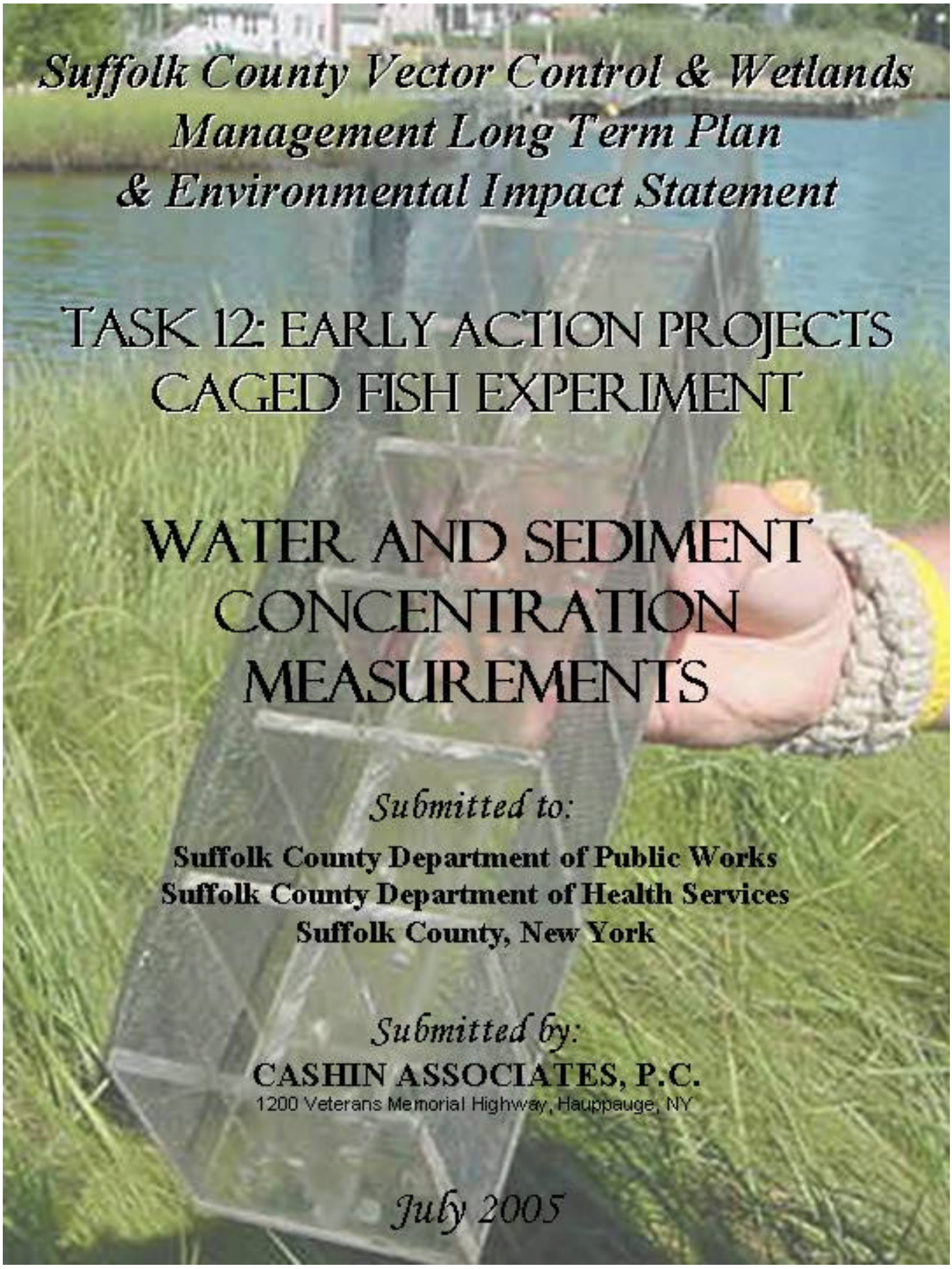

Suffolk County Vector Control & Wetlands Management Long Term Plan & Environmental Impact Statement

TASK 12: EARLY ACTION PROJECTS CAGED FISH EXPERIMENT

WATER AND SEDIMENT CONCENTRATION MEASUREMENTS

Submitted to:

Suffolk County Department of Public Works
Suffolk County Department of Health Services
Suffolk County, New York

Submitted by:

CASHIN ASSOCIATES, P.C.
1200 Veterans Memorial Highway, Hauppauge, NY

July 2005

SUFFOLK COUNTY VECTOR CONTROL AND WETLANDS MANAGEMENT LONG - TERM PLAN AND ENVIRONMENTAL IMPACT STATEMENT

PROJECT SPONSOR

Steve Levy
Suffolk County Executive

Department of Public Works
Charles J. Bartha, P.E.
Commissioner
Richard LaValle, P.E.
Chief Deputy
Leslie A. Mitchel
Deputy Commissioner

Department of Health Services
Brian L. Harper, M.D., M.P.H.
Commissioner
Vito Minei, P.E.
Director, Division of Environmental Quality

PROJECT MANAGEMENT

Project Manager: Walter Dawydiak, P.E., J.D.
Chief Engineer, Division of Environmental Quality, Suffolk County Department of Health Services

Suffolk County Department of Public Works, Division of Vector Control
Dominick V. Ninivaggi
Superintendent
Tom Iwanejko
Principal Environmental Analyst
Mary E. Dempsey
Biologist

Suffolk County Department of Health Services, Office of Ecology
Martin Trent
Acting Chief
Kim Shaw
Bureau Supervisor
Robert M. Waters
Bureau Supervisor
Laura Bavaro
Senior Environmental Analyst
Phil DeBlasi
Environmental Analyst
Jeanine Schlosser
Principal Clerk

SUFFOLK COUNTY LONG TERM PLAN CONSULTANT TEAM

Cashin Associates, P.C.	**Hauppauge, NY**
Subconsultants	
Cameron Engineering, L.L.P.	Syosset, NY
Integral Consulting	Annapolis, MD
Bowne Management Systems, Inc.	Mineola, NY
Kamazima Lwiza, PhD	Stony Brook University, Stony Brook, NY
Ducks Unlimited	Stony Brook, NY
Steven Goodbred, PhD & Laboratory	Stony Brook University, Stony Brook, NY
RTP Environmental	Westbury, NY
Sinnreich, Safar & Kosakoff	Central Islip, NY
Bruce Brownawell, PhD & Laboratory	Stony Brook University, Stony Brook, NY
Anne McElroy, PhD & Laboratory	Stony Brook University, Stony Brook, NY
Andrew Spielman, PhD	Harvard School of Public Health, Boston, MA
Richard Pollack, PhD	Harvard School of Public Health, Boston, MA
Masahiko Hachiya, PhD	Harvard School of Public Health, Boston, MA
Wayne Crans, PhD	Rutgers University, New Brunswick, NJ
Susan Teitelbaum, PhD	Mount Sinai School of Medicine, NY
Zawicki Vector Management Consultants	Freehold, NJ
Michael Bottini, Turtle Researcher	East Hampton, NY
Robert Turner, PhD & Laboratory	Southampton College, NY
Christopher Gobler, PhD & Laboratory	Southampton College, NY
Jerome Goddard, PhD	Mississippi Department of Health, Jackson, MS
Sergio Sanudo, PhD & Laboratory	Stony Brook University, Stony Brook, NY
Suffolk County Department of Health Services, Division of Environmental Quality	Hauppauge, NY

CREDITS

This report was prepared by Bruce Brownawell, PhD, and Joseph Ruggeri (Stony Brook University). It was edited by Cashin Associates (personnel including David J. Tonjes, PhD). It was edited in response to comments by Bruce Brownawell, PhD (Stony Brook University) and Cashin Associates (personnel including David J. Tonjes). Review was provided by Suffolk County Department of Health Services and Suffolk County Department of Public Works, Division of Vector Control.

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EXECUTIVE SUMMARY

The intent of this study was to trace larvicides and adulticides used for mosquito control purposes in the salt marsh environment. The pesticides applied at the study sites for the Caged Fish experiment were methoprene (as liquid Altosid) and resmethrin (applied as Scourge, 18 percent resmethrin with 54 percent piperonyl butoxide [PBO] added as a synergist). Aerial applications of pesticides were made August 3 (Altosid at Johns Neck and Timber Point), August 10 (Altosid at Johns Neck and Timber Point), August 17 (Altosid at Johns Neck) and 18 (Scourge at Johns Neck), August 25 (Scourge at Johns Neck), and September 1 (Altosid at Timber Point). The adulticides sumithrin and malathion were also analyzed for in all water samples. Estuarine water samples near Davis Park were also analyzed following a ground spray of sumithrin (applied as Anvil 10+10, 10 percent sumithrin and 10 percent PBO) on September 14, 2004. Sediment/marsh surface samples were collected at Johns Neck, Timber Point, Havens Point, and Flax Pond.

Analyses of samples were made by the US Geological Survey and Stony Brook University (Brownawell laboratory, Marine Sciences Research Center). This report concentrates on the analyses made by Stony Brook University, as the US Geological Survey data are reported elsewhere. Extremely sensitive analytical techniques were used by both laboratories, so that pesticide detection limits in water were in the low parts per trillion and (in the case of the Stony Brook University work) high parts per quadrillion range. The detection limit for sediment samples was in the parts per billion range.

The interpretation of the results is that it appears that particulate methoprene is rapidly lost in the first two hours from the water column. After this initial phase, methoprene concentrations in water are buffered by interaction with sediment to much lower levels (lower than 25 ng/L) for several days. After that, barely detectable levels (low ng/L) are sometimes found for time periods on the order of a week. Some of the applied methoprene reaches the sediments, but at lower concentrations than those expected if methoprene were not lost from the creek bottoms by transformation or tidal flushing. Since methoprene levels in sediments do not increase despite repeated applications during late July to early September, this suggests that sediment associated methoprene is lost from sediments at timescales faster than the seven day period between

spraying. More rigorous sampling in conjunction with a closer examination of the application records for the site should illuminate sediment processes better. The short term persistence of methoprene in waters and sediments of these marsh environments is consistent with the intended action of this larvicide, given that it is applied as an encapsulated, time release formulation.

On August 18, PBO was detected in water samples collected within two hours of application at concentrations that are consistent with levels predicted based upon targeted application rates. After that initial period, PBO (which is known to be both more soluble and persistent than resmethrin) was either flushed out of the marsh, or degraded over a several day period.

On August 25, much lower concentrations of PBO and resmethrin were detected. Combined with other observations, it is possible that lower concentrations of Scourge were applied to the area of the Caged Fish experiment that evening.

With one possible exception (the interface sample collected on August 18), the high ratios of PBO to resmethrin (as compared to the source three to one ratio in Scourge) suggests preferential loss of resmethrin either in the air or in the water. No resmethrin can be detected in sediment, although its high K_{ow} (octanol-water coefficient), which is similar to that for methoprene, indicates that it theoretically might partition to particulates and so be transported to sediments.

1 OBJECTIVES AND SCOPE OF WORK

This report presents and describes the results of analyses of waters and sediments sampled in conjunction with pesticide applications over salt marshes in Suffolk County, New York, during August and September, 2004. Most of the sampling was conducted in support of an experiment testing effects of pesticides on caged organisms in the marshes (CA-SBU, 2005).

The intent of this study was to trace larvicides and adulticides used for mosquito control purposes in the salt marsh environment. The pesticides applied at the study sites for the Caged Fish experiment were methoprene (as liquid Altosid) and resmethrin (applied as Scourge, 18 percent resmethrin with 54 percent piperonyl butoxide [PBO] added as a synergist). The adulticides sumithrin and malathion were also analyzed for in all water samples. Estuarine water samples near Davis Park were also analyzed following a ground spray of sumithrin (applied as Anvil 10+10, 10 percent sumithrin and 10 percent PBO) on September 14, 2004..

The objectives of this study were:

1) to provide water column pesticide exposure data during the Caged Fish experiment, at locations at the Johns Neck, Timber Point, Flax Pond, and Havens Point marshes. Aerial applications of pesticides were made August 3, August 10, August 17 and 18, August 25, and September 1. Pesticide exposure was determined by collecting subsurface unfiltered water samples at the depth of the cages (approximately six inches below surface), following previously described methods (Zulkowsky et al., 2005).

2) to assist the US Geological Survey (USGS) in determining attenuation of pesticide levels in marsh waters immediately following applications. Lower detection limits achieved by the Stony Brook University (SBU) laboratory helped constrain the die-away studies conducted by USGS in association with four applications of the Caged Fish experiment (larvicide applications at both Timber Point and Johns Neck on August 3 and adulticide application at. Johns Neck on August 18 and August 25). Multiple sampling depths (interface and subsurface) were analyzed to support this objective.

3) to compare results obtained by SBU and USGS. Different analytical protocols and sampling methods have been used by the two groups (Zulkowsky et al., 2005). The intercomparison conducted here would enhance interpretation of the combined data sets. To accomplish this, interface and subsurface samples were analyzed along with samples collected from filter media. The intent was to determine the physical form that the pesticides were in, and to determine what factors might account for differences in results obtained using the different methods.

4) to develop, test, and employ methods to determine the possible occurrence of pesticides in marsh sediments following applications. This would test whether sediments act as a short- or long-term sink for pesticides in these settings.

5) to develop, test and employ methods to determine whether undegraded pesticides are accumulated in aquatic invertebrates at detectable levels, and determine whether tissue samples could be used to assess environmental exposures. To this end, mussel samples were collected under a variety of exposure regimes following pesticide applications in 2003 and 2004.

The first four objectives were addressed, and this report discusses the findings of the analyses discussed above. This includes analysis of approximately 90 water samples (including laboratory blanks, spike matrix samples, and laboratory intercalibration samples). Useful methods for the detection of methoprene and resmethrin in sediment samples were also developed. Nearly 50 samples (including many spiked matrix samples) were successfully analyzed. Quality assurance and control (QA/QC) determined that samples from two of the marshes provided false positive values for resmethrin. The extra effort that resulted from the method development and QA/QC difficulties did not allow sufficient time to make significant progress towards objective 5 within the time allotted for the work.

2 MATERIALS AND METHODS

2.1 Sampling

Water sampling was conducted by both by USGS and SBU personnel using the same techniques. Two types of water samples were collected:

- The primary sample used for this study (both USGS time series sampling and all sampling that supported biological exposure experiments at all four sites) were subsurface batch samples in which pre-combusted one L or four L glass bottles were hand opened below the surface (to avoid incorporation of surface films at the air-water interface). These subsurface samples have also been referred to as point samples (CA-USGS, 2005).

- Additional grab samples were obtained by USGS at selected times using a sampler that fills slowly with only water from near the air water interface (upper one to two cm), and are referred to as "interface samples." This sampling approach was utilized extensively in prior mosquito control pesticide monitoring by USGS; this combined sampling approach afforded a comparison of the methods. It should be noted that the two sampling methods collected water at different time scales; it is expected that concentrations for both types of samples are highly variable in time and space, especially immediately following the spray events. Thus, it is reasonable to assume that the comparison can be made only in a most general way, as in noting (see the results section, below) that interface samples have the potential to contain much higher concentrations than do subsurface water samples.

SBU analyzed both filtered (by USGS through 0.7 µm pre-combusted glass fiber filters) and unfiltered water samples. USGS only analyzed water samples that were filtered. In prior studies, SBU chose to monitor pesticide levels in unfiltered samples as a more integrated measure of pesticide exposure to organisms in the water column. Selected filters were also stored frozen in aluminum foil, and analyzed using methods developed for sediment samples.

SBU water samples (approximately 900 mL) were preserved and later extracted with 25 mL of hexane, either immediately upon collection in the field or after filtration by USGS, within 12 hours of collection. USGS methods are different. Samples analyzed by USGS were filtered that

day and sent on ice via overnight mail for next day processing. The USGS laboratory only extracted a portion of the collected samples (247 mL of the water samples shipped as one L samples). This has the potential to result in lower pesticide concentrations:

- pesticides may have settled out on particulate matter. Although USGS filters samples, which is intended to remove particulate matter, flocculation of organic matter can occur during shipping.

- pesticides might sorb to bottle walls.

Thus, if differences accrue due to sample handling and/or extraction, it is expected that the SBU results, because of more rapid sample preservation, long contact time with hexane, and extraction of the entire bottle rather than pouring off an aliquot, should yield higher concentrations – if samples were effectively split, and both were analyzed appropriately. In addition, unfiltered samples can be expected to yield higher concentrations than filtered samples, generally, and this is especially so for low solubility analytes such as resmethrin and methoprene. For methoprene in particular, since it is released from poorly described slow release particles (10 to 20 µm in diameter) as liquid Altosid, it is likely to be largely present in water soon after applications as filterable particles.

Sediment samples were collected with procedures designed to analyze only samples from the upper cm below the air-sediment/soil or water-sediment interface. The intent was that an inventory (pesticide mass/unit area) could be determined, and then compared to water column inventories and targeted aerial application rates. Thus, sediment cores were collected using a 2 5/8 inch I.D. acrylic core barrel which had been filed and sanded sharp along the bottom. Because the sediments in the areas of the caged fish were quite soupy, it was usually necessary to secure the cores by placing and sealing a plastic cap around the bottom by hand from underneath the core, as opposed to a preferred methodology of capping the core from the top and removing it with inherent suction holding the sample in place. In any case, the bottom stopper was removed while simultaneously seating the bottom of the core barrel on a hand made extruder; the core sample was then pushed up to the top of the core barrel. The upper cm of sample was removed with a solvent-rinsed metal spoon into a solvent-rinsed glass jar. To attempt to account for areal heterogeneity of pesticide deposition and sediment properties, a total of three samples were

collected locally and combined. The combined sample was stirred, put on ice in the field, and then frozen back at the laboratory. For subaerially exposed high marsh samples, even in the few bare spots on the marshes marsh grass rhizomes prevented coring into the soil, and so samples from bare marsh locations were obtained using a knife and spoon. A one cm depth was approximated, and checked by ruler. Selected samples at Timber Point marsh were collected from microbial/algal mat ("panne") samples from intermittently flooded puddle areas of that marsh. A knife (or scissors) and spoon were used to collect those samples. In general, sediment samples were quite heterogeneous with significant amounts of detritus (subtidal and intertidal samples) or rhizome (high marsh or supertidal) present in most of the samples. Prior to extraction, sediment/soil samples were freeze-dried and diced with a sharp knife when large pieces of organic matter were present.

2.2 Study Sites and Applications Sampled

Study sites have been well described elsewhere (Cashin Associates, 2005a) (see Figure 1 for general locations). Samples were collected preceding and following spraying at Timber Point and Johns Neck marshes surrounding aerial applications at both marshes with Altosid during the morning and early afternoon on August 3, 2005. USGS collected samples prior to spraying at Johns Neck and at both sites 0.5 hr, two hr, 24 hr, 48 hr, and 96 hr after applications. Selected samples were obtained at Flax Pond and Havens Point control marsh sites during the same period, as well as at later times. Similar Altosid spraying events were sampled at 0.5 hr and 24 hr after an application on August 10 at the Timber Point and Johns Neck sites by SBU. Time series sampling of aerial evening applications of the adulticide Scourge over the Johns Neck marsh were conducted for August 18 and August 25 applications, with Havens Point marsh serving as a reference site. A similar series of samples was collected by USGS, except that instead of a 24 hour sample, samples were collected at approximately nine hours following spraying in order to collect water samples prior to sunrise, as it was intended to determine the levels of photo-reactive resmethrin prior to direct photolysis reactions degrading this pesticide. In addition, there was an application of Altosid at Johns Neck on August 17, leading to detections of methoprene in water during the resmethrin time series sampling. A final Altosid application at Timber Point (September 1) was monitored, with both Havens Point and the channel at Johns Neck serving as control sites.

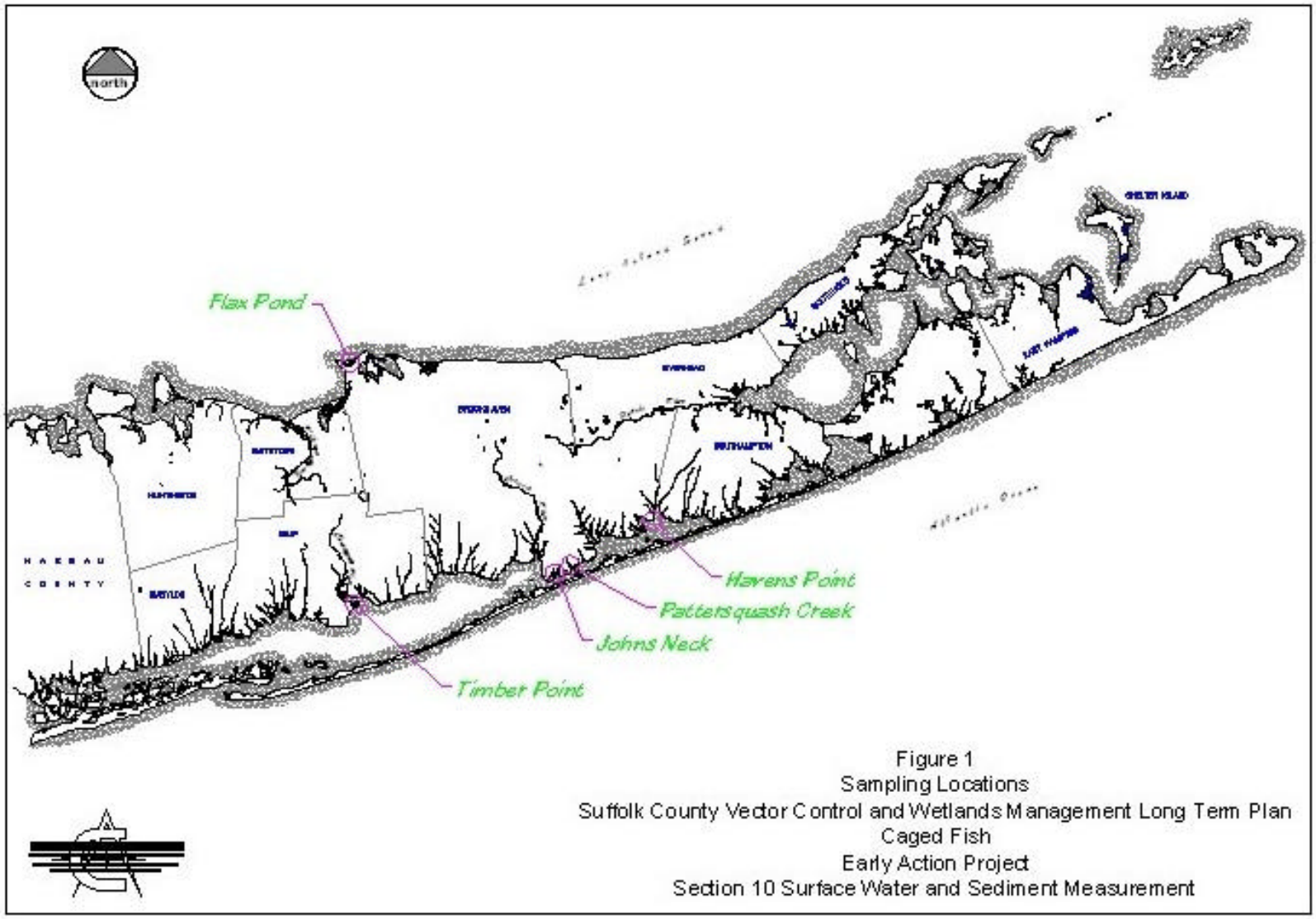

Figure 1
Sampling Locations
Suffolk County Vector Control and Wetlands Management Long Term Plan
Caged Fish
Early Action Project
Section 10 Surface Water and Sediment Measurement

The preponderance of water and sediment samples were collected in the one meter deep tidal ditches (CA-SBU, 2005; CA-USGS, 2005). Additional water samples were analyzed for pesticides from Pattersquash Creek, offsite but in the general vicinity of the Johns Neck marsh on August 18. On August 25 and 26, water samples were collected and analyzed from the larger channel at John Neck marsh, as well. Finally, three sites from the harbor and marsh at Davis Park (on the Great South Bay side of Fire Island) were sampled at mid-day on September 14 following an evening ground spray of sumithrin (see Figure 2).

Figure 2
Davis Park Sampling Locations
Suffolk County Vector Control and Wetlands Management Long Term Plan
Caged Fish
Early Action Project
Section 10 Surface Water and Sediments Measurements

2.3 Chemicals

Standards consisted of resmethrin (98 percent pure, a mixture containing 80 percent trans and 20 percent cis isomers) (note that SBU has observed large differences in isomer ratios with resmethrin standards obtained from different sources), sumithrin, PBO, malathion (98 percent pure), and methoprene (98 percent pure, racemic mixture of R and S isomers) were obtained from Crescent Chemical (Islandia, New York). Deuterated *d*-6 malathion was from utilized as an internal standard in this study and was obtained from CDN Isotopes (Quebec, Canada). All solvents were analytical grade Burdick and Jackson (VWR Scientific Products, Bridgeport, New York). All other chemicals were from Sigma Aldrich (St. Louis, Missouri).

2.4 Pesticide Analysis

Procedures for pesticide extraction and analysis are summarized in Zulkowsky et al. (2005) and detailed in the attached standard operating procedure (SOP). Briefly, a few key points are provided here, to illustrate similarities and differences in the analyses conducted at SBU and USGS. Higher volume samples were analyzed at SBU (900 mL of unfiltered water) than at the USGS (247 mL of filtered water). Two major features were shared between the methods:

1) liquid-liquid extractions (water: hexane) were employed with volume ratios between 25 to one and 40 to one, as described by Zimmerman et al. (2001). It is advantageous to use hexane as an extracting solvent because it effectively partitions the targeted analytes (even the more soluble chemicals of concern, malathion and PBO) but is relatively inefficient at extracting potential interferents (as compared to methylene chloride or solid phase extraction [SPE] sorbents), and it can be evaporated without significant co-volatilization of target chemicals.

2) The high performance liquid chromatography coupled to time-of-flight mass spectroscopy (LC-TOF-MS, used at SBU) and gas chromatography quadruple mass spectrometry (GC-MS, used at USGS, and at SBU for sediment samples) methods used were each able to sensitively and concurrently analyze all of the target analytes with very similar sensitivities (method detection limits of one to three pg injected for LC-TOF-MS, and approximately 10 pg injected for GC-MS). One advantage of LC-TOF-MS is that it

is much easier to inject a high percentage of the sample on column (SBU used 10 percent but 50 percent for off-line and 100 percent for on-line extractions and analysis are practical), whereas, routine analyses by conventional GC-MS systems rarely allow for more than one to two percent of sample to be injected.

LC-TOF-MS analyses were conducted with a Micromass LCT, equipped with a Waters 2695 HPLC and a Z-spray electrospray ionization source. The Stationary phase was a C-8 Discovery Column (dimensions = 15 cm x 2.1mm, 5µm) supplied by Supelco. The mobile phase was a mixture of methanol and water with gradient elution beginning with 40 percent methanol, held for one minute, increased to 80 percent methanol by six minutes, to 95 percent methanol by 12 minutes, held until 16 minutes, then returned to initial 40 percent by 18 minutes and held until 26 minutes to re-equilibrate the column. Both the aqueous mobile phase and the methanol contained 10 µM sodium acetate and 10 µM potassium acetate. All the pesticide analytes were analyzed and quantified as sodium adducts of parent molecules $(M+Na)^+$. Leucine enkephalin was added through post-column infusion to serve as an internal mass calibrant, in order to confirm analyte identification using accurate mass estimation (Benotti et al., 2003). The internal standards used included *d*-6-malathion. For LC-TOF-MS analysis, the hexane extract was brought to dryness with a gentle stream of nitrogen and the pesticides were redissolved in 100 µL of methanol, of which 10 µL aliquots were injected. One disadvantage of the HPLC-TOF-MS system used is the limited dynamic range of the detector. As a result samples containing more than 10 to 20 ng/L of pesticide had to be diluted and re-analyzed, sometimes two or even three times before analytical signals fell within the calibration curve.

The method for sonic probe extraction, and florisil SPE clean-up of sediment extracts is provided in the SOP for sediment analysis that is attached below. GC-MS analysis was utilized for sediment extracts, as the greater amount of co-extracted organic matrix interferes with electrospray ionization. This was completed using an HP 5890 series II GC equipped with VG Quattro mass spectrometer. The capillary column was purchased from Restek Corporation, the item is RTX-5MS, dimensions: length = 30 meters, ID = 0.25 mm, and film thickness = 0.25 µm. GC parameters were inlet = 280º C, transfer line to MS = 280º C, oven initial = 70º C, ramp 15º C/min to 190º C, ramp 5º C/min to 270º C, ramp 20º C/min to 290º C, hold at 290º C to bake out column. The mass spectrometer was operated with electron impact ionization in selected ion

monitoring mode. Various windows of acquisition were programmed specific to each compound depending upon elution time. The quantitation ions used were:

- d-10-phenanthrene = 188 m/z
- d-6-malathion = 173 m/z
- methoprene = 73 m/z
- resmethrin = 171 m/z
- piperonyl butoxide = 176 m/z
- sumithrin = 183 m/z.

Additional confirming ions (typically, two additional ions) were also monitored and this proved to be especially important for the confirmation of resmethrin in sediments. Deuterated d-6-malathion was used in this case as a surrogate standard and *d*-10-phenanthrene as an internal standard. For GC-MS analysis, the final solvent volume was adjusted to 200 µL from which one µL was injected.

Laboratory blanks with deionized water were run with each group of six to 10 samples. There were no detected pesticides in laboratory or field blank samples (processed as described by CA-USGS, 2005). Method recoveries in pure water averaged between 85 and 118 percent, and spiked matrix (water and sediment) samples yielded similar recoveries. Method detection limits were approximately 200 to 500 pg/L (parts per quadrillion) for pesticides in water and one to five ng/g (parts per billion) for pesticides in sediment samples.

An interlaboratory comparison of marsh waters spiked with known solutions containing two concentrations of the five target compounds (methoprene, resmethrin, PBO, sumithrin, and malathion) was recently conducted by three participating labs (SBU, USGS, and the Suffolk County Department of Health Services [SCDHS] Public and Environmental Health Laboratory [PEHL]); those results should be forthcoming.

3 RESULTS

Results from analyses of water samples are provided in Table 1. A distillation of time series results for pesticides following each spray event is given in Tables 2 to 5, using a combination of SBU and USGS results (averaged analyses of replicates are shown when replicates were analyzed). Table 6 summarizes the results of sediment analyses.

3.1 Blanks

There were no positive detections at 200 to 500 pg/L method detection limits for any of the analyzed for compounds (methoprene, resmethrin, sumithrin, malathion, or PBO) in any laboratory blank or field blank.

3.2 Methoprene

There were no detections of methoprene in any water sample where there was not a recent application overhead.

Methoprene was detected in pre-application samples three times:

- 1 ng/L at Timber Point on August 10, which was seven days after an application at that site
- 1.5 ng/L at Johns Neck on August 10, seven days following the previous application at that site
- 10 to 14 ng/L at Johns Neck on August 18. These detections were "pre-application" for a planned resmethrin application, but actually followed an application on August 17 of methoprene in the same marsh. These detections are actually post-application with reference to methoprene.

3.3 Resmethrin

There were no detections of resmethrin in any water sample where there was not a recent application overhead.

3.4 PBO

PBO was once detected at Havens Point (a control site) at a concentration of 1.5 ng/L (0.95 ng/L upon a subsequent re-injection), which is just above the method detection limit of 200 to 500 pg/l. PBO was also detected at Pattersquash Creek, which, although not in the application zone used for the Johns Neck marsh events, is in relatively close proximity (see Figure 2).

3.5 Sumithrin

There were no detections of sumithrin in any sample, including results from Davis Park Harbor and marsh collected between noon and 2 PM the afternoon after a ground application of sumithrin in that community the evening before.

3.6 Malathion

There were no detections of malathion in any sample.

Table 1. Pesticides measured in water samples by SBU

Date collected	Time	Filtered	Sample description	Methoprene ng/L	piperonyl butoxide ng/L	resmethrin ng/L	sumithrin ng/L	Permethrin ng/L	Malathion ng/L
Spray occurred at 11:30 on 3 August									
3-Aug	11:15	n	Subsurface	<DL	<DL	<DL	<DL	<DL	<DL
3-Aug	12:30	y	Interface	**23**	<DL	<DL	<DL	<DL	<DL
3-Aug	12:50	n	Subsurface	**1,500**	<DL	<DL	<DL	<DL	<DL
3-Aug	13:15	y	field blank	<DL	<DL	<DL	<DL	<DL	<DL
4-Aug	12:15	n	Subsurface	<DL	<DL	<DL	<DL	<DL	<DL
Spray occurred at 12:55 on 10 August									
10-Aug	12:00	n	Subsurface	**1.5**	<DL	<DL	*Interference*	<DL	<DL
10-Aug	1:25	n	Subsurface	**1,100**	<DL	<DL	<DL	<DL	<DL
11-Aug	12:00	n	Subsurface	**24**	<DL	<DL	<DL	<DL	<DL
Spray occurred at 19:30 on 18 August									
18-Aug	16:30	n	Subsurface	**3.7**	<DL	<DL	<DL	<DL	<DL
18-Aug	18:50	n	Subsurface	**14**	<DL	<DL	<DL	<DL	<DL
18-Aug	18:50	n	subsurface, replicate	**10**	<DL	<DL	<DL	<DL	<DL
18-Aug	19:30	y	field blank	<DL	<DL	<DL	<DL	<DL	<DL
18-Aug	20:00	n	Subsurface	**1.4**	**301**	**6.8**	<DL	<DL	<DL
18-Aug	20:00	n	subsurface, replicate	<DL	**110**	**8.9**	<DL	<DL	<DL
18-Aug	20:00	y	Interface	<DL	**16,000**	**300**	<DL	<DL	<DL
18-Aug	20:00	y	interface, replicate	<DL	**19,000**	**340**	<DL	<DL	<DL
18-Aug	21:20	n	Subsurface	<DL	**4,000**	**60**	<DL	<DL	<DL
18-Aug	21:50	n	Subsurface	<DL	**1,800**	**13**	<DL	<DL	<DL
19-Aug	4:30	n	Subsurface	**5.2**	**29**	<DL	<DL	<DL	<DL
19-Aug	4:30	n	subsurface, replicate	**4.5**	**19**	<DL	<DL	<DL	<DL
Spray occurred at 19:10 on 25 August									
25-Aug	17:45	n	subsurface Channel	<DL	<DL	<DL	<DL	<DL	<DL
25-Aug	17:45	n	Subsurface	<DL	<DL	<DL	<DL	<DL	<DL
25-Aug	17:45	n	subsurface, replicate	<DL	<DL	<DL	<DL	<DL	<DL
25-Aug	19:40	y	field blank	<DL	<DL	<DL	<DL	<DL	<DL
25-Aug	19:40	n	Subsurface	**1.1**	**9.7**	<DL	<DL	<DL	<DL
25-Aug	19:40	n	subsurface, replicate	<DL	**13**	<DL	<DL	<DL	<DL
25-Aug	19:40	y	interface	<DL	**26**	<DL	<DL	<DL	<DL
25-Aug	19:40	n	subsurface Channel	<DL	**17**	<DL	<DL	<DL	<DL
25-Aug	19:40	n	subsurface Channel, Rep.	<DL	**21**	<DL	<DL	<DL	<DL
25-Aug	19:40	y	interface Channel	<DL	**11**	<DL	<DL	<DL	<DL
25-Aug	21:10	n	subsurface Channel	<DL	**4.1**	<DL	<DL	<DL	<DL
25-Aug	21:10	n	subsurface Channel	<DL	<DL	<DL	<DL	<DL	<DL
26-Aug	4:30	y	Subsurface	<DL	**93**	<DL	<DL	<DL	<DL
26-Aug	4:30	y	subsurface, replicat e	<DL	**83**	<DL	<DL	<DL	<DL
26-Aug	4:30	n	subsurface Channel	<DL	**44**	<DL	<DL	<DL	<DL

Timber Point *panne sample was a small pool on marsh surface

Spray occurred at 7:05 on 3 August

3-Aug	7:35	n	Subsurface	**490**	<DL	<DL	<DL	<DL	<DL
3-Aug	7:35	y	panne, interface	**2,000**	<DL	<DL	<DL	<DL	<DL
3-Aug	7:35	y	Interface	**3,300**	<DL	<DL	<DL	<DL	<DL
3-Aug	8:20	y	field blank	<DL	<DL	<DL	<DL	<DL	<DL
3-Aug	8:50	y	Subsurface	**6.3**	<DL	<DL	<DL	<DL	<DL
3-Aug	10:10	y	offsite, interface	**0.78**	<DL	<DL	<DL	<DL	<DL
4-Aug	6:45	n	Subsurface	**17**	<DL	<DL	<DL	<DL	<DL

Spray occurred at 8:55 on 10 August

10-Aug	8:15	n	Subsurface	**1**	<DL	<DL	<DL	<DL	<DL
10-Aug	9:25	n	Subsurface	**3.3**	<DL	*interference*	<DL	<DL	<DL
10-Aug	9:30	n	Subsurface	**12**	<DL	<DL	<DL	<DL	<DL
11-Aug	10:40	n	Subsurface	**22**	<DL	<DL	<DL	<DL	<DL

Spray occurred on 1 September

2-Sep	15:30	n	Subsurface	**5.6**	<DL	<DL	<DL	<DL	<DL
5-Sep		n	Subsurface	<DL	<DL	<DL	<DL	<DL	<DL

Havens Point

3-Aug	3:47	n	Subsurface	<DL	<DL	<DL	<DL	<DL	<DL
10-Aug		n	Subsurface	<DL	<DL	*interference*	<DL	<DL	<DL
18-Aug		n	Subsurface	<DL	<DL	<DL	<DL	<DL	<DL
18-Aug		n	subsurface, replicate	<DL	<DL	<DL	<DL	<DL	<DL
18-Aug	22:30	n	Subsurface	<DL	**1.5**	<DL	<DL	<DL	<DL

Pattersquash Creek – offsite

18-Aug	22:00	y	interface - offsite	<DL	**18**	*interference*	<DL	<DL	<DL
18-Aug	22:00	y	interface - offsite, rep	<DL	**45**	<DL	<DL	<DL	<DL

Flax Pond

3-Aug		n	Subsurface	<DL	<DL	*interference*	<DL	<DL	<DL
10-Aug	15:43	n	Subsurface	<DL	<DL	<DL	<DL	<DL	<DL

Davis Park

14-Sep	13:20	n	Site #1	<DL	<DL	<DL	<DL	<DL	<DL
14-Sep	13:42	n	Site #2	<DL	<DL	<DL	<DL	<DL	<DL
14-Sep	14:14	n	Site #3	<DL	<DL	<DL	<DL	<DL	<DL
15-Sep		n	Site # 1	<DL	<DL	<DL	<DL	<DL	<DL
15-Sep		n	Site # 2	<DL	<DL	<DL	<DL	<DL	<DL
15-Sep		n	Site # 3	<DL	<DL	<DL	<DL	<DL	<DL

Table 2. Time series results for methoprene following Altosid applications August 3, 2005

Time Post Spray	Sample Type	ng/L	Lab
Timber Point- Connetquot River			
0.5 hrs	Interface	3,300	SBU
		216	USGS
	Subsurface	490	SBU
		82	USGS
2 hrs	Subsurface	6.3	SBU
		<5	USGS
24 hrs	Subsurface	17	SBU
		<5	USGS
48 hrs	Subsurface	<5	USGS
96 hrs	Subsurface	<5	USGS
Johns Neck – Uncachogue Creek			
0.5 hrs	Interface	23	SBU
		<5	USGS
	Subsurface	1,500	SBU
		10	USGS
2 hrs	Subsurface	<5	USGS
24 hrs	Subsurface	<0.5	SBU
		<5	USGS
48 hrs	Subsurface	<5	USGS
96 hrs	Subsurface	<5	USGS

All interface samples were filtered before analysis
Subsurface samples analyzed by USGS were also filtered; subsurface samples analyzed by SBU were not filtered.

Table 3. Time series for methoprene following Altosid applications on August 10, 2005

Location	Time Post Spray	Sample Type	ng/L
Johns Neck	0.5 hrs	Subsurface	1,100
	24 hrs	Subsurface	24
Timber Point	0.5 hrs	Subsurface	8
	24 hrs	Subsurface	22

All samples unfiltered and analyzed by SBU

Table 4. Time series for resmethrin and PBO following August 18, 2005 application of Scourge at Johns Neck – Uncachogue Creek

Time Post Spray	Sample Type	Resmethrin ng/L	PBO ng/L	Lab
0.5 hrs	Interface	320	18,000	SBU
		270	59,800	USGS
	Subsurface	7.8	210	SBU
		<5	1,310	USGS
2 hrs	Subsurface	36	2,900	SBU
		38	457	USGS
9 hrs	Subsurface	<.5	24	SBU
		<5	61	USGS
48 hrs	Subsurface	<5	6	USGS
96 hrs	Subsurface	<5	<5	USGS

All interface samples were filtered.
Subsurface samples analyzed by USGS were also filtered; subsurface samples analyzed by SBU were not filtered.

Table 5. Time series for resmethrin and PBO following August 25, 2005 application of Scourge at Johns Neck – Uncachogue Creek

Time Post Spray	Sample Type	Resmethrin ng/L	PBO ng/L	Lab
0.5 hrs	Interface	<.5	26	SBU
		<5	12	USGS
	Subsurface	0.8	11	SBU
		<5	15	USGS
2 hrs	Subsurface	<.5	2	SBU
		<5	28	USGS
9 hrs	Subsurface	<.5	88	SBU
		<5	113	USGS
48hrs	Subsurface	<5	<5	USGS
96 hrs	Subsurface	<5	<5	USGS

All interface samples were filtered.
Subsurface samples analyzed by USGS were also filtered; subsurface samples analyzed by SBU were not filtered.

Table 6. Pesticide concentrations measured in sediments by SBU

Date collected	Sample description	Methoprene ng/g	Piperonyl butoxide ng/g	Resmethrin ng/g
Johns Neck Creek				
8/7/04	4 day post-spray subtidal	**17**	< DL	isobaric interference
8/7/04	4 day post-spray intertidal	**25**	< DL	isobaric interference
8/11/04	Surface	**68**	< DL	< DL
8/18/04	subtidal pre-spray	< DL	< DL	< DL
8/18/04	supertidal pre-spray	**20**	< DL	< DL
8/19/04	1 day post-spray subtidal	**15**	< DL	< DL
8/19/04	1 day post-spray subtidal	**24**	**11**	isobaric interference
8/19/04	1 day post-spray supertidal	**21**	**16**	isobaric interference
8/22/04	4 day post-spray subtidal	**9.1**	< DL	< DL
8/22/04	4 day post-spray subtidal	**9.6**	< DL	< DL
8/22/04	4 day post-spray subtidal	**18**	< DL	isobaric interference
8/22/04	4 day post-spray supertidal	**57**	< DL	< DL
8/22/04	4 day post-spray supertidal	**50**	< DL	< DL
8/25/04	pre-spray subtidal	**14**	< DL	isobaric interference
8/26/04	1 day post-spray subtidal	**17**	< DL	isobaric interference
8/26/04	1 day post-spray supertidal	**21**	**5.8**	isobaric interference
8/29/04	4 day post-spray subtidal	< DL	< DL	< DL
9/5/04	subsurface ditch @ cages	< DL	< DL	isobaric interference
9/5/04	subtidal intertidal	< DL	< DL	isobaric interference
9/5/04	outer pond sed	**13**	< DL	isobaric interference
9/5/04	intertidal inter mud	< DL	< DL	isobaric interference
9/5/04	shore sed	< DL	< DL	isobaric interference
Timber Point				
8/2/04	subtidal pre-spray	**20**	< DL	< DL
8/2/04	algal mat pre-spray	**40**	< DL	< DL
8/7/04	4 day post-spray supertidal	**39**	< DL	isobaric interference
9/5/04	Panne scraping	**1200**	< DL	< DL
9/5/04	sulphur, high-marsh scraping	**58**	< DL	isobaric interference
9/5/04	deposit pond	**64**	< DL	isobaric interference
9/5/04	subsurface @ cages	**50**	< DL	isobaric interference
Flax Pond				
8/2/04	supertidal pre-spray	< DL	< DL	< DL
8/2/04	subtidal pre-spray	< DL	< DL	< DL
Havens Point				
8/2/04	supertidal pre-spray	< DL	< DL	< DL
8/2/04	subtidal pre-spray	< DL	< DL	< DL
8/18/04	subtidal pre-spray	< DL	< DL	< DL
8/18/04	supertidal pre-spray	< DL	< DL	< DL

4 DISCUSSION

4.1 Methoprene in Water

Methoprene was not detected in blanks or samples from control marshes on any dates.

Relatively high levels of methoprene could be detected in samples collected 30 minutes after spraying. The highest concentrations were found in selected samples at Johns Neck following both the August 3 and August 10 applications, and at Timber Point following the August 3 application. The results from ditch samples at Timber Point on August 10 were orders of magnitude lower than these three other sampling events.

The highest concentrations of methoprene (1,100 to 1,500 ng/L) were measured in subsurface, unfiltered water samples collected within an hour of spraying at Johns Neck. SBU analyzed only one filtered interface sample, on August 3, for comparison, with a result of 23 ng/L. In contrast to this orders of magnitude reduction for the filtered sample, at Timber Point on August 3, the filtered interface sample had a higher concentration (3,300 ng/L) than the corresponding unfiltered subsurface sample (490 ng/L). An interface sample collected August 3 from a panne on the Timber Point marsh yielded a methoprene concentration (2,000 ng/L in filtered water) similar to that measured in the creek (3,300 ng/L).

The concentrations of methoprene extracted from filter samples (data not shown) from water collected immediately after applications show that much of the methoprene inventory was removed by filtration. The methoprene levels on filters at Johns Neck (680 ng/L for interface and 250 ng/L for subsurface sample, respectively) correspond to a filtered interface sample concentration of 23 ng/L and whole water concentrations in the subsurface of 1,100 to 1,500 ng/L. For samples from Timber Point, 43,000 ng/L of methoprene was isolated from the filter for the interface sample, compared to a concentration of 3,300 ng/L that passed through the filter. For a subsurface sample (from a split sample, e.g., four L bottle), the filter sample resulted in a concentration of 1,700 ng/L compared to 490 ng/L for an unfiltered sample from the same bottle.

These kinds of results might result if the methoprene time release particles are not well-mixed in the water (patchy distributions). Filtration is likely to remove most of this material; however, a small variable fraction of small particles might pass the filter, or may be released into the

operationally defined dissolved/filtered phase soon after deposition. It is difficult to directly compare the subsurface sample results of filtered and unfiltered samples, as well as splits of samples from large bottles, or even time sequences, if variability is associated with settling of particles. It is not clear whether the higher values found in the filters compared to that found in unfiltered whole water samples resulted from sample heterogeneity (associated with particle settling) that affected how well the splits of large samples were accomplished, or whether the SBU whole water extraction procedure is inefficient in extracting methoprene from the undissolved slow release particles that likely were entrained in the unfiltered samples. Extraction efficiency tests accomplished as QA/QC for the analyses did not address this issue, as the efficiency tests were run on liquid methoprene rather than the encapsulated Altosid product.

The sets of results appear to indicate that much of the methoprene inventory is found in the near interface region initially, rather than being mixed down into variably deep water columns. Rigorous testing of this hypothesis would require a statistically-based sampling regime focusing on whether methoprene initially accumulates at the air-water interface.

The concentrations of methoprene were much lower (less than 25 ng/L in filtered and unfiltered subsurface water samples) in water collected two to 24 hours after applications at both marshes, according to the USGS die-away studies conducted by USGS on August 3, and supported by SBU data on August 11 at the two marshes, and from SBU samples from September 2 at Timber Point. There may be some indications that this is not a consistent process, as subsurface samples taken at Timber Point on August 10, 30 minutes post-application, had concentrations ranging from three to 12 ng/L whereas the 24 hour post-spray concentration (August 11) was 22 ng/L. Causes for such an increase could include variability in the amount of methoprene containing particulates collected in the samples, draining of methoprene from the marsh over time, or the result of transport of waters that received different loadings of methoprene from the helicopter spray.

Samples collected 96 hr to seven days after spraying were always less than five ng/l, resulting in non-detections (USGS) or some very low ng/l detections by SBU.

There is general agreement between the magnitude of concentrations determined by USGS and SBU, in that high and low concentrations in the same or similar samples correspond with each

other, and concentrations reported by USGS to be below detection limits (5 ng/L) are also never reported to exist as higher concentrations by Stony Brook. Tentatively (absent the intercalibration data), the absence of closer agreement of concentrations is attributable to the combination of variability in local concentration of methoprene in water collected minutes apart (hypothetically in part due to methoprene-laden particles that may be patchy) to variations in how particles were distributed when four L samples were split between laboratories, and to differences in the ways in which samples were stored and extracted following filtration.

Of all the replicate samples analyzed, only two had detectable concentrations of methoprene (both at Johns Neck in subsurface waters). The results are in good agreement.

The concentrations of methoprene measured in water samples immediately after spraying compares well to some estimates of potential concentrations based on an application rate of two oz/acre. For example, if this application rate mixed vertically into a one meter depth, the expected total concentration would be 1,400 ng/L. This is comparable to the highest levels determined in whole unfiltered subsurface samples. If most of the inventory applied to the marsh is limited to the near interface region, assuming the interface sampler integrates water from the upper two cm of water, the expected concentration would be 70,000 ng/L, which is less than twice as great as the highest concentrations measured on filter samples collected from the interface sample from Timber Point 30 minutes post-application on August 3. Thus, the highest concentrations detected are similar to those that might be expected based on operational parameters.

It needs to be noted that despite repeated applications of Altosid to these two marshes prior to and during the sampling campaigns, there were only occasional detections of very low levels of methoprene except immediately after applications, even for samples from shallow ditches.

4.2 Methoprene in Sediment Samples

Methoprene was detected in sediment samples collected from Timber Point and Johns Neck marshes at levels ranging from nine ng/g (parts per billion) (the detection limit was five ng/g) to 68 ng/g; these sites received applications of methoprene during the experiment, and at other times during the mosquito control season of 2004. Methoprene was not detectable

(concentrations below five ng/g) in sediments collected at Flax Pond and Havens Point, sites where methoprene applications were not conducted. Supertidal (high marsh) soil samples yielded concentrations similar to those in subtidal and intertidal samples. A higher methoprene concentration (1,200 ng/g) was found in an algal mat sample (the September 5 "panne scraping" in Table 6);

Detection of methoprene in these samples was supported by:

- the shape and retention time of the mass chromatographic peaks
- the absence of significant baseline peaks at other nearby retention times
- the reasonable agreement of ratio of confirming (m/z = 111 and 153) to quantitation ions (m/z = 73)
- the consistency of all of the above with spiked matrix experiments with sediments from each location

The detected concentrations in sediments (9 – 68 ng/g) can be compared to a theoretical loading. The following assumptions were made:

- all of the application went to sediments
- deposition to the marsh was consonant with a two oz/acre application rate
- there is no net focusing of methoprene to the sediments (e.g., washoff from the marsh surface to sediments)
- all of the inventory is preserved in the upper one cm sample of sediment collected
- the bulk dry density of solid sediments are in the range of 0.2 to 1.0 g/cm^3 (bulk density was not determined, but professional experience with such soupy sediments suggests that it was much closer to the lower end of this range for subtidal and intertidal samples)

The resulting predicted concentrations range from 80 to 400 ng/g; the variability is generated by the range of bulk densities, with the upper limit derived from the lower, more reasonable bulk

densities. The measured concentrations of methoprene are thus lower than the potential loading, but are within a factor of 10 to 50 (which seems acceptable, given the overly conservative assumptions for the predicted values).

The Johns Neck and Timber Point marshes received multiple applications of methoprene over the sampling time period. Although the sampling data shows some variation over the time, the lack of any definitive upward trend in sediment associated methoprene concentrations suggests that degradation of methoprene (or other loss from the system such as resuspension and tidal flushing) occurs over timescales at least as fast as the weekly period between applications. This is emphasized by considering that the inventories (concentrations) that are found in the upper one cm of sediment are much lower than the amounts applied over a season. The cumulative predicted inventory from multiple applications most likely is in excess of 1,000 ng/g, if no degradation or loss from the system occurred.

This conceptual model is supported by sampling at Johns Neck September 5 (where no immediately preceding application occurred). Measured concentrations were lower then (three ditch samples were ND [concentrations less than five ng/g] and a sample from a nearby pond was measured at 13 ng/g) compared to the range of concentrations of nine to 57 ng/g for six subtidal sediment samples collected between August 22 and 26. This is also suggestive methoprene is lost from surface sediments over a several week time frame. Rapid loss of methoprene from sediments is further supported by the before mentioned nondetectable or very low ng/L concentrations measured in shallow waters measured seven days post spray on multiple occasions; the lack of increase in methoprene in sediments over the spraying season; and the much lower measured methoprene sediment concentrations (less than five to 68 ng/g) than those predicted (greater than 1,000 ng/g) to occur at the end of the season if methoprene accumulated in sediment without loss..

4.3 Resmethrin and PBO in water samples

Two adulticide (Scourge) applications (August 18 and August 25) at Johns Neck marsh were studied. There were no aerial applications of Scourge at the other sites, and neither resmethrin nor PBO were detected at sites other than at Johns Neck. One exception to this blanket statement was a single 0.95 ng/L detection of PBO from a sample at Havens Point marsh. There is no

definitive reason for this single likely false positive detection; however, laboratory or field contamination of the sample would not be surprising, given that PBO was measured at Johns Neck the same day at concentrations up to 19,000 ng/L.

PBO was measured more frequently and at much higher levels than resmethrin by both USGS and SBU. This is consistent with earlier findings that monitored applications of pyrethroid adulticides on Long Island and elsewhere (Zulkowsky et al., 2005).

Resmethrin was only detected in water samples following the August 18 application. Following that event, resmethrin was only found in samples collected in the first two hours post-spray, and were undetectable by the nine hour sample collection. The greatest resmethrin concentrations were detected in filtered interface samples collected 0.5 hour post-application (replicate analysis concentrations of 300 and 340 ng/L) and were much lower in unfiltered subsurface samples (seven to 60 ng/L, measured 0.5 to 2 hours after the application). The highest levels of PBO were also measured in filtered interface samples collected just after the application was made. PBO could be detected at low levels in the larger channel that communicates hydraulically with the ditches; this was not the case for resmethrin (all samples below detection levels).

The much lower levels (up to three orders of magnitude lower) of both ingredients detected following the August 25 application (as compared to the August 18 event) are consistent with other observations. These included slightly lower mosquito abatement responses (there was a delay in mortality for the caged mosquitoes in open area nets compared to the complete and rapid mortality on August 18). In addition, peak PBO levels in deposition pans near the Caged Fish site were slightly lower on August 25 as compared to August 18 (Cashin Associates, 2000b). This suggests that the aerial application of Scourge resulted in lower levels of pesticide in the Caged Fish site vicinity on August 25.

Neither PBO nor resmethrin were measurable at Johns Neck in samples collected prior to spraying on either date. There was clearly no carryover from prior years' applications, and even none from spraying a week before sampling (the pre-August 25 samples showed no effect from the August 18 application). Neither ingredient appears to be persistent in this marsh for periods greater than a few days, therefore.

The ratios of PBO to resmethrin were much higher in the aqueous samples than the ratios for the parent Scourge formulation. The ratio in Scourge is three to one. For the eighteen samples with the highest PBO concentrations, the ratio of PBO to resmethrin (using the detection limit when resmethrin was not detected) ranged from nine to one to 260 to one (the nine to one ratio was actually greater than that, as resmethrin was ND for that sample). The median PBO to resmethrin ratio was 46 to one. The only plausible explanation for these results is that resmethrin is either not reaching the marsh surface (transformation or differential transport in the night-time air) or is being rapidly transformed in the water column. These results are consistent with earlier SBU and USGS findings from water column monitoring (Zulkowsky et al., 2005; Abbene et al., 2005), and from results of deposition collectors used by Suffolk County (CA-CE, in press). It is well documented that resmethrin is readily photo-oxidized, but the loss of resmethrin under night-time conditions is a mystery. Any hypothesis concerning possible transformations would be purely speculative. However, it is interesting and potentially relevant that nitro-PAHs (poly-aromatic hydrocarbons) can be formed at night from reactions of PAHs with photochemically formed nitrogen oxide radicals. These radicals can persist in night-time air in urban atmospheres, so it is not impossible for such gas phase reactions to occur at night.

Five filters were analyzed for resmethrin and PBO from times when Scourge had not been applied; no detections ensued. Results from a two-hour post-application subsurface sample filter sample showed four ng/L resmethrin and no detectable PBO. Unfiltered subsurface water samples collected at or near the same time had much greater concentrations (13 to 60 ng/L for resmethrin and 1,800 to 4,000 ng/L for PBO. The results for an interface sample collected 0.5 hours post-application showed 24,000 ng/l of resmethrin and 46,000 ng/L for PBO. The filtered water from that same sample showed 300 to 340 ng/l of resmethrin and 16,000 to 19,000 ng/L for PBO. It is intriguing that the combination of the filtered water and filter results result in a 64,000 to 24,000 ratio of PBO to resmethrin, which is somewhat close to the source three to one ratio of the compounds. As most of the samples analyzed in this work were unfiltered, it is clear that the "missing" resmethrin is not simply a function of particulate partitioning, however, and more work is needed to resolve the issue.

PBO results are generally consistent between USGS and SBU. There was not a consistent bias between the laboratories for reporting higher results.

For resmethrin, there were so few detections for USGS (given the higher detection limit) that no comparison between the laboratories was possible.

The concentrations of PBO in water determined just after the August 18 application are in reasonable agreement with predicted levels based on an application rate of 9.6 g/acre, computed analogously as with methoprene, above. The resultant concentration is 790 ng/L, which is intermediate between unfiltered water sample measurements for samples collected at 0.5 hr (110 to 300 ng/L) and two hours (1,800 to 4,000 ng/L) post-application. If most of the inventory applied to the marsh were limited to the air-water interface region, and the interface sample integrated water from the upper two cm of water, the expected PBO concentration would be 40,000 ng/L, which is similar to the highest concentrations measured (64,000 ng/L) on combined dissolved and filter results from the same interface sample collected 0.5 hours post-application. For August 25, the concentrations of PBO are much lower than these predicted levels.

4.4 Resmethrin and PBO in sediment samples

Neither PBO nor resmethrin could be reliably be detected in sediments. A number of samples from both Johns Neck and Timber Point marshes (where resmethrin was not applied in 2004) initially yielded positive results. Selected ion chromatograms of resmethrin using m/z=123 resulted in a peak and a very close retention time match for the second (trans isomer) of the two cis/trans resmethrin peaks; otherwise, the baseline of the reconstructed ion chromatogram was very flat. This was originally interpreted as a isomer selective fractionation of resmethrin in the environment. Selected ion chromatograms of the same samples of the confirming ion m/z= 171 showed a similar peak in the same sample, but peak shape/retention time were not quite identical. MS/MS experiments yielded a peak with the same transition, but a different quantitative estimate of concentration. In the end, because the ratios of the two confirming ions (171 to 123) that were two to more than 10 times lower in samples than in standards, resmethrin detections were ruled out. In spiked matrix experiments (using 60 ng/g), the ratio of the two confirming ions and peak shapes agreed well with standards. Therefore, conservatively, if the peaks were to indicate resmethrin presence, the concentrations must be much less than 60 ng/g. The interpretation of the results, although the weight of evidence requires classifying the peaks as isobaric interference, is made more difficult since the peaks were only found for extracts of

sediment samples collected at the Johns Neck and Timber Point marshes, and not at the Havens Point and Flax Pond reference sites.

The inferred maximum concentrations of resmethrin in sediments (from less than five ng/g to much less than 60 ng/g for the samples with the isobaric interferences) are much lower than levels predicted to accumulate in sediments if the targeted application rate (3.2 g/acre) all reached the upper one cm of sediment. Following the assumptions used above for methoprene, the range sediment concentrations that could be predicted would be between 80 and 400 ng/g from a single application.

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Appendix A

STANDARD OPERATING PROCEDURE FOR ANALYSIS OF PESTICIDES IN WATER SAMPLES

1 Purpose

To investigate the aquatic fate of pesticides used against mosquitoes and the West Nile virus. Current sampling objectives include identifying how much of the pesticides are present in the environment following spray events, as well as monitoring quantities of pesticide in experimental exposure tanks.

This method is for extraction from aqueous samples. A method for extraction of pesticides from sediment samples is under development.

2 Summary

Environmental samples are to be collected in amber sampling bottles. They will be transported to the Marine Sciences Research Center at Stony Brook University (MSRC). Samples will be extracted and prepared for instrumental analysis. The extracts will be analyzed by LC-MS. Data will be evaluated to confirm presence of internal standards and identify samples containing pesticides. The integrated areas of mass chromatograms will then be used to calculate pesticide concentrations.

The methods used are modified from existing methods in order to achieve very low detection limits from a one liter sample that is relatively easy to collect, transport, and store. Larger sample volumes may be collected if lower detection limits are required. Chemical analysis will be carried out using a research grade mass spectrometer for greater sensitivity. The mass spectrometer is capable of determining an accurate mass measurement that is used for confirmation of analyte.

3 Procedure

3.1. Sample Collection

Environmental samples will be collected into one liter amber jars and sealed with lids using a teflon liner. The location and time of sampling shall be recorded. They will be kept chilled in an ice packed cooler until transfer to refrigerated storage at the MSRC. There must be sufficient headspace in the jar to allow for the addition of 25.0 ml of solvent and still have room for mixing. As soon as practical, a known portion of hexane must be added, either in the field or within 12 hours back in the laboratory, to the sample to preserve the pesticides.

3.2. Environmental Sample Preparation and Extraction

3.2.1 A record will be kept to indicate details of the extraction.

3.2.2 Each group of samples being extracted will be referred to as a batch. Each batch must include blank samples for quality control. Water may also be spiked with pesticide to produce positive controls as well; these are referred to as Laboratory Control Samples (LCS).

3.2.3 Place a mark on the sample jar to indicate the surface height of the sample

3.2.4 Identify the quantity of hexane that was added as a preservative and add hexane, if needed, so that the total hexane volume is 25.0ml.

3.2.5 Seal extraction vessel then agitate/mix for 30 seconds, pause, agitate for 10 seconds, pause, and then agitate for 10 more seconds.

3.2.6 Add purified lab water to fill the sample jar, such that most of the hexane layer resides in the neck of the bottle, facilitating removal.

3.2.7 Quantitatively remove half of the hexane layer and transfer to a concentrator tube. Record the amount of hexane removed. Be sure not to

take any of the aqueous phase. In the case of an emulsion, transfer the emulsion to a disposable vial and centrifuge at 3,000rpm for 5 minutes.

3.2.8 Take the extract to just to dryness at 35ºC with a stream of nitrogen.

3.2.9 Add 0.100 ml methanol/water (40:60) to the dried extract.

3.2.10 Transfer 0.090 ml to an autosampler vial containing a low volume insert.

3.2.11 Add 0.010 ml internal standard.

3.2.12 Store below 0ºC until analysis.

3.2.13 When extraction is completed remove the remaining hexane and any emulsion that was formed and add to the non-halogenated solvent waste jar. Then adjust the remaining water to the mark which indicates the initial sample volume. Pour this into a 1.0 graduated cylinder to determine the volume. Record the volume on the extraction record.

3.3. Procedure for extraction of small volume samples collected during laboratory exposures with high pesticide levels (>50 ng/L)

3.3.1 Amber 12.0 ml vials are solvent washed and dried in the laboratory. They are filled with 1.0 ml hexane.

3.3.2 Experimental samples are acquired using a quantitative pipette and added to the 12.0 ml vial containing hexane.

3.3.3 The vial is shaken for 30 seconds, pause, agitate for 10 seconds, pause, and then agitate for 10 more seconds.

3.3.4 Remove 0.100 ml from the hexane portion and place directly to a vial containing a low volume insert. Be sure not to collect any of the aqueous layer.

3.3.5 Take to dryness using a gentle stream of nitrogen.

3.3.6 Add methanol/water (50:50) to bring sample to proper volume, allow for IS addition.

3.3.7 Add internal standard so the final concentration is 0.1µg/ml d6 malathion.

3.3.8 Store below 0ºC until analysis

3.4. Sample Analysis

The details regarding operation of the analytical system are specified in the Instrument Control Procedure for LC-MS TOF. This section will present the specific instrumental settings which apply to the mosquito pesticides. Included are discussion of the chromatography conditions and the recommended settings for the electrospray source. Other details such as preparation of quantitative standards and interpretation of results are not discussed.

Using these settings will elute the analytes in 15 minutes and the system will increase to high organic and then return to initial conditions to equilibrate the column. The electrospray will be fixed to ES+ during the run. The pesticides will be observed as [M+Na]+ using ES+.

3.4.1 Liquid Chromatography

The extracts are to be eluted at 0.2 ml/minute through Discovery C-8 column with gradient elution. The mobile phase initial condition is 40% methanol and 60% water with 10 microMolar sodium acetate and 10 microMolar potassium acetate. The gradient has variable slopes, but ultimately results in 95% methanol at 12 minutes. This is held and then returned to 40% methanol to re-equilibrate the column for 8 minutes before the next injection occurs.

Solvents must be free of organic contaminants. Trace analysis grade methanol is obtained commercially, transferred to a muffle-treated LC solvent bottle and sparged with helium for 20 minutes prior to use. The solvent must be dry primed through the Waters LC.

Sodium acetate is added to Milli-Q filtered water and brought to volume. It is then passed through an activated C-18 extraction disk and transferred to a muffle-treated LC solvent bottle. It is sparged with helium for 20 minutes prior to use. The solvent must be dry primed through the Waters LC. The system must be wet primed and then the column may be installed, but not yet connected to the spectrometer. The solvent may be pumped through the column to equilibrate it while the spectrometer is calibrated and prepared for use. Be sure that the solvent is directed to a waste container.

3.4.2 Mass Spectrometry

Routine service must be performed prior to use.

- Ballast the rough pump for 15 minutes
- Confirm the vacuum reading is less than $5x10^{-6}$
- Check the sample cone for tarnishing, remove and clean if needed
- Check the nitrogen supply

Tune the system and complete Mass Calibration as described in the Instrument Control Procedure for LC-MS TOF. Determine the need for Lock-Mass and connect plumbing if it is required. Then program the MS acquisition to the appropriate mass range and be sure to indicate the current calibration file.

Program the tune file with the same settings used to generate the Mass Calibration. Then modify the settings for the electrospray source to those indicated below. Different settings may be used, as long as the same settings that are in place to analyze the quantitative calibration standards are also used to analyze the samples.

To acquire for pesticides as [M+Na]+

• ion mode	ES+	• desolvation temp	300 C
• capillary voltage	2600 V	• source temp	150 C
• sample cone	20 V	• desolvation gas	500 L/h
• extraction cone	7 V	• cone gas	0 L/h

3.4.3 Quantitative Calibration Curve

There is a five point calibration curve utilized. The analytes are quantified by calculating a response factor from the internal standard. The compounds are quantified within the linear range of the calibration curve, typically observed to be from 10 pg to 200 pg of each analyte.

Secondary calibration curve may also be generated using the C13 isotope mass for each analyte. This is useful, particularly in the case of piperonyl butoxide, when the response exceeds the linear range of the calibration curve. When standards are evaluated beyond the curve, the isotope mass has demonstrated a response which provides an "extended" linear range that is used to determine analyte concentrations.

3.4.4 Quality Control Requirements

Each day the accuracy of the calibration curve shall be evaluated. A standard shall be used that contains analytes at a concentration near the mid-point of the calibration curve. This sample shall be analyzed and processed as an unknown sample. The results obtained for the internal standard and analytes are to be examined. If the results are within 20% then the calibration is concluded to remain valid and analysis of samples may be carried out with confidence in the accuracy of the results. If the results are not acceptable then service the LC-MS

system accordingly and re-analyze the calibration check sample or proceed to analyze the calibration standards.

A blank sample must be evaluated in order to demonstrate that the chromatography system is free of contaminants.

3.4.5 Analysis of Samples

Chromatography parameters used for sample analysis shall be the same as those used to evaluate the quantitative calibration standards. The mass spectrometer parameters will have different settings at the source for mass calibration, but the same settings must be used that are in place when quantitative standards are analyzed.

The response measured for the internal standard and each compound shall be evaluated; the retention time must be within 0.5 minutes and peak shape must be Gaussian. The analyte results will be determined by the software. Results must be within limits of calibration curve.

4 Quality Control – Quality Assurance

4.1. Sample Collection and Handling

Amber bottles shall be used for collecting environmental samples.

Hexane is added to each sample to preserve the analytes.

Each sample is labeled as to location and date of collection.

Samples are stored near 4ºC, either in a cooler with ice or ice packs and in the lab using refrigeration.

4.2 Extraction

Each batch of sample extracts includes a blank to demonstrate that there was neither carryover nor contamination in the laboratory.

Positive control samples will be analyzed to determine the efficiency and reproducibility of the extraction.

4.3 Analysis

The accuracy of the spectrometer is verified prior to analysis of each batch. The mass calibration is verified and adjusted, if necessary, using polyalanine.

The chromatography system is evaluated by analysis of a mid-point from the calibration curve. If variation is observed the instrument is serviced and a new calibration is analyzed.

The extraction controls are analyzed with the batch to determine their accuracy.

D5 malathion is added to each extract as an Internal Standard to monitor the instrument performance and to normalize data for determination of concentration.

Samples with high analyte responses that exceed the upper limit of the calibration curve are diluted and reanalyzed. The sample following the high response is evaluated for possible carryover and reinjected if the same analyte is present.

References

Zimmerman, LR; AP Strahan, and EM Thurman. 2001. *Methods 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 Using Liquid-Liquid Extraction and Gas Chromatography/Mass Spectrometry*. U.S. Geological Survey Open File Report 01-273, Lawrence, KS. 11p.

Appendix B

STANDARD OPERATING PROCEDURE FOR ANALYSIS OF PESTICIDES IN SEDIMENT

1 Purpose

To investigate the aquatic fate of pesticides used against mosquitoes and the West Nile virus. Current sampling objectives include identifying how much of the pesticides are present in the environment following spray events and determining the quantity of pesticide ending up in the sediment, as well as its residence time in the sediment.

This method is for extraction from sediment samples. This method will also be expanded to include extraction from tissue samples.

2 Summary

Sediments and organisms are to be collected in solvent washed sample jars. They will be transported to the Marine Sciences Research Center at Stony Brook University (MSRC). Samples will be extracted and prepared for instrumental analysis. The extracts will be analyzed by GC-MS. Data will be evaluated to confirm presence of internal standards and identify samples containing pesticides; then the results will be used to calculate pesticide concentrations.

The methods used are modified from existing methods in order to achieve very low detection limits from a two gram sample that has been freeze dried. Chemical analysis will be carried out using a research grade mass spectrometer for greater sensitivity. The mass spectrometer is capable of MS-MS analysis, further fragmentation using a collision cell for formation of daughter ions that is used for confirmation of an analyte when matrix interferences raise any degree of uncertainty.

3 Procedure

3.1 Sample Collection

Samples will be collected into two or four ounce jars. The location and time of sampling shall be recorded. They will be kept chilled in an ice packed cooler until transfer to freezer storage at the MSRC.

3.2 Sediment Sample Preparation and Extraction

3.2.1 A record will be kept to indicate details of the extraction

3.2.2 A portion of the collected sample is transferred to a screw cap vial, using yellow light

3.2.3 The vial is frozen and freeze dried until moisture is removed.

3.2.4 Each group of samples being extracted will be referred to as a batch. Each batch must include blank sample for quality control. Sediment may also be spiked with pesticide to produce positive controls as well; this sample is referred to as a Matrix Spike (MS).

3.2.5 Transfer a portion of the dried sample to a solvent washed Teflon centrifuge tube. Record the mass of the sample.

3.2.6 Add 50 µl, corresponding to 50 ng of surrogate mixture to each sample, blank, and all control samples.

3.2.7 Add pesticide spiking solution to the Laboratory Control Spike (LCS) and the Matrix Spike (MS).

3.2.8 Prepare 50/50 (methylene chloride: acetone) mixture for extraction, 250 ml is enough to extract a batch of 12.

3.2.9 Use 5.0 ml portions of extraction mixture to each sediment.

3.2.10 Sonicate for 5 minutes using sonication probe with pulse.

3.2.11 Pour off solvent through funnel packed with sodium sulfate and collect in a vial.

3.2.12 Add a second 5 ml portion of extraction mixture, sonicate and pour solvent through same funnel and collect with previous portion.

3.2.13 Repeat with a third 5 ml portion of extraction mixture, sonicate and pour solvent through same funnel and collect with previous portion.

3.2.14 Place vial under stream of nitrogen and submerge vial in water bath heated to 35ºC.

3.2.15 Take to dryness. Remove quickly, do not allow to sit in water bath under nitrogen more than is necessary, or recovery of pesticides will be compromised.

3.2.16 Add 2.0 ml hexane to each vial and mix with vortex mixer.

3.2.17 May store samples overnight at this point using a glass stopper to seal the vial. Place in refrigeration.

3.3 Sediment Sample Clean-Up

3.3.1 Florisil SPE cartridge from Supelco is utilized. A different cartridge is used for each sample, blank, and control sample

3.3.2 Prepare a mixture of ethyl acetate and water. An 80 ml portion of ethyl acetate may be wetted with 1.2 ml milli-Q water. Check for complete mixing, may use sonication bath.

3.3.3 Pass 4.0 ml of ethyl acetate/water mixture through each SPE. Do not allow SPE to go to dryness from this point forward, until the sample is eluted.

3.3.4 Pass 5.0 ml hexane through each SPE.

3.3.5 Pass 2.0 ml portion of each sample extract through the SPE.

3.3.6 Add a second 2.0 ml portion of hexane to each sample, vortex, and then pass this through the SPE as well.

3.3.7 Repeat with a third 2.0 ml portion of hexane.

3.3.8 Prepare eluting solvent using 35 ml hexane, 15 ml ethyl ether, plus 2.5 ml methanol. Mix using sonication bath.

3.3.9 Place a small vial under each SPE to collect the sample.

3.3.10 Elute every sample, blank and control using a 5.0 ml portion of the eluting solvent.

3.3.11 Apply vacuum and take SPE to dryness.

3.3.12 Remove the sample vials and place under a stream of nitrogen in a water bath heated to 35ºC.

3.3.13 Take to dryness. Remove quickly, do not allow to sit in water bath under nitrogen more than is necessary, or recovery of pesticides will be compromised.

3.3.14 Add 200 µl hexane to each and mix using the vortex mixer

3.3.15 Transfer 100 µl to a microvolume insert in a 2 ml screw cap vial.

3.3.16 Add internal standard so the final concentration is 50 ng/ml.

3.3.17 Store below 0ºC until analysis.

3.4 Sample Analysis

The details regarding operation of the analytical system are specified in the Instrument Control Procedure for GC-MS Quattro. This section will present the specific instrumental settings which apply to the mosquito pesticides. Included are discussion of the chromatography conditions and the recommended settings for the electron impact source. Other details such as preparation of quantitative standards and interpretation of results are not discussed.

Using these settings will elute the analytes in 25 minutes and the system will bake at a higher temperature for 5 minutes. The mass spectrometer is operated in SIM mode, focusing only on ion fragments of interest. Several discrete acquisition windows are utilized to maximize sensitivity for each analyte.

3.4.1 Gas Chromatography

. The system is fitted with a 5% diphenyl low-bleed capillary column. The currently installed column was supplied by Restek, model #RTX-5MS; length = 30 meters, inner diameter = 0.25 mm, and film thickness = 0.25 micron.

The head pressure is set to 18 psi. There is no system for electronic pressure control.

Initial temperatures include: inlet = 280ºC, column = 70ºC, transfer line to MS = 280ºC. The oven ramp is programmed to heat at 15ºC/ min to 190ºC, followed by a more gradual increase of 5ºC/min to 290ºC then hold at 290ºC for 5 minutes.

Upon injection the split vent is closed, but it opens after 0.5 minute to purge the inlet.

3.4.2 Mass Spectrometry

Routine service must be performed prior to use.

- Check supply of helium carrier gas
- Confirm the system is under proper vacuum conditions

Typical readings:	source	= 1 x 10^{-5} mbar
	analyzer	< 2 x 10^{-6} mbar
	inlet	= 3 x 10^{-3} mbar
	gas cell	= 2 x 10^{-4} mbar

- Inject and evaluate mass spectra from DFTPP for key ions and abundances

To acquire for pesticides as fragments

• ion mode	EI+	• source temp	200ºC
• emission current	80 µA	• aperture	open
• electron energy	70 V	• photo multiplier 1	550 V
• repeller	12 V	• photo multiplier 2	500 V

Tune the system and complete Mass Calibration as described in the Instrument Control Procedure for GC-MS Quattro.

Program the tune file with the same settings used to generate the mass calibration. Then modify the settings for the electron impact source to those indicated below. Different settings may be used, as long as the same settings that are in place to analyze the quantitative calibration standards are also used to analyze the samples.

For improved sensitivity the Mass Spectrometer is operated in Single Ion Recording (SIR) mode, collecting only those ions of interest. When using the SIR mode the instrument has been found to provide the best peak shape with a dwell time = 0.2 seconds with no more than four different ions for each SIR window.

3.4.3 Quantitative Curve Calibration

There is a five point calibration curve utilized. The analytes are quantified by calculating a response factor from the internal standard. The compounds are quantified within the linear range of the calibration curve, typically observed to be from 25 pg to 500 pg of each analyte.

3.4.4 Quality Control Requirements

Each day the accuracy of the calibration curve shall be evaluated. A standard shall be used that contains analytes at a concentration near the mid-point of the calibration curve. This sample shall be analyzed and processed as an unknown sample. The results obtained for the internal standard and analytes are to be examined. If the results are within 20% then the calibration is concluded to remain valid and analysis of samples may be carried out with confidence in the accuracy of the results. If the results are not acceptable then service the GC-MS system accordingly and re-analyze the calibration check sample or proceed to analyze the calibration standards.

A blank sample must be evaluated in order to demonstrate that the chromatography system is free of contaminants.

3.4.5 Analysis of Samples

Chromatography parameters used for sample analysis shall be the same as those used to evaluate the quantitative calibration standards. The mass spectrometer parameters will have different settings at the source for mass calibration, but the same settings must be used that are in place when quantitative standards are analyzed.

The response measured for the internal standard and each compound shall be evaluated; the retention time must be within 0.5 minutes and peak shape must be Gaussian. The analyte results will be determined by the software. Results must be within limits of calibration curve.

4 Quality Control-Quality Assurance

4.1 Sample Collection and Handling

Glass jars shall be used for collecting samples. They shall be rinsed with acetone and hexane prior to use.

Each sample is labeled as to location and date of collection.

Samples are stored below 0C, either in a cooler with ice or ice packs and in the lab using a freezer.

4.2 Extraction

Each batch of sample extracts includes a blank to demonstrate that there was neither carryover nor contamination in the laboratory.

Positive control samples will be analyzed to determine the efficiency and reproducibility of the extraction.

D6 malathion is added to each sample prior to extraction as a surrogate compound and utilized to determine the efficiency of the extraction.

D10 phenanthrene is added to each extract after completion of extraction and is utilized to normalize the data for the determination of analyte concentration

4.3 Analysis

The accuracy of the spectrometer is verified prior to analysis of each batch. The mass calibration is verified and adjusted, if necessary, using PFTBA.

The chromatography system is evaluated by analysis of a mid-point from the calibration curve. If variation is observed the instrument is serviced and a new calibration is analyzed.

The extraction controls are analyzed with the batch to determine their accuracy.

D10 phenanthrene is added to each extract as an Internal Standard to monitor the instrument performance.

Samples with high analyte responses that exceed the upper limit of the calibration curve are diluted and reanalyzed. The sample following the high response is evaluated for possible carryover and reinjected if the same analyte is present.

References

You, J., DP Weston, and MJ Lydy. 2004. A sonication extraction method for the analysis of pyrethroid, organophosphate, and organochlorine pesticides from sediment by gas chromatography with electron-capture detection. *Archives of Environmental Contamination and Toxicology* 47(2):141-147.

Zimmerman, LR; AP Strahan, and EM Thurman. 2001. *Methods 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 Using Liquid-Liquid Extraction and Gas Chromatography/Mass Spectrometry*. U.S. Geological Survey Open File Report 01-273, Lawrence, KS. 11p.