Investigating the Presence of Pesticides in American Lobster from Long Island Sound

The Connecticut Agricultural Experiment Station

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Overview of the 2014 Long Island Sound Lobster Pesticide Study

A large and well documented lobster die-off occurred in Long Island Sound during late 1999. Since then, mortality events continue to be an annual fall occurrence in the Sound. Many lobstermen, concerned citizens and elected officials have expressed concern that pesticides might be somehow related to these lobster die-off events.

In September 2011, following reports of dead and dying lobsters in the western basin, 13 weak and lethargic lobsters were collected and held on ice overnight prior to necropsy. The next day, all 13 lobsters were lively and submitted to the CT Veterinary Medical Diagnostic Laboratory. Pathology results showed that all of those lobsters had a variety of local tissue abnormalities (lesions), which were indicative of normal active immune system responses to infection or disease. Such responses would not have been expected if their immune systems had been compromised by pesticides.

House Bill 5260 was introduced by the Connecticut General Assembly in 2012, intending to prevent the application of malathion or methoprene into any waterway, catch basin or storm drain. Following this and using legislative funds made available through the Lobster Restoration account (PA 05-281), the Connecticut Department of Energy and Environmental Protection developed a laboratory assessment and monitoring strategy in 2012 to document the health of lobster from Long Island Sound. This involved seasonal collections of lobster from the three basins of Long Island Sound and testing for the presence of bifenthrin, cyhalothrin, permethrin, and resmethrin, and methoprene. A total of 91 lobsters were collected between July and December of 2012.

Analyses of those lobster tissues by the UCONN Center for Environmental Science and Engineering (UCONN CESE), indicated there may have been pesticides present. In laboratory experiments, impaired immune system function has been documented as a sub-lethal effect of pesticide exposure. However, pathology results for those 91 animals collected in 2012 were not indicative of exposure to pesticides, as they exhibited normal active immune system responses to infection or disease.

Ultimately, the analytical results for those animals were considered inconclusive when a second laboratory did not detect the presence of pesticides and sample quality came into question. These findings warranted the funding of a second, more comprehensive study to both determine the levels at which these pesticides could be confidently detected using advanced techniques and to test additional lobsters from Long Island Sound for the presence of pesticides. As with the first study, the pesticides of concern were bifenthrin, cyhalothrin, permethrin, and resmethrin (pyrethroid pesticides), and methoprene (insect growth regulator). Before this study was completed, the Connecticut General Assembly passed House Bill 6441 in 2013. Public Act 13-197 restricted the use of resmethrin and methoprene in the state.

A steering committee was formed to design and guide this study. Their job was to identify the best scientific methods necessary to achieve reliable, consistent and accurate results for testing the presence of the five aforementioned pesticides in lobster tissue. The steering committee was comprised of analytical chemists from: the Federal Environmental Protection Agency (EPA), pesticide industry, UCONN, and Connecticut Agricultural Experiment Station (CAES). Additionally, the committee included a member of an environmental advocacy group with a focus on LIS issues and staff from the Marine Fisheries and Pesticide Divisions of the CT DEEP.

Prior to testing lobster samples collected from Long Island Sound, an extensive method validation study was completed where clean lobster tissues were fortified with measurable levels of each pesticide (using standards provided by the pesticide manufacturers). These samples were then analyzed by both laboratories contracted to do the work using conventional, state of the art Gas Chromatography Tandem Mass Spectrometry (GC/MS/MS) procedures. The results of the fortification study from each laboratory were demonstrated to be within acceptable accuracy established for this project. The percent recoveries for each pesticide for both laboratories were within the industry standard for environmental work. The acceptance criteria, consistent with that used in standard EPA methods in the method validation phase, were employed during testing of the wild samples from Long Island Sound.

Comprehensive quality assurance / quality control steps were applied during actual sample analysis. These steps included analyzing blank samples (preparation and calibration), sample duplicates and triplicates, matrix spikes and matrix spike duplicates, and laboratory controls. These processes were taken to ensure that none of the target chemicals inadvertently contaminated the samples during preparation and analysis, as well as to make sure the method was performing as expected. Both laboratories used a "standard addition" technique as part of their comprehensive quality assurance protocol. Standard addition is a powerful method used to rule out false positives. Both laboratories used multiple ions to identify compounds. The use of multiple ions increases ability to confirm the presence or absence of the compound. The laboratories used different sample preparation and clean-up methods for the GC/MS/MS analysis but found similar results for each sample. The results of quality control samples for each laboratory were within acceptable parameters. Though CAES did find contamination in one of their sample blanks they were able to identify the source of contaminant and eliminate it.

In October 2014, a total of forty five lobsters were collected (15 from each of the three basins of LIS) during an ongoing lobster mortality event. To ensure there would be no sample quality issues, and at the recommendation of the steering committee, tissues were stored at -80oC until the time they were processed. Each of the 45 LIS lobsters had hepatopancreas (tomalley) and claw/tail muscle evaluated for the presence of methoprene, resmethrin, bifenthrin, cyhalothrin and permethrin using methods previously identified by the steering committee. These analytical tests employed GC/MS/MS techniques capable of detecting the subject compounds in trace quantities. Detection limits ranged from 6 parts per billion (ppb) to 20 ppb, depending on the pesticide.

No detectable levels of any of the five pesticides at or above their established detection limits were found by either laboratory in any of the hepatopancreas or claw/tail muscle samples. Based on the quality control results of this study, these data are considered to be of high quality.

Final reports, detailing the methodology and results, from both the CAES (Appendix 1) and UCONN CESE (Appendix 2) can be found in the appendices that follow.

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Founded 1875 *Putting science to work for society*

Examination of Long Island Sound Lobsters for Pesticide Residues
February 3, 2016 Final Report
The Connecticut Agricultural Experiment Station (CAES)

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I. Background and History

In 2013, a CT Department of Energy and Environmental Protection (DEEP) funded University of CT Center for Environmental Sciences and Engineering (UConn) study (results of which were presented to the legislature) reported the presence of the pyrethroid insecticides resmethrin, bifenthrin, cyhalothrin and permethrin and the juvenile hormone analog methoprene in wild lobster harvested from Long Island sound. Archived tissue samples and sample extracts were sent to CAES for confirmatory analysis. The CAES could not confirm the presence of any residues in the samples or the extracts.

In 2014, DEEP assembled a Lobster Pesticide Steering Committee consisting of members from UConn, CAES, experts from EPA and industry. The goals of the committee were 1) to validate (at CAES and UConn) a method for the analysis of 5 analytes in lobster meat and hepatopancreas, 2) to harvest lobsters from Long Island Sound, 3) to provide split samples to each laboratory for analysis, 4) to have each laboratory provide results back to DEEP.

In early 2016, CAES completed the validation study and the analysis of harvested lobster samples. This document contains a technical summary of that work.

II. Multi-Laboratory Validation

A. Sample Extraction and Clean-up

1) CAES used the Quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction method. Briefly, 5 g of lobster tissue was combined with 10 g of water, 6 g of magnesium sulfate and 1.5 g of sodium acetate. The mixture was shaken and partitioned with 15 g of acetonitrile and centrifuged.
2) The acetonitrile was decanted and added to a tube containing 1.5 g magnesium sulfate, 0.5 g silica bonded primary-secondary amine (PSA) clean-up sorbent and 2g toluene. The mixture was shaken, centrifuged and concentrated. The concentrate was re-constituted to 1 g with toluene and analyzed directly.

B. Sample Analysis

CAES analyzed for the 5 analytes utilizing tandem gas chromatography (GC) with triple

quadrapole (QQQ) mass spectrometry detection. Specifically, the instrument was a Thermo Scientific Trace GC Ultra connected in tandem to a TSQ Quantum XLS Ultra triple quadrapole mass spectrometer. To perform the analysis with the desired analyte specificity both CAES and UConn: 1) used identical GC oven conditions, 2) monitored 2 separate MS-MS transitions, 3) monitored the ion ratios between these transitions, 4) monitored the retention times for each analyte. This information is summarized below

GC Conditions:

- Constant flow helium carrier gas 2.0 ml/min with vacuum compensation.
- PTV splitless injection 280 ^{o}C with 2mm x 2.75 x 120 Siltek deactivated baffle liner treated with Surfacil.
- MS transfer line 300 ^{o}C.
- Oven Initial 100 ^{o}C, hold 1 min, ramp 15 ^{o}C/min to 280 ^{o}C, hold 17 min. Total run time 30 min.

MS Conditions:

- MS source heater 300 ^{o}C.
- Emission current 60 µA – 0 min OFF – 8 min ON.
- Collision Gas Pressure (mTorr): 1.5.
- Cycle Time (s): 0.300.
- Chrom Filter Peak Width (s): 5.0.
- Q1 Peak Width (FWHM): 0.70.
- Q3 Peak Width (FWHM): 0.70.

Table 1: GC-QQQ Transitions Monitored

Compound	Parent	Product	SRM Collision Energy	Ion Ratio (%) n = 7	Retention Time(s) (min)
Methoprene	153.00	111.00	5	6.68	13.27
	235.19	147.12	10		
Resmethrin	171.00	128.00	12	29.04	15.73
	171.10	143.10	9		
Bifenthrin	181.20	166.10	15	48.53	16.31
	165.00	139.00	25		
Cyhalothrin	181.04	152.03	23	67.84	17.61
	197.10	141.10	10		
Permethrin	183.04	153.03	15	87.93	19.07, 19.29
	183.04	168.03	15		
TPP (method ISTD)	326.07	325.07	10	109.10	15.86
	325.07	169.04	25		
PCB28 (instr. ISTD)	255.96	186.02	20	63.58	12.17
	257.96	186.02	20		
d6-Cyhalothrin (IS)	455.00	203.00	25	62.33	17.08, 17.28, 17.56, 17.81
	203.00	146.00	15		
d6-Permethrin (IS)	169.00	96.00	12	84.26	19.07, 19.29

	169.00	133.00	15		
d6-Esfenvalerate (Surrogate)	425.2	173	15	8.89	23.69, 24.36
	173.00	96.00	25		
D6-Fenpropathrin (Surrogate)	131.00	103.00	15	9.56	16.5
	355.2	131.00	15		

C. Standards

Analytical standards used in this work were obtained as neat stocks from the EPA Pesticide Repository. The d6-internal standards and surrogates were obtained from members of industry represented on the Lobster Pesticide Steering Committee. Method development work at CAES during 2014 indicated a significant matrix effect which dictated the use of matrix matched standards in the analysis. For matrix matching, clean (unspiked) meat and hepatopancreas samples were processed through the QuEChERS extraction. The combined final extract was used to dilute the calibration standards. Stock standards of analytes were prepared in toluene. Individual calibration standards for the points on the calibration curve corresponding to 25, 50, 100, 250, 500, 750, 1000 and 2500 ng-g were made using the stocks solutions which were diluted to final volume(s) using the above extract.

D. Spiked Samples

1) Background

In October of 2015, the CAES received spiked lobster samples from UConn. There were 21 meat and 21 hepatopancreas samples, each in 15 ml tubes. There was one blank sample of each meat and hepatopancreas; there were 5 replicate spikes at each of four levels corresponding to 50, 150, 500 and 1000 ng-g in tissue. To perform the QuEChERS extraction at CAES, the material needed to be weighed and spiked in 50 mL tubes such that solvent, water and reagents could be added directly to the samples. Attempts to quantitatively transfer material from one tube to another were not successful. Oily residues were often left behind. It was impossible to ensure that all matrix and spiked analytes were quantitatively transferred. Further, the transfer would lead to greater overall method uncertainty. The CAES was fortunate to have frozen excess supplies of both meat and hepatopancreas remaining from previous method development work. The meat was utilized in the spiking study; however, the quality of the hepatopancreas had degraded to a point that it was unusable. Fresh hepatopancreas was obtained from DEEP.

2) Analysis

Approximately 5 g of lobster meat was weighed into twenty-one 50 mL tubes. This was repeated for the hepatopancreas. Five meat samples were spiked with the analytes at each of four spiking levels corresponding to 50, 150, 500 and 1000 ng-g in tissue. The remaining meat sample was used as a blank. This was repeated for the hepatopancreas samples. All lobster meat samples were processed through the QuEChERS extraction as a single batch, as were the hepatopancreas samples. Samples were extracted and analyzed as described above.

E. Quality Control

In addition to spiking analytes into the two matrices, d6-esfenvalerate and d6-fenpropathrin were added to each sample at 500 ng-g. Two internal standards, d6-cyhlaothrin and d6-permethrin, were added to each sample by the GC autosampler. In addition to the quality elements used in this study, the CAES added triphenylphosphate method internal standard in the acetonitrile extraction solution at the beginning of the method, and PCB28 instrument internal standard directly prior to injection by the GC autosampler. The following pre-defined and agreed upon quality control parameters were employed:

- Matrix spike @ 500 ng-g in tissue — recovery 50-130%
- Matrix spike duplicate — 20% relative percent difference
- Preparation blank — below 50 ng-g
- Laboratory control sample — recovery 50-130%
- Triplicate sample — 20% relative percent difference
- Calibration blanks — below 50 ng-g
- Calibration Verification — recovery 85-115%
- Surrogate standards — Recovery 50-130%

F. Reporting and Detection Limits

This study has been designed such that the lowest spiking level (50 ng-g) is the reporting limit. The GC-QQQ instrument used in this study are capable of determining values of the analytes below this reporting level. Approximate CAES detection limits were:

- Methoprene 20 ng-g
- Resmethrin 40 ng-g
- Bifenthrin 10 ng-g
- Permethrin 25 ng-g
- Cyhalothrin 30 ng-g

G. Results of Validation

Accuracy (as percent recovery) and linearity (correlation coefficient, r) data from the spiking experiments are presented in tables 2 (meat) and 3 (hepatopancreas) below. The data is based upon the Triphenylphosphate method internal standard added at the beginning of the method. Regulatory pesticide residue work conducted at the CAES has shown this use of internal standard to be highly robust and accurate. The recovery data obtained were acceptable, with the exception of the data for bifenthrin. Trace bifenthrin contamination was found in an internal standard added at the autosampler throughout validation work. This contamination was eliminated during the analysis of the wild-caught lobster samples below. The average percent recoveries ranged from 78.0 to 124.7%, with bifenthrin at 148% owing to contamination. The relative standard deviation (RSD) in all the groups (with the exception of bifenthrin) were 20% or less. Overall method uncertainty ranged from 6.4 to 41.0 percent (bifenthrin was at 53.8% in hepatopancreas and 66.3% in meat). All calibration curves were

found to be linear with correlation coefficients of at least 0.99. No background contamination was found in any of the blanks.

Table 2: Lobster Meat Spike-Recovery Summary – Triphenylphosphate ISTD

Spiked Conc. (ng-g) - ppb	Matrix	Methoprene % Recovery	Resmethrin % Recovery	Bifenthrin % Recovery	Cyhalothrin % Recovery	Permethrin % Recovery
1000	Muscle	132.8	102.2	118.8	101.3	101.7
500		132.3	88.5	142.7	92.7	94.3
150		120.6	72.4	130.4	83.6	82.7
50		113.3	48.9	198.1	82.2	77.4
Avg. (n=20)		124.7	78.0	147.5	89.9	89.0
RSD (n=20)		10.5	20.0	33.1	9.5	10.5
Expanded Uncertainty (%) (k=2)		21.0	41.0	66.3	18.9	21.0
Correlation Coefficient, (r)		0.9983	0.9917	0.9902	0.9930	0.9954

Table 3: Lobster Hepatopancreas Spike-Recovery Summary – Triphenylphosphate ISTD

Spiked Conc. (ng-g) - ppb	Matrix	Methoprene % Recovery	Resmethrin % Recovery	Bifenthrin % Recovery	Cyhalothrin % Recovery	Permethrin % Recovery
1000	Hepato	92.7	85.8	103.4	102.7	75.9
500		95.1	84.7	92.4	108.0	76.7
150		94.2	83.2	80.1	140.0	82.7
50		85.2	79.7	38.7	107.7	90.0
Avg. (n=20)		91.8	83.3	78.6	105.6	81.3
RSD (n=20)		7.2	3.2	26.9	4.4	9.4
Expanded Uncertainty (%) (k=2)		14.3	6.4	53.8	8.9	18.9
Correlation Coefficient, (r)		0.9942	0.9992	0.9901	0.9925	0.9951

Surrogate recoveries were acceptable throughout the study. Average recoveries of d6-fenpropathrin were 96.9% (n=20) in meat and 92.7% (n=20) in hepatopancreas. Average recoveries of d6-esfenvalerate were 96.8% (n=20) in meat and 95.8% (n=20) in hepatopancreas.

H. Conclusion

The results of the work summarized above were presented to the Lobster Pesticide Steering Committee in early January 2016. The committee members reviewed the validation

work, as well as that performed by UConn. It was concluded that the two laboratories had appropriately demonstrated that the five analytes could be quantitated in both meat and hepatopancreas. The committee gave their approval to test the wild caught lobsters from Long Island Sound.

III. Wild Caught Lobster Analysis

A. Background

Forty-five (45) lobsters were harvested from Long Island Sound by DEEP in the Fall-Winter of 2014. Meat and hepatopancreas were harvested from each individual resulting in a total of 90 samples for analysis. Meat from each lobster sample was divided into two portions. One portion destined for analysis by the CAES and one for UConn. The same was done with the hepatopancreas samples. Within these samples there were two lobster samples for which triplicates were provided to each laboratory for quality assurance. The samples were all frozen and maintained at -80 ^{o}C until analysis. Prior to lobster harvest, the Lobster Pesticide Steering Committee agreed that all samples were to be stored at -80 ^{o}C until analysis. This would stop all enzymatic activity and preserve any potential pesticide residues in the samples. The 90 tissue samples were extracted using QuEChERS (as described above) in 4 separate batches in January of 2016. A triplicate sample was run with each batch, as were all other the pre-defined QC.

B. Results

None of the analytes were found in any of the 90 wild caught lobster samples. A breakdown by sample and batch of the results for each sample is provided in Appendix I. The following quality control elements were tested as part of the four analytical batches run. Appendix II contains two tables which provide average values for the following parameters over 1) the combined meat batches, and 2) the combined hepatopancreas batches:

Recovery QC Elements:

- Matrix spike @ 500 ng-g in tissue — recovery 50-130%
- Matrix spike duplicate — 20% relative percent difference
- Laboratory control sample — recovery 50-130%

Calibration QC Elements

- Calibration Verification (CCV) — recovery 85-115%
- Independent Calibration Verification (ICV) — recovery 50-130%
- Preparation blank — below 50 ng-g
- Calibration blanks — below 50 ng-g

The average quality control over the course of the analysis was acceptable. The one exception was the average percent recovery of methoprene in the laboratory control samples of 134%, which was slightly above the pre-defined criteria maximum of 130.0%. In the wild caught lobster sample triplicates, there is a QC element that addresses the relative percent deviation between these samples (less than 20% relative percent difference). However, this QC

element was not used because none of the samples contained any of the analytes. Alternatively, it could be stated that the relative percent difference in all cases was zero (0).

The surrogates d6-esfenvalerate and d6-fenproathrin were added to all samples; recovery was to be 50-130%. Recoveries of d6-esfenvalerate ranged from 84 – 123% (Avg. 96.1%) in meat and 63 – 114% (Avg. 88.2%) in hepatopancreas. Recoveries of d6-fenpropathrin ranged from 74 – 109% (Avg. 91.1%) in meat and 68 – 102% (Avg. 83.2%) in hepatopancreas.

C. Conclusions

The method validation work summarized above shows that the CAES extraction and analysis protocol is fit for purpose for the current study and for future similar work. Analysis of the 90 tissue samples from the 45 wild caught lobsters showed that none of the analytes of interest (pyrethroids, methoprene) were detected in any of the analyzed tissues.

Appendix I

Results by Sample and Batch for Long Island Sound Wild Caught Lobster Meat and Hepatopancreas Samples Harvested in 2014

Results by Sample and Batch for Wild Caught Lobster Meat Analysis (ND = Not detected)

Retention Time (m)	16.2	17.5	13.2	18.9 & 19.1	15.6
Analyte	Bifenthrin (ng-g)	Cyhalothrin (ng-g)	Methoprene (ng-g)	Permethrin (ng-g)	Resmethrin (ng-g)
Lobster Meat Samples Batch 1					
140318-001 M	ND	ND	ND	ND	ND
140318-002 M	ND	ND	ND	ND	ND
140318-003 M	ND	ND	ND	ND	ND
140318-004 M	ND	ND	ND	ND	ND
140318-005 M	ND	ND	ND	ND	ND
140318-006 M	ND	ND	ND	ND	ND
140318-007 MA	ND	ND	ND	ND	ND
140318-007 MB	ND	ND	ND	ND	ND
140318-007 MC	ND	ND	ND	ND	ND
140318-008 M	ND	ND	ND	ND	ND
140318-009 M	ND	ND	ND	ND	ND
140318-010 M	ND	ND	ND	ND	ND
140318-011 M	ND	ND	ND	ND	ND
140318-012 M	ND	ND	ND	ND	ND
140318-013 M	ND	ND	ND	ND	ND
140318-014 M	ND	ND	ND	ND	ND
140318-015 M	ND	ND	ND	ND	ND
140342-001 M	ND	ND	ND	ND	ND
140342-002 M	ND	ND	ND	ND	ND
140342-003 M	ND	ND	ND	ND	ND
140342-004 M	ND	ND	ND	ND	ND
140342-005 M	ND	ND	ND	ND	ND
140342-006 M	ND	ND	ND	ND	ND
140342-007 M	ND	ND	ND	ND	ND
140342-008 M	ND	ND	ND	ND	ND

Lobster Meat Samples Batch 2					
140342-009 M	ND	ND	ND	ND	ND
140342-010 M	ND	ND	ND	ND	ND
140342-011 M	ND	ND	ND	ND	ND
140342-012 M	ND	ND	ND	ND	ND
140342-013 M	ND	ND	ND	ND	ND
140342-014 M	ND	ND	ND	ND	ND
140342-015 M	ND	ND	ND	ND	ND
140315-001 MA	ND	ND	ND	ND	ND
140315-001 MB	ND	ND	ND	ND	ND
140315-001 MC	ND	ND	ND	ND	ND
140315-002 M	ND	ND	ND	ND	ND
140315-003 M	ND	ND	ND	ND	ND
140315-004 M	ND	ND	ND	ND	ND
140315-005 M	ND	ND	ND	ND	ND
140315-006 M	ND	ND	ND	ND	ND
140315-007 M	ND	ND	ND	ND	ND
140315-008 M	ND	ND	ND	ND	ND
140315-009 M	ND	ND	ND	ND	ND
140315-010 M	ND	ND	ND	ND	ND
140315-011 M	ND	ND	ND	ND	ND
140315-012 M	ND	ND	ND	ND	ND
140315-013 M	ND	ND	ND	ND	ND
140315-014 M	ND	ND	ND	ND	ND
140315-015 M	ND	ND	ND	ND	ND

Results by Sample and Batch for Wild Caught Lobster Hepatopancreas Analysis (ND = not detected)

Retention Time (m)	16.2	17.5	13.2	19.3 & 19.6	15.6
Analyte	Bifenthrin (ng-g)	Cyhalothrin (ng-g)	Methoprene (ng-g)	Permethrin (ng-g)	Resmethrin (ng-g)
Lobster Hepatopancreas Samples Batch 1					
140318-001 H	ND	ND	ND	ND	ND
140318-002 H	ND	ND	ND	ND	ND
140318-003 H	ND	ND	ND	ND	ND
140318-004 H	ND	ND	ND	ND	ND
140318-005 H	ND	ND	ND	ND	ND
140318-006 HA	ND	ND	ND	ND	ND
140318-006 HB	ND	ND	ND	ND	ND
140318-006 HC	ND	ND	ND	ND	ND
140318-007 H	ND	ND	ND	ND	ND
140318-008 H	ND	ND	ND	ND	ND
140318-009 H	ND	ND	ND	ND	ND
140318-010 H	ND	ND	ND	ND	ND
140318-011 H	ND	ND	ND	ND	ND
140318-012 H	ND	ND	ND	ND	ND
140318-013 H	ND	ND	ND	ND	ND
140318-014 H	ND	ND	ND	ND	ND
140318-015 H	ND	ND	ND	ND	ND
140315-001 H	ND	ND	ND	ND	ND
140315-002 H	ND	ND	ND	ND	ND
140315-003 H	ND	ND	ND	ND	ND
140315-004 H	ND	ND	ND	ND	ND
140315-005 H	ND	ND	ND	ND	ND
140315-006 H	ND	ND	ND	ND	ND
140315-007 H	ND	ND	ND	ND	ND
140315-008 H	ND	ND	ND	ND	ND
Lobster Hepatopancreas Samples Batch 2					
140342-001 H	ND	ND	ND	ND	ND
140342-002 H	ND	ND	ND	ND	ND
140342-003 H	ND	ND	ND	ND	ND
140342-004 H	ND	ND	ND	ND	ND
140342-005 H	ND	ND	ND	ND	ND
140342-006 HA	ND	ND	ND	ND	ND
140342-006 HB	ND	ND	ND	ND	ND
140342-006 HC	ND	ND	ND	ND	ND

140342-007 H	ND	ND	ND	ND	ND
140342-008 H	ND	ND	ND	ND	ND
140342-009 H	ND	ND	ND	ND	ND
140342-010 H	ND	ND	ND	ND	ND
140342-011 H	ND	ND	ND	ND	ND
140342-012 H	ND	ND	ND	ND	ND
140342-013 H	ND	ND	ND	ND	ND
140342-014 H	ND	ND	ND	ND	ND
140342-015 H	ND	ND	ND	ND	ND
140315-009 H	ND	ND	ND	ND	ND
140315-010 H	ND	ND	ND	ND	ND
140315-011 H	ND	ND	ND	ND	ND
140315-012 H	ND	ND	ND	ND	ND
140315-013 H	ND	ND	ND	ND	ND
140315-014 H	ND	ND	ND	ND	ND
140315-015 H	ND	ND	ND	ND	ND

Appendix II

Averaged
Quality Control Parameters

Recovery QC Elements:

	Matrix Spike Avg. Percent Recovery (50 – 130 %)		Matrix Spike Duplicate Avg. Rel. % Dev. (RPD) (< 20%)		Lab Control Sample Avg. Percent Recovery (50 – 130%)	
	Meat	Hepato.	Meat	Hepato.	Meat	Hepato.
Methoprene	112.1	127.1	5.0	3.1	101.1	**134.0**
Resmethrin	92.3	96.1	4.9	4.2	106.4	88.3
Bifenthrin	93.3	107.8	4.6	3.5	101.8	104.2
Permethrin	106.5	74.6	4.9	2.8	116.5	107.8
Cyhalothrin	58.7	92.6	4.9	3.4	67.9	93.5

Calibration QC Elements

	Calibration Verification CCV (500 ng-g) (85 - 115 %)		Independent Calibration Verification ICV (500 ng-g) (50 – 130 %)		Preparation Blanks & Calibration Blanks	
	Meat	Hepato.	Meat	Hepato.	Meat	Hepato.
Methoprene	96.5	111.9	105.7	106.6	ND	ND
Resmethrin	97.7	93.8	88.7	110.0	ND	ND
Bifenthrin	94.7	100.2	99.4	105.4	ND	ND
Permethrin	98.8	97.5	109.6	105.9	ND	ND
Cyhalothrin	82.3	87.1	76.3	101.1	ND	ND

Appendix 2.

Final Project Report

Investigating the Presence of Pesticides in American Lobster from Long Island Sound

Michael Willig, Christopher Perkins, and Anthony Provatas

3/4/16

Method Summary

Spiked Sample Preparation

The Center for Environmental Sciences and Engineering (CESE) separately homogenized the hepatopancreas and muscle from each of 15 individual lobsters, using clean technique. The composites of each lobster were then transferred into 20 individual aliquots of approximately 0.2 and 5 g for CESE and the Connecticut Agriculture Experiment Station (AES), respectively. Each individual aliquot was spiked with the target analyte mixture and mixed thoroughly. The spiking levels for the SRM were at the equivalent of 50, 150, 500 and 1000 ppb in tissue. Since each sample was spiked individually and sample mass varied, method performance was compared against the actual spiked concentration.

Wild-caught Lobster Preparation

CESE was provided individual muscle and hepatopancreas samples from 45 lobsters collected and submitted by the Connecticut Department of Energy and Environmental Protection (CTDEEP). CESE separately homogenized the hepatopancreas and muscle from each of 15 individual lobsters, using clean technique. The composites for each lobster were then transferred into 20 individual aliquots of approximately 0.2 g.

Extraction

CESE utilized a modified QuEChERS process (dispersive extraction) to prepare tissue for analysis coupled with a solid phase extraction (SPE) clean-up process.

CESE weighed 0.2 g of tissue in an 8 ml vial and 5 ml of extraction solvent (acetonitrile and formic acid) was added. The samples were vortexed and 0.5 g of the QuEChERS powder added. The samples were then centrifuged, and 2 ml of the extract was loaded into the wells of an OSTRO SPE plate. The sample was passed through the plate, followed by a wash of 1 ml acetonitrile. The samples were then evaporated to dryness using a Genevac auto evaporator, 190 ul of acetonitrile was then added, and followed by the addition of 10 ul internal standard.

Analysis

CESE analyzed the extracts using a Waters, Inc. (Milford, MA) gas chromatograph with a tandem mass spectrometer (GC/MS/MS) equipped with a 30m DB-5 column. The instrument operating parameters, including transitions monitored were equivalent between CESE and the AES.

The points on the matrix matched calibration curve were: 25, 50, 100, 250, 500, 1000, and 2500 ng/mL for all pesticides. There were 2 surrogate standards, Esfenvalerate-d6 and

Fenpropathrin-d6, and 2 labeled, internal standards, Cyhalothrin-d6 and Permethrin-d6, used in this analysis.

Quality Control

There were 2 internal standards, Esfenvalerate and Fenpropathrin, and 2 labeled, surrogate standards, Cyhalothrin and Permethrin, used in this analysis. Second source calibration standards from the USEPA, were used for the calibration verification. A set of predetermined quality control assessments were used in the analysis with proposed acceptance criteria, and included:

- Matrix Spike at the equivalent of 500 ppb in tissue: 50-130% recovery
- Matrix Spike Duplicate: 20% relative percent difference
- Preparation Blank: below detection limit
- Laboratory Control Sample: 50-130% recovery
- Triplicate Sample: 20% relative percent difference
- Calibration Blanks: below detection limit
- Calibration Verification: 85-115% recovery
- Surrogate Standards: 50-130% recovery

Detection and Reporting Limits

The reporting limits for this study were based on the lowest point on the calibration curve, at 50 ng/g. Detection limits were determined using standard EPA methods and determined during the method development process:

- Methoprene: 0.020 ug/g
- Resmethrin: 0.060 ug/g
- Bifenthrin: 0.006 ug/g
- Permethrin: 0.080 ug/g
- Cyhalothrin:0.030 ug/g.

All results that were measured below the detection limit are reported as non-detect (ND).

Results

Method Development and Spiking Study

The results of the spiking study were all within acceptable tolerances and with the exception of 3 spiked concentrations; the average of the five composites were all within 15% of the expected concentration (Table 1). For each individual sample, concentrations were within 35% of the expected concentration with highest recovery of 133.8% and the lowest recovery of 71.0% (See Appendix). The preparation utilized by CESE resulted in excellent

chromatograms, with minimal background noise (see Appendix), providing very good analytical separation and quantification.

Table 1. Average percent recovery of the 5 replicates, spiked with the target compounds.

Spiked conc. (ppb)	Matrix	Methoprene % recovery	Resmethrin % recovery	Bifenthrin % recovery	Cyhalothrin % recovery	Permethrin % recovery
1000	Muscle	88.6	96.9	93.5	106.5	89.1
500	Muscle	103.4	98.4	102.4	93.5	75.7
150	Muscle	98.3	90.8	102.1	98.2	96.4
50	Muscle	91.0	91.9	100.7	98.4	99.6
1000	Hepato	98.7	112.4	105.5	81.4	111.1
500	Hepato	98.3	111.5	105.4	90.5	114.1
150	Hepato	109.3	107.3	106.4	99.6	107.5
50	Hepato	125.2	103.5	100.8	100.4	109.1

All of the data quality was within acceptable parameters, and no background contamination was found in any of the lobster blanks. Surrogate recoveries, with an exception detailed below, were within 30 percent, and over 80% were within 15%. We did encounter a challenge with recovery of the surrogate Fenpropathrin-d6 in the hepatopancreas samples due to a matrix issue or impurity that co-eluted, so all recoveries for this surrogate were greater than 150%. There were no co-eluting compounds that interfered with surrogate recovery for Fenpropathrin-d6 in muscle samples.

Wild-caught Lobsters from Long Island Sound

CESE analyzed 45 hepatopancreas and 45 muscle sample composites using the same method and quality assurance criteria developed during the spiking study. All samples concentrations were below the detection limit (Appendix A).

The vast majority of the quality control was within acceptable limits. CESE detected some degradation of the cyhalothrin calibration check standard, but all other quality control was within acceptable parameters. For muscle tissue analysis, surrogate recoveries for 5 muscle samples were outside the acceptance criteria (3 high and 2 low). For permethrin analysis of muscle tissue, the calibration verification standards were lower than acceptable; however, all other QA/QC was within acceptable parameters. The first preparation batch of hepatopancreas saw the matrix spike concentration fall outside of the acceptance criterion, at 148%, however, both the MS and MSD were within acceptable tolerances. Due to the agreement in sample results between CESE and AES, the Lobster Pesticide Steering Committee determined that reanalysis of the samples that fell outside of the quality assurance parameters was not required.

Summary

There were no detectible concentrations of target pesticides in any of the lobster tissues collected from Long Island Sound. The vast majority of the quality assurance and quality control was within acceptable limits, except as noted. The Lobster Steering Committee determined that reanalysis of the samples that fell outside of the quality assurance parameters was not required.

Appendix

Appendix A. Concentration of the target comounds in each of the lobster homogenates.

		Concentration in Muscle Tissue (ug/g wet weight)					Concentration in Hepatopancreas (ug/g wet weight)				
Lab ID	**Field ID**	**Bifenthrin**	**Cyhalothrin**	**Methoprene**	**Permethrin**	**Resmethrin**	**Bifenthrin**	**Cyhalothrin**	**Methoprene**	**Permethrin**	**Resmethrin**
140315-001	N14001	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140315-002	N14002	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140315-003	N14003	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140315-004	N14004	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140315-005	N14005	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140315-006	N14006	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140315-007	N14007	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140315-008	N14008	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140315-009	N14009	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140315-010	N14010	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140315-011	N14011	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140315-012	N14012	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140315-013	N14013	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140315-014	N14014	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140315-015	N14015	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-001	S14001	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-002	S14002	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-003	S14003	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-004	S14004	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-005	S14005	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-006	S14006	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-007	S14007	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-008	S14008	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-009	S14009	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-010	S14010	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-011	S14011	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-012	S14012	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-013	S14013	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-014	S14014	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140318-015	S14015	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-001	B14001	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-002	B14002	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-003	B14003	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-004	B14004	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-005	B14005	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-006	B14006	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-007	B14007	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-008	B14008	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-009	B14009	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-010	B14010	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-011	B14011	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-012	B14012	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-013	B14013	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-014	B14014	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
140342-015	B14015	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Average Detection Limit		**0.006**	**0.030**	**0.020**	**0.080**	**0.060**	**0.006**	**0.030**	**0.020**	**0.080**	**0.060**
Reporting Limit		**0.050**	**0.050**	**0.050**	**0.050**	**0.050**	**0.050**	**0.050**	**0.050**	**0.050**	**0.050**