through the analysis of a method blank for each matrix type, that all glassware and reagents are interference free. Each time there is a change of reagents, a method blank must be processed as a safeguard against laboratory contamination. A QC check solution must be prepared and analysed with each sample batch that is processed. Prepare this solution, at a concentration of 2 mg/L of each analyte, from the 40 mg/L mixed QC standard solution (Section 10.7.7.3). The acceptable response range is 1.7 to 2.3 mg/L for each analyte. Negative interference due to quenching may be examined by spiking the extract with the appropriate standard, at an appropriate concentration, and examining the observed response against the expected response. Confirm any detected analytes by substituting the NaOH and OPA reagents in the post column reaction system with deionised water, and reanalyse the suspected extract.

Continued fluorescence response will indicate that a positive interference is present (since the fluorescence response is not due to the post column derivatisation). Exercise caution in the interpretation of the chromatogram. Analyse a solvent blank (20 µL of methanol) to ensure that the system is clean. Analyse the calibration standards (Section 10.7.7.3), starting with the 0.5 mg/L standards and ending with the 5 mg/L standard. If the percent relative standard deviation (%RSD) of the mean response factor (RF) for each analyte does not exceed 20%, the system is calibrated and the analysis of samples may proceed. If the %RSD for any analyte exceeds 20%, recheck the system and/or recalibrate with freshly prepared calibration solutions. Using the established calibration mean response factors, check the calibration of the instrument at the beginning of each day by analysing the 2 mg/L mixed standard. If the concentration of each analyte falls within the range of 1.7 to 2.3 mg/L (i.e., within $\pm$15% of the true value), the instrument is considered to be calibrated and the analysis of samples may proceed. If the observed value of any analyte exceeds its true value by more than $\pm$ 15%, the instrument must be recalibrated. After 10 sample runs, or less, the 2 mg/L standards must be analysed to ensure that the retention times and response factors are still within acceptable limits. Significant variations (i.e., observed concentrations exceeding the true concentrations by more than $\pm$ 15%) may require a re-analysis of the samples.

10.7.7.9 Interferences

Fluorescent compounds, primarily alkyl amines and compounds which yield primary alkyl amines on base hydrolysis, are potential sources of interferences. Co-eluting compounds that are fluorescence quenchers may result in negative interferences. Impurities in solvents and reagents are additional sources of interferences. Before processing any samples, the analyst must demonstrate daily, through the analysis of solvent blanks, that the entire analytical system is interference-free.

10.7.7.10 Safety

Refer section 10.7.5.10. The parameter covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogen. Primary standards of these toxic compounds should be prepared in a hood.

10.7.7.11 Bibliography

1. Standard Methods for the Examination of Water and Wastewater; APHA, AWWA and WEF, 21st Edition, 2005.

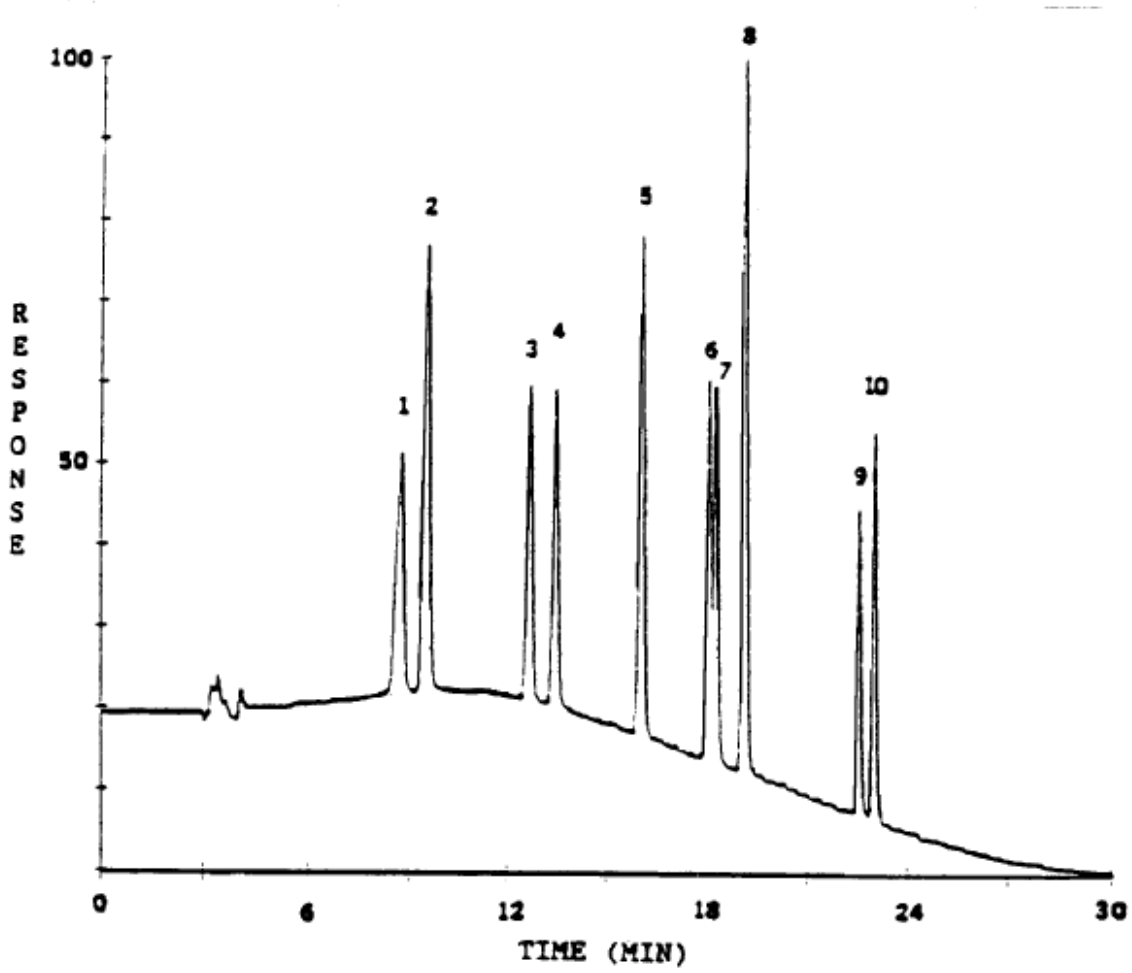
2. Okamoto, H.S., D. Wijekoon, C. Esperanza, J. Cheng, S. Park, J. Garcha, S. Gill, K. Perera. Analysis for N-Methylcarbamate Pesticides by HPLC in Environmental Samples, Proceedings of the Fifth Annual USEPA Symposium on Waste Testing and Quality Assurance, July 24-28, 1989, Vol. II, 57-71.

Table 10.7.1: Elution order, retention times and single operator method detection limits

Compound	Retention Time (min)	Method Detection Limits (µg/kg)
Aldicarb sulphone	9.59	1.9
Methomyl (Lannate)	9.59	1.7
3-Hydroxycarbofuran	12.70	2.6
Dioxacarb	13.50	2.2
Aldicarb (Temik)	16.05	9.4
Propoxur (Baygon)	18.06	2.4
Carbofuran (Furadan)	18.28	2.0
Carbaryl (Sevin)	19.13	1.7
Naphthold	20.30	-
Methiocarb (Mesurol)	22.56	3.1
Promecarb	23.02	2.5

Table 10.7.2: Single operator average recovery and precision data

Compound	Recovered	%Recovery	SD	%RSD
Aldicarb sulphone	225	75.0	7.28	3.24
Methomyl (Lannate)	244	81.3	8.34	3.42
3-Hydroxycarbofuran	210	70.0	7.85	3.74
Dioxacarb	241	80.3	8.53	3.54
Aldicarb (Temik)	224	74.7	13.5	6.03
Propoxur (Baygon)	232	77.3	10.7	4.57
Carbofuran (Furadan)	239	79.6	9.23	3.86
Carbaryl (Sevin)	242	80.7	8.56	3.54
Methiocarb (Mesurol)	231	77.0	8.09	3.50
Promecarb	227	75.7	9.43	4.1



1.00 mg/mL each of:

1. Aldicarb sulphone
2. Methomyl
3. 3-Hydroxycarbofuran
4. Dioxacarb
5. Aldicarb
6. Propoxur
7. Carbofuran
8. Carbaryl
9. Methiocarb
10. Promecarb

Figure 10.7.1: Chromatogram of target carbamate compounds on 25 cm x 4.6 mm C-18 reverse phase HPLC column with fluorescence detector

River Data Compilation-2 Directorate

Central Water Commission

New Delhi