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Method of Analysis and Quality-Assurance Practices by the U.S. Geological Survey Organic Geochemistry Research Group—Determination of Four Selected Mosquito Insecticides and a Synergist in Water Using Liquid-Liquid Extraction and Gas Chromatography/ Mass Spectrometry

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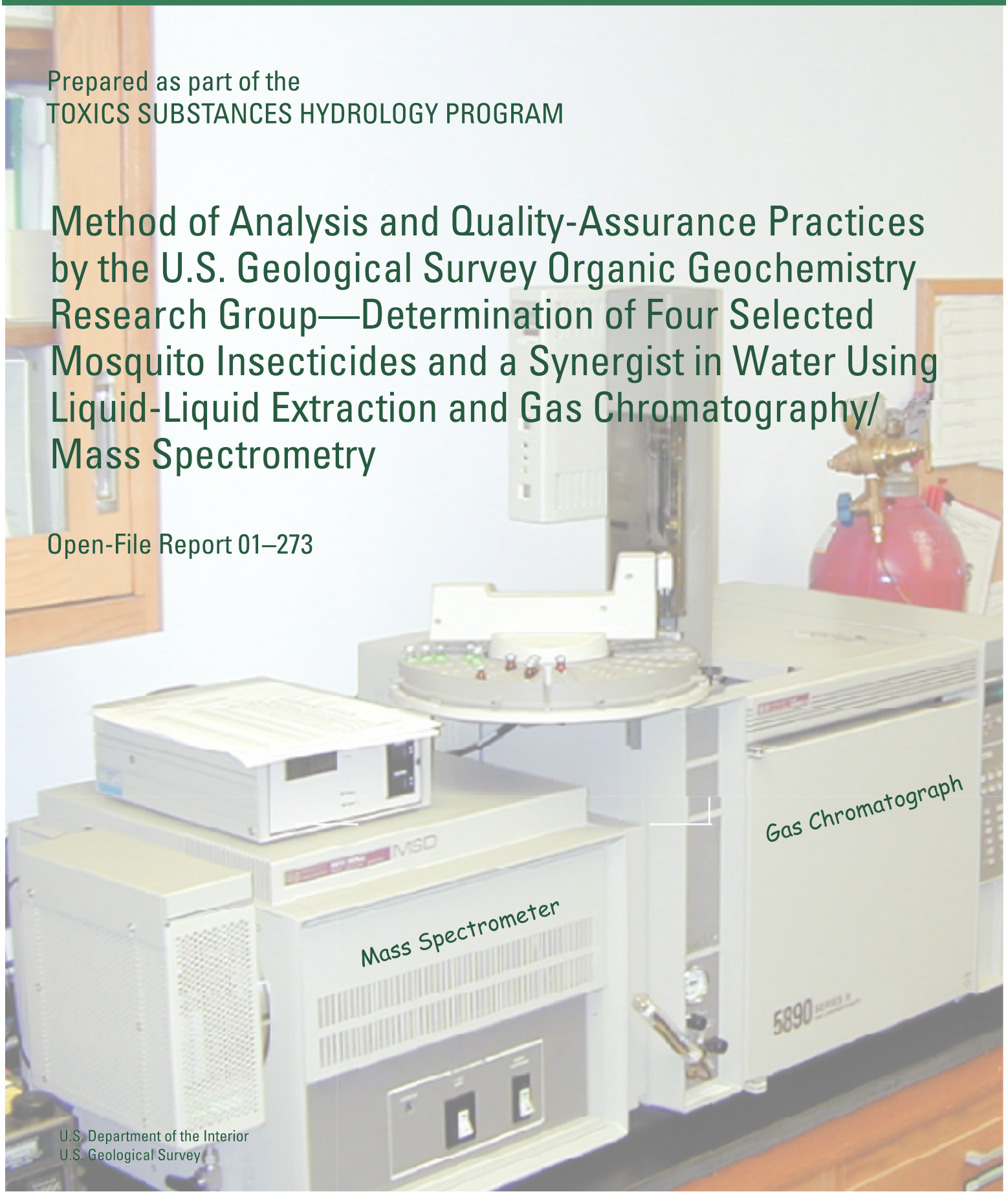

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CONVERSION FACTORS, MISCELLANEOUS ABBREVIATIONS, AND ABBREVIATED WATER-QUALITY UNITS

Conversion Factors

Multiply	By	To obtain
foot (ft)	0.3048	meter (m)
gram (g)	0.002205	pound (lb)
liter (L)	33.82	ounce (oz)
ounce (oz)	0.02957	liter (L)
kilopascal (kPa)	0.1450377	pound per square inch (lb/in^2)

Temperature can be converted to degrees Celsius (^oC) or degrees Fahrenheit (^oF) by the equations:

$$^oC = 5/9\ (^oF - 32)$$
$$^oF = 9/5\ (^oC) + 32.$$

Miscellaneous Abbreviations

atomic mass units (amu)
mass to charge (m/z)
meter (m)
micrometer (µm)
milligram (mg)
millimeter (mm)
millisecond (ms)
minute (min)
second (sec)
nanogram (ng)

Abbreviated Water-Quality Units

liter (L)
microgram per liter (µg/L)
microliter (µL)
milligram per milliliter (mg/mL)
mililiter (mL)
nanogram per liter (ng/L)
nanogram per microliter (ng/µL)

Method of Analysis and Quality-Assurance Practices by the U.S. Geological Survey Organic Geochemistry Research Group—Determination of Four Selected Mosquito Insecticides and a Synergist in Water Using Liquid-Liquid Extraction and Gas Chromatography/Mass Spectrometry

By L.R. Zimmerman[1], A.P. Strahan[2], *and* E.M. Thurman[2]

Abstract

A method of analysis and quality-assurance practices were developed for the determination of four mosquito insecticides (malathion, methoprene, phenothrin, and resmethrin) and one synergist (piperonyl butoxide) in water. The analytical method uses liquid-liquid extraction (LLE) and gas chromatography/mass spectrometry (GC/MS). Good precision and accuracy were demonstrated in reagent water, urban surface water, and ground water. The mean accuracies as percentages of the true compound concentrations from water samples spiked at 10 and 50 nanograms per liter ranged from 68 to 171 percent, with standard deviations in concentrations of 27 nanograms per liter or less. The method detection limit for all compounds was 5.9 nanograms per liter or less for 247-milliliter samples. This method is valuable for acquiring information about the fate and transport of these mosquito insecticides and one synergist in water.

INTRODUCTION

The persistence of organic pesticides in water is of great importance because of concerns over water quality. Pesticides that find their way into lakes, streams, or drinking-water supplies may pose a potential health threat to wildlife and humans. The U.S. Geological Survey (USGS), as part of the Toxic Substances Hydrology Program, has been studying the fate and transport of four mosquito insecticides and a synergist in the New York City metropolitan area.

Recently, there has been concern in the Northeastern United States about the appearance of the West Nile virus. The West Nile virus was first identified in Africa (Center for Disease Control, 2001a) and has since spread to temperate regions of Europe and North America. It is generally not dangerous to healthy humans but can develop into a deadly form of encephalitis (inflammation of the brain) in the elderly, children, and people with compromised immune systems. In the United States, West Nile virus is transmitted by infected mosquitoes, primarily members of the *culex* species (Center for Disease Control, 2001b).

A direct way to combat the spread or prevent a recurrent outbreak of West Nile virus is to control the mosquito population. One of the methodologies for control is the use of insecticides, either larvicides or adulticides.

Larvicides for mosquito control include methoprene, an insect growth regulator. Methoprene controls mosquito larva populations by mimicking the natural juvenile growth hormone, JHIII. This hor-

[1]University of Kansas, Center for Research, Inc., Lawrence, Kansas.

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mone inhibits developing mosquito pupae from molting and passing into the adult stage where they could reproduce. Methoprene is available in suspension, emulsifiable, and soluble concentrate formulations, as well as in briquette, aerosol, and bait form. Methoprene was introduced in the late 1970s as a means of flea and mosquito control.

Adulticides, which may be used in the chemical control of mosquitos, include the organophosphate malathion and the pyrethroids phenothrin, also called sumithrin, and resmethrin. In addition, a synergist compound commonly is applied with pyrethroids to overcome resistance that pests develop with use of insecticides.

Malathion is a nonsystemic, wide-spectrum organophosphate insecticide. It was one of the earliest organophosphate insecticides developed (introduced in 1950). Malathion is used for the control of mosquitoes, flies, household insects, animal parasites (ectoparasites), and head and body lice.

Pyrethrins are natural insecticides in the flowers of certain species of the chrysanthemum plant. Semisynthetic derivatives of the chrysanthemumic acids have been developed as insecticides. These are called pyrethroids and tend to be more effective than natural pyrethrins, and they are less toxic to mammals. The most frequently used pyrethroids for adult mosquito control are phenothrin, also called sumithrin, and resmethrin.

Insects possess an enzyme system called the mixed-function oxidases (MFOs) that give them the ability to rapidly detoxify and become resistant to many insecticides, especially pyrethroids. Piperonyl butoxide (PBO) inhibits the action of MFOs, which allows the applicator to use less active ingredient to obtain the mortality rate desired or to prolong the usefulness of insecticides by overcoming MFO resistance. As is common with pyrethroid insecticides, the synergist compound piperonyl butoxide (PBO) is applied with phenothrin and resmethrin.

An analytical method and quality-assurance practices were developed for the determination of four mosquito insecticides and one synergist at nanogram-per-liter levels in water samples. The method involves using liquid-liquid extraction (LLE) to isolate the compounds from water samples and gas chromatography/mass spectrometry (GC/MS) to identify and quantify these compounds. Quality-control practices include evaluation of laboratory blank and spiked samples, instrument performance, and corrective actions. Method detection limits (MDLs) are calculated on the basis of procedures recognized by the U.S. Environmental Protection Agency (USEPA) (1992). Mean recoveries of the targeted insecticides and synergist from reagent, surface, and ground water also are presented.

The LLE-GC/MS method of analysis described in this report and used at the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas, has been assigned the method number “O–2134–01” by the USGS Office of Water Quality in Reston, Virginia. At the Organic Geochemistry Research Laboratory, the method of analysis described herein has been given the analysis code "GCM." This unique analysis code can be used to identify the method.

METHOD OF ANALYSIS

Scope and Application

The method described in this report and used by the USGS Organic Geochemistry Research Laboratory is suitable for the determination of nanogram-per-liter concentrations of four mosquito insecticides and a synergist in filtered, natural water samples. Registry numbers and molecular weights are shown in table 1 for each compound determined by the method. This method is applicable to compounds that are (1) efficiently partitioned from the water phase by hexane liquid extraction and (2) sufficiently volatile and thermally stable for gas chromatography. Suspended particulate matter is removed from the samples by filtration, so this method is suitable only for dissolved-phase compounds.

Compounds were selected because of their potential use in controlling mosquitoes in the New York City metropolitan area. The calibration range for the method is equivalent to concentrations from 5 to 100 ng/L without dilution.

Summary of Method

Water samples are filtered at the collection site using glass-fiber filters with 0.7-µm nominal pore diameter to remove suspended particulate matter. In the laboratory, a surrogate compound is added, and a small volume of sample is removed from the bottle. Then hexane is added directly to the remaining sample in the bottle and mixed. The hexane extract is

Table 1. Compound name, class, molecular weight, water solubility, and registry number for compounds determined using method 0–2134–01

[water-solubility data from Kidd and James (1991) except where noted; amu, atomic mass units; mg/L, milligrams per liter; ˚C, degrees Celsius; CAS, Chemical Abstract Service; <, less than]

Compound	Class	Molecular weight (amu)	Water solubility [mg/L (°C)]	CAS registry number
Malathion	organophosphate	330	[1]145 (25)	121–75–5
Methoprene	insect growth regulator	310	1.4 (25)	40596–69–8
Phenothrin	pyrethroid	350	<1 (30)	26002–80–2
Piperonyl butoxide (PBO)	synergist	338	[2]<.001	51–03–6
Resmethrin	pyrethroid	338	<1 (30)	10453–86–8

[1]Tomlin (1997).
[2]Nature Conservation Council of New South Wales (2001).

removed, spiked with an internal standard, and evaporated under nitrogen. The sample components are separated, identified, and measured by injecting an aliquot of the concentrated extract into a high-resolution, fused-silica capillary column of a GC/MS system under selected-ion mode (SIM). Compounds eluting from the GC column are identified by comparing their measured ions and retention times to reference ions and retention times obtained by the measurement of control samples under the same conditions used for the water samples. The concentration of each identified compound is measured by relating the MS response of the quantitation ion produced by that compound to the MS response of the quantitation ion produced by the surrogate standard.

Interferences

Organic compounds having identical mass ions and GC retention times to those of the compounds of interest may interfere.

Apparatus and Instrumentation

- *Analytical balances*—Capable of accurately weighing 0.0100 g ± 0.0001 g.
- *Volumetric glassware*—With volumes of 50 mL and 2 L.
- *Autopipettes*—20- to 200-µL, variable-volume autopipettes with disposable tips (Rainin, or equivalent, Woburn, MA).
- *Repeating pipette*—100-µL dispensing volume (Repeater Pipettor Plus, or equivalent, Eppendorf, Hamburg, Germany).
- *Automated solvent evaporator*—The heat-bath temperature needs to be maintained at 45 ˚C and the nitrogen gas pressure at 103 kPa (Turbovap LV, or equivalent, Zymark, Inc., Hopkinton, MA).
- *Fused-silica capillary column*—Cross-linked methyl siloxane capillary column (12 m x 0.2 mm inside diameter, 0.33-µm film thickness) (HP Ultra 1, or equivalent, Hewlett Packard, Wilmington, DE).
- *GC/MS benchtop system*—Hewlett Packard (Wilmington, DE), model 5890 series II Plus, or equivalent, GC with autoinjector connected to a Hewlett Packard, model 5970, or equivalent, MS detector.
- *GC conditions*—Oven, 70 ˚C (hold 1 min), then ramp to 190 ˚C at 10 ˚C/min, then 5 ˚C/min to 270 ˚C, and hold for 2 min; injection port, 250 ˚C; carrier gas, helium; injection volume, 2 µL, splitless injection.
- *MS conditions*—Multiplier, 400 over autotune; detector, 280 ˚C; dwell time, 50 ms; mass ions monitored are listed in table 2 (see section on "Calibration Curve").
- *Moisture sieve and oxygen scrubber for carrier gas.*
- *Data system*—Computer and printer compatible with the GC/MS system used.
- *Software*—HP DOS ChemStation Software, 1030A version C (Hewlett Packard, Wilmington, DE), is used to acquire and store data and for peak integration.

Reagents and Consumable Materials

- *Sample bottles*—Baked 8-oz amber glass bottles (Boston round) with Teflon-lined lids.

- *Reagent water*—Generated by purification of tapwater through activated charcoal filtration and deionization with a high-purity, mixed-bed resin, followed by another activated charcoal filtration, and finally distillation in an autostill (Barnstead, or equivalent, Dubuque, IA).
- *Analytical standards*—Standards of the insecticides and synergist, surrogate standard, and the internal standard.
- *Disposable serological pipette*—25 mL (Kimble, or equivalent, Vineland, NJ).
- *Bottle-top liquid dispensers*—For measuring and dispensing 7 mL of hexane and 1 mL reagent water (BrandTech Scientific, Inc., or equivalent, Wertheim, Germany).
- Solvents—
  - Hexane, American Chemical Society (ACS) and high-performance liquid chromatography (HPLC) grade.
  - Ethyl acetate, HPLC grade.
  - Methanol, ACS and HPLC grade.
- *Disposable snap-cap finish centrifuge tubes*—10 mL (Kimble, or equivalent, Vineland, NJ).
- *Gas for evaporation*—Nitrogen, ultrapure grade.
- *Pasteur pipettes*—(Kimble, or equivalent, Vineland, NJ).
- *0.1-mL autosampler vials*—Amber plastic vial with glass cone insert and cap (Wheaton, Millville, NJ).
- *GC carrier gas*—Helium, ultrapure grade.

Sampling Methods

Following USGS protocol, sampling methods capable of collecting water samples that accurately represent the water-quality characteristics of the surface water or ground water at a given time or location are used. Detailed descriptions of sampling methods used by the USGS to obtain surface-water samples are given in Edwards and Glysson (1988) and Ward and Harr (1990). Similar descriptions of sampling methods for obtaining ground-water samples are given in Hardy and others (1989).

Briefly, sample-collection equipment is free of tubing, gaskets, and other components made of nonfluorinated plastic material that might leach interferences into water samples or sorb organic compounds from the water. The water samples from each site are composited in a single container and filtered through a 0.7-µm glass-fiber filter using a peristaltic pump (Sandstrom, 1995). Filters are leached with about 200 mL of sample prior to filtration of sample. The filtrate for analysis is collected in baked 8-oz amber glass bottles with Teflon-lined lids. Samples are chilled immediately and shipped to the laboratory via an overnight carrier. At the laboratory, samples are logged in, assigned identification numbers, and extracted on the day they arrive.

Standards and Controls

- *Stock standard solutions*—Obtain the insecticide, synergist, and internal- and surrogate-standard compounds as pure materials from commercial vendors or chemical manufacturers. Prepare solutions of 1.0 mg/mL (corrected for purity) by accurately weighing, to the nearest 0.001 g, 50 mg of the pure material in a 50-mL volumetric flask and dilute with methanol. Store at less than 0 ˚C. This solution is stable for about 24 months.
- *Primary fortification standard*—Prepare a 0.5-ng/µL concentration, primary fortification standard by combining appropriate volumes of the individual insecticide and synergist stock solutions in a 100-mL volumetric flask. Dilute with methanol. Store at less than 0 ˚C. This solution is stable for about 24 months.
- *Internal standard solution*—Prepare a solution of phenanthrene-d_{10} in ethyl acetate at a concentration of 0.2 ng/µL. The internal standard may be purchased as a 100-µg/mL solution in methylene chloride rather than preparing a stock standard solution from pure material. Dilute 800 µL in 4 L of ethyl acetate. Smaller volumes may be prepared. Store at less than 0 ˚C. This solution is stable for about 24 months.
- *Surrogate standard solution*—Prepare a solution of terbuthylazine in methanol at a concentration of 1.23 ng/µL using the stock standard solution. Store in a freezer at less than 0 ˚C. This solution is stable for about 24 months.
- *Calibration and control standards*—Prepare a series of solutions using the primary fortification standard in reagent water at concentrations ranging from 5.0 to 100 ng/L (5.0, 10.0, 25.0, 35.0, 50.0, and 100 ng/L). Prepare these in 2-L volumetric flasks and then transfer aliquots to individual 8-oz bottles. This yields eight calibration and control standards at each concentration. Blank (0 ng/L)

calibration and control standards are prepared using unspiked reagent water. The calibration and control standards are processed through the extraction procedure (described in the "Extraction" section).

- *Matrix spike control*—The primary fortification solution is used for spiking a replicate of an actual sample. Matrix spike controls usually are spiked to a concentration of 25 ng/L (12.35 µL of primary fortification solution is added to a 247-mL sample), but other concentrations may be used. This is prepared immediately prior to beginning the extraction procedure.

Extraction

- *Extraction set-up*—An extraction set consists of as many as six samples. In addition to the samples, each extraction set has at least one replicate sample, a matrix spike control, one laboratory blank control, a high-concentration control, and a low-concentration control. All the bottles in the extraction set are processed identically.
- *Sample preparation*—Samples and controls are prepared in 8-oz amber glass bottles filled to the base of the neck of the bottle. The volume of sample processed is 247 mL. Should a sample contain less than 247 mL, distilled water is added to bring the volume to the required 247 mL. Any volume added is recorded.
- *Spiking of surrogate standard*—Each sample and control is spiked with 100 µL of surrogate standard (1.23 ng/µL terbuthylazine in methanol). Spiking is performed using a repeating pipetter with a dedicated syringe tip. All samples, the replicate sample, and controls then are capped and shaken by hand to assure that the surrogate standard is well mixed.
- *Removal of excess liquid*—Approximately 25 mL of sample are removed from each sample and control using a 25-mL disposable serological pipette. This allows space for the sample to be extracted in its original sampling bottle.
- *Transferring of compounds to organic phase*—7 mL of hexane are added to each sample and control using a bottle-top dispenser. Each sample bottle then is capped and agitated by vigorously shaking by hand for at least 30 sec. A mechanical wrist-action shaker may be used. Agitation then is repeated for an additional 10 sec two times to assure that there has been sufficient mixing to allow any insecticides and synergist to be transferred into the organic hexane phase.
- *Removal of hexane*—Distilled water is added to each bottle to bring the level of sample and hexane to the top of the bottle. This allows for easier removal of the hexane. Each bottle is allowed to stand for 10 min so that the organic hexane phase can separate from the aqueous phase. The organic hexane layer is removed from each sample and control using a pasteur pipette and transferred to a labeled test tube that has been prespiked with 100 µ L of internal standard (0.2-ng/µL phenanthrene-d_{10} in ethyl acetate).
- *Evaporation*—The spiked extracts are evaporated to a volume of approximately 60 µL using a solvent evaporator with 103 kPa nitrogen and a 45-˚C water bath. Each extract then is transferred to a 0.1-mL autosampler vial using an autopipette with disposable tips and capped. The extracts are stored at less than 0 ˚C until analysis by GC/MS.

Calibration Curve

- Initial calibration curves are prepared using freshly prepared calibration standards that are extracted using the same procedure as samples (described previously).
- Data are acquired from a GC/MS that meets all performance criteria using the same procedure and method as samples.
- Calculate the relative retention time (RRT_c) for each selected compound and the surrogate compound in the calibration solution or in a sample as follows:

$$RRT_c = \frac{RT_c}{RT_i}, \quad (1)$$

where RT_c = uncorrected retention time of the quantitation ion of the selected compound or surrogate compound, in minutes, and

RT_i = uncorrected retention time of the quantitation ion of the internal standard (phenanthrene-d_{10}), in minutes.

See table 2 for an example of retention times, relative retention times, quantitation ions, and qualification ions.

- Initial calibration data are entered into a computer spreadsheet (Microsoft Excel, Microsoft, Inc., Seattle, WA), and ratios are calculated for each quantitation ion relative to the surrogate standard (terbuthylazine). Graphs are made from the GC/MS data by plotting the terbuthylazine ratios of a single ion on the x axis and the concentrations of the calibration standards used on the y axis. The spreadsheet determines a trend line for the data points using a quadratic curve fit forced through the origin. The equation of the trend line and the correlation coefficient value (r^2) appear on each compounds' graph.
- Initial calibration data are acceptable if the correlation coefficient (r^2) value for all curves is greater than or equal to 0.99 for all compounds.
- Subsequent daily response factors calculated for the majority of compounds need to agree within $\pm$ 20 percent of the mean response factor for the compounds analyzed. A response factor is equal to the area of the quantitation ion for the selected compound or surrogate divided by the area of the quantitation ion for the internal standard.

Table 2. Retention times, relative retention times, quantitation ions, and qualification ions for selected mosquito insecticides, synergist, and internal and surrogate standards analyzed using gas chromatography/mass spectrometry

[min, minute; m/z, mass to charge; --, not applicable]

Compound	Retention time (min)	Relative retention time (dimensionless)	Quantitation ion (m/z)	Qualification ion(s) (m/z)
Insecticides and synergist (in order of increasing retention time)				
Malathion	15.720	1.170	173	127, 93, 158
Methoprene	17.320	1.289	73	111, 153, 191
Pieronyl butoxide (PBO)	21.710	1.615	176	177, 149, 119
Resmethrin	21.800	1.622	123	171, 143, 128
Phenothrin I	23.380	1.740	123	183, 81
Phenothrin II	23.580	1.754	123	183, 81
Internal standard				
Phenanthrene-d_{10}	13.440	1.000	188	--
Surrogate standard				
Terbuthylazine	13.520	1.006	214	173, 229

- Analyze at least one laboratory blank control with each sample set, one low calibration standard ranging from 5.0 to 25.0 ng/L, and one high standard ranging from 35.0 to 100.0 ng/L to verify instrument response in each range.

Evaluation of Mass Spectrometer Performance

Mass spectrometer performance is evaluated by assessing isotopic ratios, contamination, electron multiplier sensitivity, and abundance.

- Tune the mass spectrometer before each GC/MS sample set (approximately 43 injections or three extraction sample sets) using the procedure and software supplied by the manufacturer. Parameters in the tuning software are set to give $\pm$0.15-amu resolution at masses 69, 219, and 502 in the spectrum of perfluorotributylamine (PFTBA). With the resolution of the 69 ion at 100-percent abundance, the mass 219 ion should be 35 $\pm$20 percent, and the mass 502 ion should be more than 3 percent relative abundance; however, the relative abundances may vary depending on the mass spectrometer used. Check mass assignments to ensure accuracy to $\pm$ 0.15 amu and that mass peak widths measured at one-half the peak height range from about 0.50 to 0.60 amu.
  - Also, during the tuning of the mass spectrometer, check the mass spectrometer for the presence of excessive water and air, which indicate leaks in the vacuum. If detected, locate and fix leaks.
- Initially adjust the electron multiplier of the mass spectrometer to ensure that the established reporting level for each selected compound can be achieved.

Calculation and Reporting of Results

Qualitative Identification

- The expected retention time *(RT)* of the peak of the selected insecticide or synergist of interest needs to be within $\pm$6 sec of the expected retention time on the basis of the RRT_c obtained from the internal-standard analysis. Calculate the expected retention time as follows:

$$RT = (RRT_c)(RT_i), \quad (2)$$

where RT = expected retention time of the selected compound, in minutes;

RRT_c = relative retention time of the selected compound, dimensionless; and

RT_i = uncorrected retention time of the internal standard, in minutes.

- Mass-spectral verification for each selected compound is done by comparing the relative abundance values of the quantification and qualification ion(s) to the same values obtained from the control standard samples. The relative ratios of the ions need to be within ±20 percent of the relative ratios obtained in the absence of any obvious interferences.

Quantitation

- Calculate the dilution factor to correct for the volume of sample processed as follows:

$$DF = \left(\frac{247}{247 - V_a}\right), \quad (3)$$

where DF = dilution factor; and

V_a = volume added = milliliters of distilled water added to a sample that contains less than 247 mL.

The dilution factor is incorporated into the calculation for determining final concentrations of samples.

- If a selected insecticide or synergist has passed the aforementioned qualitative identification criteria, calculate the concentration in the sample as follows:

$$C = \left((a)\left(\frac{A_c}{A_i}\right)^2 + (b)\left(\frac{A_c}{A_i}\right) + 0\right) \times (DF) \times (SC), \quad (4)$$

where C = concentration of the selected insecticide or synergist in the sample, in nanograms per liter;

a = coefficient of x^2 in the quadratic curve fit;

A_c = area of the quantitation ion of the selected insecticide or synergist identified;

A_i = area of the quantitation ion of the surrogate standard, terbuthylazine;

b = coefficient of x in the quadratic curve fit;

DF = dilution factor as calculated in equation 3; and

SC = slope correction.

Reporting of Results

The four insecticides and the synergist are reported in concentrations ranging from 5 to 100 ng/L. If the concentration is greater than 100 ng/L, the sample is reextracted with a 1:10 dilution (sample:distilled water) and reanalyzed for those compounds that were greater than 100 ng/L.

METHOD PERFORMANCE

A reagent-water sample, a surface-water sample collected from the Kisco River below Mt. Kisco, New York, and a ground-water sample collected from a 27-ft deep well near Halstead, Kansas, were used to test the method performance. The surface- and ground-water samples were collected in 45-L carboys. Aliquots of each sample were fortified with either 10 or 50 ng/L of primary fortification standard. Then they were split into eight 247-mL samples at each concentration (10 and 50 ng/L). In addition, unfortified samples of reagent, surface, and ground water were extracted and analyzed to determine background concentrations of the pesticides. All samples were analyzed in one laboratory (the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas) using one GC/MS system. Each sample set was extracted and analyzed on different days from April through May 2001, so comparison of different matrices and concentrations included bias from day-to-day variation. Accuracy and precision data from the analyses are listed in tables 3, 4, and 5.

Corrections for background concentrations—Neither the surface- nor ground-water sample required correction for background concentrations of insecticides or synergist. The reagent-water sample also had no detections of insecticides or synergist.

Method detection limits (MDLs)—An MDL is defined as the minimum concentration of a substance that can be identified, measured, and reported with 99-percent confidence that the compound concentration is greater than zero. MDLs were determined according to procedures outlined by the U.S. Environmental Protection Agency (1992) using fortified reagent water. Two liters of reagent water were fortified with 5.0 ng/L of primary fortification standard and split into eight 247-mL samples. These were extracted and analyzed to determine MDLs (table 6). Each sam-

Table 3. Accuracy and precision data from eight determinations of mosquito insecticides and a synergist in a fortified reagent-water sample

[ng/L, nanograms per liter]

Compound	Samples spiked at 10 ng/L				Samples spiked at 50 ng/L			
	Mean observed compound (ng/L)	Standard deviation (ng/L)	Relative standard deviation (percent)	Mean accuracy (percentage of true concentration)	Mean observed concentration (ng/L)	Standard deviation (ng/L)	Relative standard deviation (percent)	Mean accuracy (percentage of ftrue concentration)
Malathion	11.4	1	12	114	52.1	9	17	104
Methoprene	17.1	3	20	171	65.5	13	21	131
Phenothrin, total	15.6	3	18	156	54.7	27	49	109
Piperonyl butoxide (PBO)	12.2	1	10	122	59.6	10	17	119
Resmethrin	13.4	4	26	134	48.3	18	38	97

Table 4. Accuracy and precision data from eight determinations of mosquito insecticides and a synergist in a fortified surface-water sample

[ng/L, nanograms per liter]

Compound	Samples spiked at 10 ng/L				Samples spiked at 50 ng/L			
	Mean observed concentration (ng/L)	Standard deviation (ng/L)	Relative standard deviation (percent)	Mean accuracy (percentage of true concetration)	Mean observed concentration (ng/L)	Standard deviation (ng/L)	Relative standard deviation (percent)	Mean accuracy (percentage of true concentration)
Malathion	10.2	1	14	102	47.8	9	18	96
Methoprene	10.4	3	28	104	47.0	16	33	94
Phenothrin, total	12.9	2	13	129	42.9	9	21	86
Piperonyl butoxide (PBO)	11.4	1	6	114	55.5	8	14	111
Resmethrin	11.8	3	25	118	41.3	14	33	83

Table 5. Accuracy and precision data from eight determinations of mosquito insecticides and a synergist in a fortified ground-water sample

[ng/L, nanograms per liter]

Compound	Samples spiked at 10 ng/L				Samples spiked at 50 ng/L			
	Mean observed concentration (ng/L)	Standard deviation (ng/L)	Relative standard deviation (percent)	Mean accuracy (percentage of true concetration)	Mean observed concentration (ng/L)	Standard deviation (ng/L)	Relative standard deviation (percent)	Mean accuracy (percentage of true concentration)
Malathion	10.8	2	15	108	51.5	10	19	103
Methoprene	10.2	3	34	102	50.4	22	43	101
Phenothrin, total	11.9	4	33	119	33.8	10	30	68
Piperonyl butoxide (PBO)	11.2	2	16	112	54.9	8	15	110
Resmethrin	10.8	3	29	108	40.0	19	47	80

ple was analyzed on different days during April through May 2001, so day-to-day variation is included in the results.

The MDL was calculated using the following equation:

$$MDL = (S)(t_{(n-1,1-\alpha)=0.99}), \quad (5)$$

where S = standard deviation of replicate analysis, in nanograms per liter, at the fortified concentration;

$t_{(n-1,\ 1-\alpha=0.99)}$ = Student's *t*-value for the 99-percent confidence level with *n*-1 degrees of freedom (U.S. Environmental Protection Agency, 1992); and

n = number of replicate analyses.

The estimated mean MDLs ranged from 1.7 to 5.9 ng/L (table 6). According to the U.S. Environmental Protection Agency (1992) procedure, the fortified concentrations should be no more than five times the estimated MDL. The fortified concentrations were within five times the MDL.

Mean accuracy—Mean accuracy in reagent-, surface-, and ground-water samples was determined by comparing the mean observed concentration (see "Quantitation" section) from eight replicate samples to the spiked concentration. Mean accuracy as a percentage of the true concentration was best in surface water fortified at 50 ng/L (table 4). The mean accuracy of all compounds spiked at the concentrations in tables 3, 4, and 5 were averaged to calculate the mean recovery for the three matrixes. Mean recoveries in reagent-water samples were farther from 100 percent than the mean recoveries in surface- and ground-water samples. The mean recovery in reagent water was 139 and 112 percent at 10 and 50 ng/L, respectively. The mean recovery in surface water was 113 and 94 percent at 10 and 50 ng/L, respectively. The mean recovery in ground water was 110 and 92 percent at 10 and 50 ng/L, respectively.

Extraction absolute recovery—Absolute recovery of each insecticide and synergist was determined by comparing standard curves (0 to 50 ng/L) prepared internally and externally to the extraction procedure. The same mass of compound from the primary fortification standard was added either to a reagent-water sample or directly to a test tube spiked with internal standard (phenanthrene-d_{10}). The internal standard curve samples were processed using the aforementioned extraction procedure. Then both standard curves were injected on the GC/MS. For each compound in each standard curve, a graph was made with the ratio of the area of the compounds'quantitation ion divided by the area of the quantitation ion of the internal standard. A linear best-fit trend line was calculated for each graph. Finally, the slope of the internal standard curve was divided by the slope of the external curve for each compound to determine the absolute recovery for that compound. Absolute recoveries are listed in table 7. Absolute recovery is different than mean accuracies listed in tables 3–5 in that mean accuracies are calculated from an initial calibration curve that is processed in the same manner as the samples, thus correcting for routine analyte losses.

Table 6. Method detection limits calculated at the 5.0-nanograms-per-liter concentration in reagent water

[ng/L, nanograms per liter; MDL, method detection limit]

Compound	Mean observed concentration (ng/L)	Mean standard deviation (ng/L)	MDL (ng/L)
Malathion	5.5	1.2	3.7
Methoprene	8.6	1.6	4.8
Phenothrin, total	8.6	2.0	5.9
Piperonyl butoxide (PBO)	5.8	.6	1.7
Resmethrin	7.1	1.8	5.3

QUALITY-CONTROL DATA

Quality-control data are produced to quantitatively check the measurement process for environmental samples. The types of quality-control data collected include results of the analysis of duplicate samples, matrix-spiked samples, laboratory blank samples, and controls of differing concentrations.

Duplicate Samples

Each extraction set of as many as six samples contains a minimum of one duplicate sample. The duplicate samples are analyzed concurrently and reanalyzed if agreement of the calculated concentration for any detected insecticide or synergist is not within 40 percent, as determined by the relative percentage difference.

$$RPD = \left| \frac{X_1 - X_2}{\overline{X}} \right| \times 100, \qquad (6)$$

where *RPD* = relative percentage difference;

Table 7. Absolute recovery for mosquito insecticides and synergist in reagent water

Compound	Absolute recovery (percent)
Malathion	77
Methoprene	64
Phenothrin, total	84
Piperonyl butoxide (PBO)	98
Resmethrin	86

$|X_1-X_2|$ = absolute value of the difference between the two values; and

$\overline{X}$ = mean of the two values.

Matrix-Spiked Samples

Recovery of all target compounds is determined for each matrix-spiked sample. After the water sample is received in the laboratory, 12.35 µL of the primary fortification standard are added prior to extraction. Any compounds present in the unspiked sample are subtracted from the matrix-spiked sample's values. These final concentration values are reported.

Laboratory Blank Samples

Laboratory blank samples are used to demonstrate that laboratory equipment or instruments are cleaned adequately and that no contamination is contributed by the laboratory procedures. A laboratory blank consists of reagent water that is processed exactly like samples. If any insecticide or synergist is detected at any concentration greater than the MDL in the laboratory blank control, the source of the problem is determined and corrected. Samples analyzed in that extraction set then are reevaluated for contamination.

Calibration Verification

Low and high concentration controls are used to verify the calibration curve being used for quantification. The recoveries for each insecticide and synergist are determined. A new calibration curve is prepared if the recovery is outside the control limits in two consecutive runs. Control limits are initially set at ±20 percent until an adequate number of controls have been analyzed to calculate a relevant standard deviation. Control warning limits are set at ±1.5 standard deviations from the mean and the control limits at ±2 standard deviations from the mean.

Surrogate Recoveries

Recovery of the surrogate, terbuthylazine, is determined for each sample, including all control samples. Control charts for the terbuthylazine recovery are constructed using the mean; the warning limits are set at 1.5 standard deviations from the mean and the control limits at ±2 standard deviations from the mean. The control charts are constructed using all previous sample terbuthylazine recoveries. A sample is reextracted and reanalyzed on the GC/MS if the recovery is outside the control limits.

CONCLUSIONS

This report presents a method of analysis and quality-assurance practices for the determination of four mosquito insecticides and one synergist in natural water samples. From the data presented in this report, liquid-liquid extraction with gas chromatography/mass spectrometry detection are shown to be a sensitive and reliable method for the determination of nanogram-per-liter concentrations. Good precision and accuracy were demonstrated. Method detection limits ranged from 1.7 to 5.9 ng/L. The mean accuracies of the mosquito insecticides and synergist from water samples spiked at 10 and 50 ng/L ranged from 68 to 171 percent, with relative standard deviations of 6 to 49 percent. Information about the fate and transport of the four mosquito insecticides and one synergist in water can be acquired from the analysis of surface- and ground-water samples. These methods also can be useful for water-quality determinations and analytical verification in toxicological studies.

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