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Fate of methoprene in temperate salt marsh ditches following aerial applications

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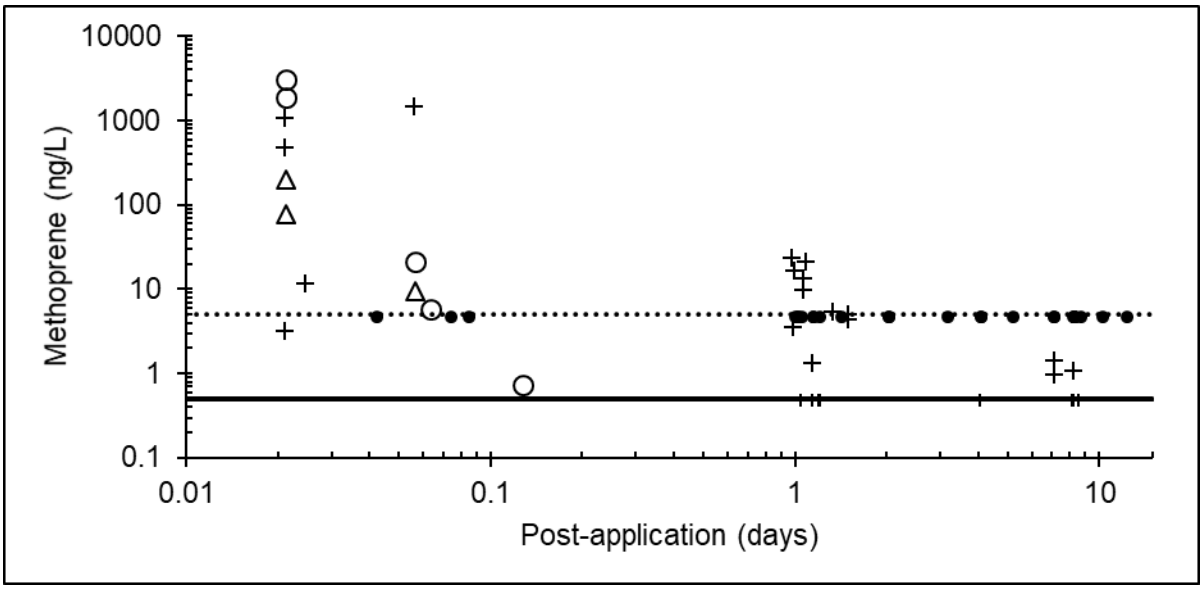
Abstract

Aerial applications of liquid methoprene are used in salt marshes to control mosquitoes by preventing adult emergence. Despite concern about toxicity to non-target organisms, little is known about environmental concentrations after applications, nor methoprene's persistence in salt marsh environments. Aqueous and sediment samples were collected from two marshes receiving weekly applications. Aqueous samples were collected as early as 30 minutes after applications and as long as nine days afterwards; sediment samples were taken within hours of application and as long as 19 days post-application. Use of time-of-flight liquid chromatography – mass spectral analysis allowed for ultra low detection limits (0.5 ng/L) in water samples. The data show loss of nearly all methoprene from 1 m deep marsh ditches within 1 day and presence but not accumulation of methoprene in marsh sediments despite repeated applications. Methoprene concentrations observed in salt-marsh mosquito ditches were below those found to be of toxicological significance in other studies.

Graphical abstract:



Aqueous sampling data from salt marsh ditches on the South Shore of Long Island (+ = Stony Brook University whole water detections; ○= Stony Brook University filtered water detections; Δ = USGS filtered water detections; dotted line = USGS ND, with points indicating ND results; solid line = Stony Brook University ND, with slashes indicating ND results)

Highlights:

- Methoprene is detectable (at low ng/L) in salt marsh ditches 8 days after applications
- Concentrations decrease by two orders of magnitude over two tide cycles
- Methoprene is detectable but does not accumulate in sediments
- Aqueous concentrations were below toxicological significance

Key words: mosquito control, pesticides, persistence, aquatic environments, sediments, estuarine

Abbreviations

Bti: *Bacillus thuringienesis* var. *israelensis*

DDT: dichlorodipheyltrichloroethane

DE: Delaware

DL: detection limit

DP: Davis Point

FP: Flax Pond

GC: gas chromatograph

HP: Havens Point

JN: Johns Neck

LC-TOF-MS: liquid chromatography time-of-flight mass spectrometry

LD_{50}: 50% lethal dose (dose at which 50% mortality occurs)

KS: Kansas

MO: Missouri

MS: mass spectrometry

NWIS Web: National Water Information System (Web version)

NY: New York

PC: Pattersquash Creek

SBU: Stony Brook University

TP: Timber Point

USGS: United States Geological Survey

WNV: West Nile virus

1.0 Introduction

Salt marsh mosquitoes, especially the New Jersey salt marsh mosquito (*Aedes sollicitans*), are said to have inhibited development along the mid-Atlantic coast of the United States until control measures were applied (Smith 1904; Richards 1938). These mosquitoes have multiple broods throughout summer, triggered by the recession of higher lunar tides in the high marsh. Eggs must dry after being laid on mud; development commences when the eggs are wetted by tides, but those not in isolated puddles are washed off the marsh or are consumed by fish and other predators (Crans 2004; Harwood and Horsfall 1959). Mosquito control has relied on habitat alteration, including marsh ditching as early as 1900 in Long Island, NY (Tonjes 2013) and other more sophisticated manipulations beginning in the 1960s (Ferrigno et al. 1975). Pesticides have been heavily relied on, initially with DDT until it was banned (locally and temporarily in Suffolk County in 1966 and nationally in 1973) (Wurster 2015), and more recently using a variety of second generation pesticides based on natural compounds, hormone mimics and more rapidly degrading synthetic compounds (Becnel and Floore 2007).

It is less efficient to spray a pesticide into the air to control flying adult insects than it is to prevent their emergence (Pimental 1995), so larval control is generally considered to be more effective than adult control (Becnel and Floore 2007). *Bacillus thuringienesis* var. *israelensis* (*Bti*) and methoprene are two commonly used larvicides (Lacey 2007; Henrick 2007). *Bti* is bacterial-based, and derived from natural compounds; few objections are made to its use (Cashin Associates 2006), although effects on some non-target organisms have been found (Boisvert and Boisvert 2000). Methoprene is synthetic, generally causes more public concern (Cashin Associates 2006a), and its potential non-target impacts have been more widely studied (Glare and O'Callaghan 1999; Stark 2005). *Bti* is most effective on Stages I-III larvae which feed on the

bacteria that is toxic to the target insect (Lacey 2007). Methoprene is absorbed by larvae and then mimics juvenile hormone, blocking receptor sites, preventing further development to adulthood; it is very effective on Stage IV larvae (Henrick 2007). Formulated methoprene products result in pesticide release over longer times, enabling applications to be made when larvae are in younger stages. When higher summer temperatures reduce development times, making forecasts of brooded mosquito larval staging uncertain, or when intermittent hatching results in a mixture of larval stages, so-called duplex applications (*Bti* plus methoprene) can be used to ensure control of the target mosquito (Cashin Associates 2006b).

Methoprene is extremely toxic to salt marsh mosquitoes (LD_{50} = 5 to 150 ng/L) (Glare and O'Callaghan 1999) and surviving larvae may have deformities as adults (Sawby et al. 1992). Methoprene is acutely toxic to 12 orders of other insects, at doses as low as 50 µg/L (Glare and O'Callaghan 1999). Testing on benthic organisms has been limited (Levy and Miller 1978; Reish et al. 1985; Kikuchi et al. 1992; Hoss and Weltje 2007); isopods, the most sensitive, were affected at 300 µg/L (Kikuchi et al. 1992). Methoprene functions as an insect hormone analog, affects gene transcription (Zhao 2013), and developmentally-related impacts to non-target organisms have been reported, which are perceived by many as endocrine system disruptions. Experiments using different exposure approaches and doses have resulted in reported effects on fish, amphibians, crustaceans, and insects, sometimes at less than µg/L concentrations (Appendix A).

Pesticide use causes many ecological concerns, both when it is applied where the target organism is and also when applications reach areas where the pesticide will not achieve its intended effect. For instance, an impetus for this study was reports of fish kills in open waters temporally and physically in the vicinity of pesticide applications made to control mosquitoes

(Cashin Associates 2004). Several studies have tested for non-target impacts in areas where mosquitoes develop, with significant insect population effects in four instances: Norland and Mulla (1975) found mayfly and dytiscid beetle populations were reduced; Breaud et al. (1977) found reductions in scud, some shrimp and prawn populations, flies, midges, and chionomidae larvae, and several species of beetles, while water boatmen nymphs, moth fly larvae, diving beetles, and crawfish populations increased; Hershey et al. (1998) found general impacts to insects generally, especially predatory insects, and most especially to chironmidae; Pauley et al. (2015) found a decrease in gray treefrog tadpole survival. Two others did not find impacts (Lawler et al. 2000; Pinckney et al, 2000), and one measured significant impacts to springtails that varied (increases at one site, decreases at the other), and non-significant effects on dipterans, heteropternans, and hymenopterans (effects from applications were less than other population variations over the sampling period) (Russell et al. 2009). The 1999 collapse of the Long Island Sound lobster fishery was linked by litigants and in the press to widespread pesticide use following the initial West Nile virus (WNV) outbreak in the New York City area. It was suspected that the pesticides applied in urban settings and marshes washed into open waters where it would not affect mosquitoes but could impact other organisms, such as lobsters. An investigation of the die-off did not find methoprene or other mosquito pesticides to be important factors (but did not rule out potential contributory effects) (Pearce and Balcom 2005).

Theoretical calculations of maximal environmental concentrations in standard meter deep water bodies resulting from label-compliant applications are mostly low μg/L concentrations (<4 μg/L [USEPA 2001]), but values >1,000 μg/L have been published (Appendix B). Few environmental measurements of methoprene have been made. Most recent reports in the past decade were on solid formulations (most conducted circa 2010) because of increased use of

methoprene in catch basins and other subterranean structures (Appendix C). These solid formulation studies had detection limits tended to range from about 50 ng/L to 200 ng/L, and fewer than half of all samples resulted in detectable concentrations. However, a few stray samples found concentrations up to three orders of magnitude above the detection limits. In comparison, liquid methoprene, after initial testing in mesocosm experiments (Ross et al. 1994), has only occasionally been tested for following applications (Siemering 2004; Abbene et al 2005; Zulkowsky et al. 2005; Johnson and Kinney 2006), with most samples finding no detectable concentrations. Methoprene has been analyzed for even more rarely in sediments (Siemering 2004); once, organic matter collected following a laboratory mesocosm test of methoprene briquets was analyzed (Butler et al. 2010).

Mosquito control undergoes scrutiny with emergence of novel mosquito-borne diseases. The recent Western Hemisphere Zika virus outbreak drew more attention to control programs, just as WNV did nearly two decades ago. The 1999 emergence and subsequent perseverance of WNV caused Suffolk County (Long Island, NY, US) to re-evaluate its mosquito control program. New York State officials required the County to determine if operational pesticide applications affected fish and aquatic invertebrates, partly because of the reports of fish kills (Cashin Associates 2004). Aerial applications of liquid Altosid (methoprene) were part of that required study. Thus, in the summer of 2004 aqueous sampling that focused on ditches in salt marshes, where mosquitoes do not develop but which may be important fish habitat, was conducted following methoprene applications to the salt marshes by the United States Geological Survey (USGS) and Stony Brook University (SBU), jointly and separately, and sediment sampling was conducted by SBU. The USGS has published its data previously in a technical report (Abbene et al. 2005); however, the entire joint set of sampling data has not been presented

in the peer-reviewed scientific literature and the analysis presented here is more comprehensive than the earlier report.

2.0 Materials and methods

Sampling was conducted by the USGS and SBU as lead investigators, with assistance from Suffolk County Department of Health Services, Suffolk County Vector Control, and Cashin Associates. Samples were analyzed separately by USGS and SBU.

2.1 Sampling

Water and sediment sampling occurred primarily in mosquito control ditches in two tidal marshes along Great South Bay (Figure 1) which retained sufficient water and appeared to have adequate dissolved oxygen concentrations to support the biological exposure studies being conducted at the same time. The sites received multiple methoprene applications: seven applications at Johns Neck (JN) and eight at Timber Point (TP). Control sites, which also contained ditches which retained enough water to support the biological testing, were Havens Point (HP), and Flax Pond (FP), a tidal marsh on the north shore of Long Island. Sampling sites in the ditches had mean tidal depths of 1 - 1.5 m. Two samples after one application at JN were taken in Pattersquash Creek (PC), ~ 3 km by air and 6 km by water from JN and outside the spray zone and away from the main channel from JN. Samples were also collected at a marina at Davis Park (DP) on Fire Island two weeks after mainland methoprene applications ended; DP received a dozen weekly applications of sumithrin over the summer, but no methoprene was applied.

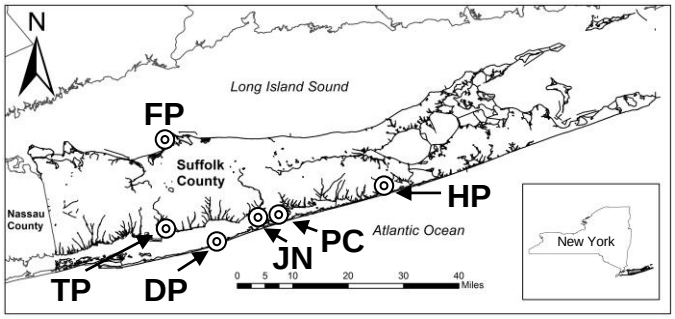


Figure 1. Location Map (TP = Timber Point, FP = Flax Pond, DP = Davis Park, JN = Johns Neck, PC = Pattersquash Creek, HP = Havens Point)

2.1.1 Aqueous Sampling

The collection times of sampling events and types of aqueous samples collected are provided in Table 1. The first two rounds by SBU (following applications August 3 and 10) at both JN and TP aimed at characterizing the initial impact of the application and one day later; USGS sampling following August 3 applications extended over four days. Samples were taken at JN after August 17 to explicitly track the effects from adulticide (resmethrin) applications on August 18 and 25, but these samples also produced data relevant to a methoprene application there on August 17. Monitoring at TP following a September 1 application was conducted with less intensity. Control samples (pre-application) at treatment marshes (which also served as a measure of residual methoprene from previous applications) were collected 15 min (August 3) and 55 min (August 10) before the applications at JN and 40 min prior to the application at TP on August 10. Samples taken from open waters outside of the mosquito ditches occurred August 18 at PC, and September 14 and 15 at DP. Sampling of control marshes occurred on August 3 and 10 (FP and HP) and again at HP on August 18. Replicate samples were taken 10 times (three on August 18 at JN, one on August 19 at JN, three on August 25 at JN, one on August 26 at JN, one on August 18 at the control site HP, and one from the off-site PC sampling).

240

Applications	**Sampling**										
Date, Site, Time	**JN**				**TP**				**Other**		
	Date	**Time**	**Org.**	**Type**	**Date**	**Time**	**Org.**	**Type**	**Date**	**Location**	**Type**
8/3, TP, 7:05					8/3	7:35	SBU USGS	S, I, I_{panne} S, I	8/3	HP	S
						8:50	SBU USGS	S S		FP	S
						10:10	SBU	$I_{offsite}$			
					8/4	6:45	SBU USGS	S S			
					8/5	6:45	USGS	S			
					8/7	6:45	USGS	S			
8/3, JN, 11:30	8/3	11:15	SBU USGS	S S, I							
		12:30	SBU	I							
		12:50	SBU USGS	S S, I							
		14:25	USGS	S							
	8/4	12:15	SBU USGS	S S							
	8/5	12:15	USGS	S							
	8/7	12:15	USGS	S							
8/10, TP, 8:55					8/10	8:15	SBU	S	8/10	HP	S
						9:25	SBU	S		FP	S
						9:30	SBU	S			
					8/11	10:40	SBU	S			
8/10, JN, 12:55	8/10	12:00	SBU	S							
		13:25	SBU	S							
	8/11	12:00	SBU	S							
8/17, JN, 17:00	8/18	16:30	SBU USGS	S S, I					8/18	HP	S* S
		18:50	SBU	S*					8/18	PC	I*
		20:00	SBU USGS	S*, I* S, I							
		21:20	SBU	S							
		21:30	USGS	S							
		21:50	SBU	S							
	8/19	4:30	SBU USGS	S* S							
	8/20	20:15	USGS	S							
	8/22	20:15	USGS	S							
	8/25	17:45	SBU USGS	S*, $S_{channel}$ S, I							
		19:40	SBU USGS	S*, I, $S_{channel,}$ $I_{channel}$ S, I							
		21:10	SBU USGS	$S_{channel}$ S							
	8/26	4:30	SBU USGS	S*, $S_{channel}$ S							
	8/27	19:40	USGS	S							
	8/29	18:40	USGS	S							
9/1, TP, 10:00					9/2	15:30	SBU	S	9/14	DP	S, S, S
					9/4	12:00	SBU	S	9/15	DP	S, S, S

Table 1. Aqueous sampling (* = replicated sample; f = filtered, S = subsurface, I = interface; JN = Johns Neck, TP = Timber Point, HP = Havens Point, FP = Flax Pond, PC = Pattersquash Creek, DP = Davis Park) (note that sampling for the 8/17 application was in response to an aerial adulticide application 8/18)

USGS samples included seven interface samples (termed grab samples in Abbene et al. 2005), using a sampler that collects primarily the upper 1-2 cm of water. Other samples were collected in clean 1 L amber bottles opened approximately 15 cm below the air-water interface (called point samples in Abbene et al. 2005 but labelled subsurface samples here). All USGS samples were filtered within three hours on collection using baked glass 0.7 μm filters, with the filters pre-leached with 200 mL of sample water. The filtered water was stored in dark glass 32 oz. (1L) bottles. Samples were preserved on ice and shipped overnight to the USGS laboratory in Lawrence, KS for analysis. Sample types and locations are described in figures in Appendix D.

The SBU water samples generally were collected as described in Zulkowsky et al. (2005). Most whole water samples were collected in clean 1 L amber jars as subsurface samples. Six additional samples were collected for SBU by USGS personnel as interface samples. Subsurface samples (approximately 900 mL) were preserved with 25 mL of hexane added in the field; thus, the analysis of subsurface samples was of combined dissolved and particulate phases. In contrast, the SBU interface samples were processed by USGS: filtered within three hours of collection and placed on ice for preservation. Hexane was added to SBU samples post filtering. Some samples were also taken from certain marsh surface water bodies, either as surface interface samples or subsurface samples from as deep in the water body as practical.

2.1.2 Sediment Sampling

SBU sampling of salt marsh sediments was conducted over approximately one month at treatment marshes, and on one (FP) or two (HP) dates at control sites (Table 2). The intensity of sampling at JN to track the August 18 and 25 adulticide applications was greater (these samples also measured the August 17 methoprene application). Three types of samples were collected: subtidal samples (0-1 cm deep) from the water sampling sites at depths well below mean low tide; samples above the mean tide line (intertidal samples) (0-1 cm deep); and several scrapings of surface sediments from a variety of marsh surface environments in the high marsh. The latter samples came from areas with very hard surfaces; sample depths ranged from a few mm to a cm. Sample types and locations are described in figures in Appendix D.

Subtidal and most intertidal sediments were collected with polyacrylate tubes and extruded from below to collect only the upper sediment layer. Each sample was drawn from three cores; the sediments were combined in a solvent rinsed glass bowl and homogenized before transferring them to glass jars that were iced and then frozen within hours on return to the laboratory. For some intertidal sediments, where marsh grass rhizomes were present, samples were obtained by knife and spoon, approximating a 1 cm depth. Due to the fibrous nature of the rhizomes these samples were diced with a stainless steel knife after freeze-drying and prior to extraction. No replicate sediment samples were taken.

Date (relation to application)	Marsh	Samples taken
8/2 (6 d post, 1 d pre)	TP	S, $PS_{algal\ mat}$
	HP	I, S
	FP	I, S
8/7 (4 d post)	JN	I, S
(4 d post)	TP	I
8/11 (1 d post)	JN	I
8/18 (1 d post)	JN	I, S
	HP	I, S
8/19 (2 d post)	JN	I, S (2)
8/22 (5 d post)	JN	I (2), S (3)
8/25 (8 d post)	JN	S
8/26 (9 d post)	JN	I, S
8/29 (12 d post)	JN	S
9/5 (4 d post)	TP	S, PS, $PS_{marsh\ pond}$, $PS_{sulfur\ panne}$
(19 d post)	JN	S (2), I, PS_{shore}, $PS_{outer\ pond}$

Table 2. SBU sediment samples (TP = Timber Point, JN = Johns Neck, HP = Havens Point (control site), FP = Flax Pond (control site), S = subtidal, I = intertidal, PS = panne scraping)

2.2 Sample Analysis

USGS analysis procedures are described in Zimmerman et al. (2001). Briefly, hexane was added to the delivered sample. Liquid-liquid extraction with hexane followed, which was evaporated and reconstituted at a known volume. Analysis was by Hewlett Packard (Wilmington, DE) model 5890 series II Plus GC with autoinjector connected to a Hewlett Packard model 5970 MS detect. USGS followed its internal rules for blanks and calibration as described in Zimmerman et al. (2001).

SBU analysis of water samples followed Zulkowsky et al. (2005). Briefly, extraction was performed by liquid-liquid extraction with hexane, which was evaporated and reconstituted in 100 µL of methanol for analysis by HPLC-time-of-flight mass spectrometry (LC-TOF-MS) with a Micromass LCT, equipped with a Waters 2695 HPLC and a Z-spray electrospray ionization

297 source. SBU sediment analysis is described in Appendix E in more detail. Briefly, solid
298 extraction was achieved using a 50:50 acetone:dichloromethane solvent followed by liquid-liquid
299 extraction with hexane and analysis with an HP 5890 series II GC equipped with VG Quattro
300 mass spectrometer.

301 There were no detected pesticides in laboratory (SBU and USGS) or field blanks (SBU).
302 SBU method recoveries in pure water averaged between 85 and 118 percent, and spiked matrix
303 (water and sediment) samples yielded similar recoveries. USGS method recoveries were not
304 specifically documented for these samples. SBU method detection limits in sample matrix
305 (signal:noise > 3) were $\leq$ 0.5 ng/L in water and $\leq$ 5 ng/g for sediments. The USGS reporting
306 level was 5 ng/L.

307 The SBU standard was 98% pure, racemic mixture of R and S isomers methoprene
308 (Crescent Chemical, Islandia, NY). Deuterated *d*-6 malathion was utilized as an internal standard
309 (CDN Isotopes, Quebec, Canada). All solvents were analytical grade Burdick and Jackson
310 (VWR Scientific Products, Bridgeport, NY). All other chemicals were from Sigma Aldrich (St.
311 Louis, MO). USGS chemical stocks are described in Zimmerman et al. (2001).

312 **3.0 Results**

313 **3.1 Aqueous samples**

314 Detectable levels of methoprene were found in only three of 30 USGS samples (Figure
315 2), from samples taken immediately after the applications on August 3. One was an interface
316 sample (216 ng/L) at TP; the others were a concurrent subsurface sample (82 ng/L) at TP, and a
317 subsurface sample at JN (10 ng/L), again taken soon after the application. No other sample
318 contained detectable levels >5 ng/L of methoprene (non-detections in Figure 2 may represent
319 more than one sample).

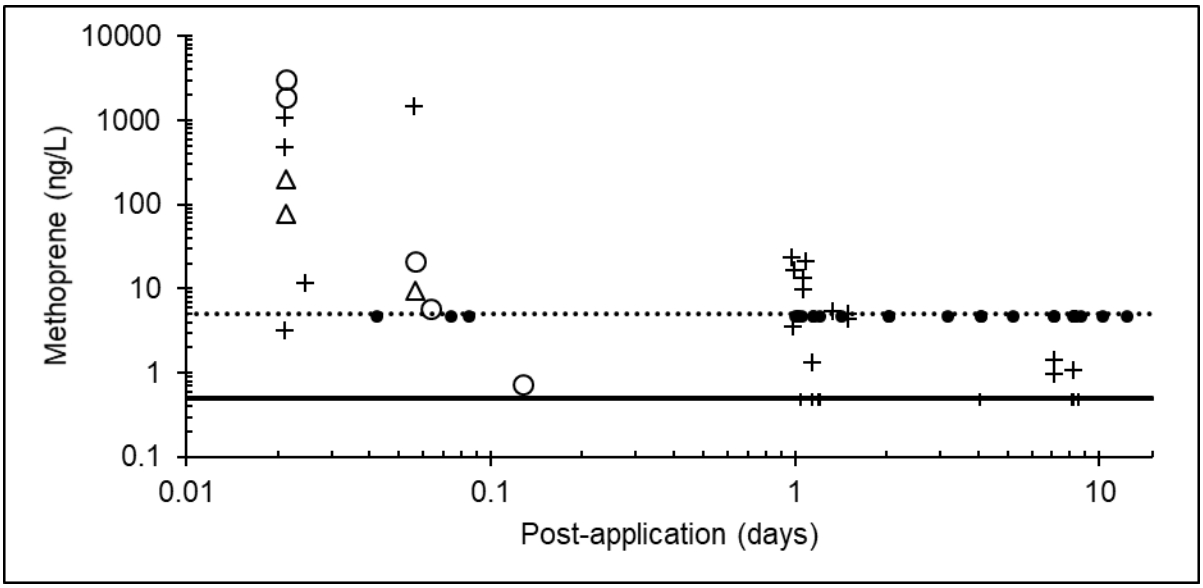


Figure 2. Aqueous sampling data from JN and TP marsh ditches, all events (+ = SBU whole water detections; ○= SBU filtered water detections; Δ = USGS filtered water detections; dotted line = USGS ND, with points indicating ND results; solid line = SBU ND, with slashes indicating ND results)

There were a greater number of detections from the larger number of aqueous samples analyzed by SBU (Figure 2). Some unfiltered samples taken < 90 min after application had concentrations of methoprene in the vicinity of 1,000 ng/L, such as two subsurface samples at JN on August 3 and 10 (1,100 ng/L and 1,500 ng/L). Concentrations of methoprene in some of the initial filtered interface samples from TP on August 3 also exceeded 1,000 ng/L, including an interface sample (3,300 ng/L), and an interface panne sample (2,000 ng/L); another TP interface sample that day was 490 ng/L. This pattern of high initial concentrations was not found for the August 10 event at TP, where initial unfiltered subsurface samples were 3.3 ng/L and 12 ng/L, and on August 3 for a filtered interface sample at JN, which was 23 ng/L.

Methoprene concentrations in water samples decreased rapidly post application. None of concentrations reported for the 30 samples (including replicates) taken 23 hrs or more after

applications exceeded 25 ng/L. Similarly, none of day old or older filtered samples had detectable methoprene (0.5 ng/L detection limit). Methoprene was detected in 13 of 24 day old or older whole water samples. Combined whole water results from SBU analyses for both sites and all sampling events show that although concentrations decreased rapidly post-application, detectable levels of methoprene were found over one week after some applications were made at JN (non-detection symbols in Figure 2, which all occurred 24 hr or more after the applications, sometimes represent more than one sample).

Four August 3 filtered aqueous samples (two analyzed by USGS and two analyzed by SBU) could be retroactively coupled with SBU analyses of filter residues to reconstruct whole water samples (Table 3). The label application rate for methoprene is the equivalent to 280 ng/cm^2; if it is assumed the interface sampler collected water from the top 2-3 cm of water only (it aims for the top 1-2 cm of water), then the theoretical maximum concentration associated with an application would be ~75,000 - 150,000 ng/L. The sum of filtered aqueous fraction pesticide and filtrate fraction pesticide for the interface sample at TP of 46,000 ng/L is perhaps 50% of the theoretical calculation; the combined concentrations were much lower in the JN interface sample.

Location	Time post-spray (min)	Sample type	Removed concentrations: filter results in ng/L equivalents	Aqueous concentration (ng/L)	Sum (ng/L)
TP	30	Interface	43,000	3,300	46,000
JN	60	Interface	680	23	700
TP	30	Subsurface	1,700	82*	1,800
JN	60	Subsurface	250	10*	260

*USGS results

Table 3. Combination of SBU methoprene results from filter material and filtered water concentrations on August 3, 2004

There were no detections of methoprene in two interface (filtered) samples collected from PC the day after an application at nearby JN marshes, or in any of the six unfiltered samples collected at DP approximately two weeks after applications ceased in the Great South Bay basin. There were no detections of methoprene in any of the seven unfiltered samples collected from the control marshes (HP and FP).

3.2 Sediment Samples

Methoprene was detected in SBU sediment samples collected from TP and JN ditches at levels ranging from 9.1 ng/g to 68 ng/g. Methoprene concentrations from the intertidal sediments ranged between 20 ng/g and 68 ng/g, with a median detected concentration of 32 ng/g (Figure 3). Subtidal sediments generally had lower concentrations of methoprene than intertidal sediments, ranging from 9.1 ng/g to 50 ng/g, with a median detected concentration of 17 ng/g and only one sample above 25 ng/g (Figure 3). Only five of 20 total ditch sediment samples had no detections, and most of the NDs were from subtidal sediments collected 12 or more days after applications. There were six high marsh scrapings (Table 4); one was non-detectable, and a September 5 TP

panne scraping had a 1,200 ng/g; the other four results ranged from 13 ng/g to 64 ng/g, with a median detected concentration of 49 ng/g with the lowest results associated with samples taken 19 days after an application at JN. None of the six sediment samples taken at the control marshes had detectable concentrations.

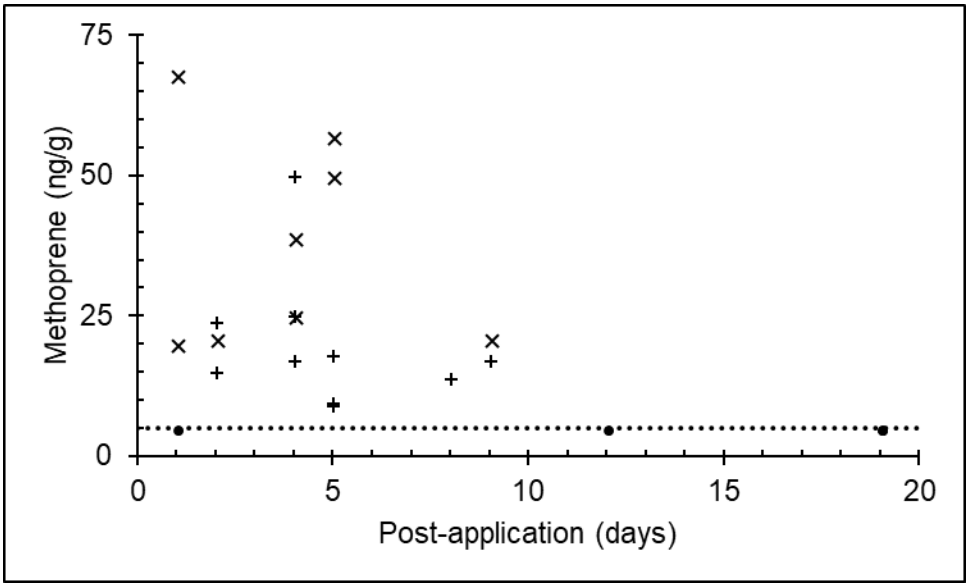


Figure 3. Sediment sampling results from JN and TP ditch sediments (x = intertidal detections; + = subtidal detections, dotted line = ND, with points indicating ND results)

Date	Marsh	Relation to application	Sample type	Result (ng/g)
8/2	TP	(6 d post, 1 d pre)	algal mat	40
9/5	TP	4 d post	panne	1200
			marsh pond	58
			sulfur panne	64
	JN	19 d post	shore	13
			outer pond	<5

Table 4. SBU marsh surface sediment sampling results

4.0 Discussion

4.1 Aqueous samples discussion

Whole water samples (and sample concentrations reconstituted from filtered samples and analyses of the filtrate material) found detectable concentrations of methoprene in mosquito

ditches in a salt marsh, sometimes at levels greater than 1,000 ng/L. Concentrations varied considerably between applications and sites; in particular, the results suggest TP on August 10 may have received less pesticide, although helicopter records indicate the application was standard. Highest concentrations were found in samples collected within one hour of the application, either as filtered surface film (interface samples) samples or as unfiltered subsurface samples. Comparisons of replicate SBU unfiltered and USGS filtered samples and analyses of filtrate materials suggest that significant amounts of methoprene are removed by filtering. Therefore, the concentrations from unfiltered samples that are similar to filtered sample results from the surface film actually indicate large reductions in overall pesticide concentrations.

Methoprene concentrations in the mosquito ditches decreased rapidly after spraying. One day after an application, subsurface samples were more than an order of magnitude lower, and in general were in the low ng/L range. These marsh ditches had been selected because they did not dry out at low tide, and so one day post-application meant two tidal cycles had mixed estuarine waters into these ditches, and potentially advected pesticide out of the system. Reduced concentrations thus result from degradation of the pesticide (half-life of 10-14 d, attributed to microbial and photolytic processes [USEPA 2001]), settlement or sorption to sediments, removal by tidal flushing, and dilution by estuarine waters containing no (or very small) amounts of pesticide. After several days, methoprene was only detectable at near 1 ng/L concentrations in the ditches.

USGS filtered water sample concentrations and detection percentages were much lower than SBU whole water results at least partially because filtering removes particulate matter that methoprene may have sorbed to and any still-encapsulated methoprene (liquid methoprene is formulated as micro-encapsulated particles so as to release the pesticide over a week or so), as

409 indicated by the SBU filtrate sample analyses. Methoprene is strongly hydrophobic; its log K_{ow}
410 is reported to be 5.5 (Hanch and Hoekman 1995) and Steuckle et al. (2008) and Jordao et al.
411 (2016) both report finding it difficult to maintain methoprene concentrations in laboratory
412 exposures (apparently because of sorption onto containers). Current sampling procedures for
413 other high K_{ow} chemicals such as per- and polyfluoroalkyls call for use of polypropylene or high
414 density polypropylene bottles to avoid sorbtion to glass containers and standard bottle caps
415 (NGWA 2017).

416 Analytical capabilities of the laboratories were shown to be approximately similar
417 through an intercalibration exercise (Appendix F). The lower detection limit achieved by SBU
418 meant eight of 23 SBU detections were made below the USGS reporting level of 5 ng/L; six of
419 the 13 whole water detections of methoprene made 23 hours or more after the applications were
420 below 5 ng/L. The lower SBU detection limit therefore strengthened the finding that methoprene
421 is present in ditches for some time (and several tide cycles) after applications. USGS did not
422 detect any methoprene more than 80 min after an application. Two filtered SBU samples
423 detected methoprene later than this; one result was nearly an order of magnitude lower than the 5
424 ng/L USGS detection limit. SBU did not detect any methoprene in filtered samples more than 3
425 hrs. after any application.

426 Filtered samples therefore imply that methoprene rapidly disappears entirely from the
427 mosquito control ditches. However, whole water samples resulted in continued detections of the
428 pesticide, albeit at concentrations ~1 ng/L, up to 9 days after applications.

429 The lack of detections at PC and DP provide some evidence that methoprene applications
430 in marshes are unlikely to result in methoprene concentrations as much as 1 ng/L in the open
431 waters of Great South Bay. Similarly, in 2002 SBU summer sampling of open waters in Long

Island Sound and the East River in New York City that were drainage basins for methoprene application areas found no methoprene (Zulkowsky et al. 2005). Methoprene was detectable in whole water samples from the mosquito ditches for some time, however.

Aqueous sample data generated by SBU and USGS in these marshes in 2004 are similar to the sparse other reports for liquid methoprene (see Appendix G), where aqueous concentrations of methoprene were all found to be less than 1,000 ng/L with one notable exception: an interface sample more than 9,000 ng/L that was collected by USGS in 2003 at one Long Island marsh immediately after an application. This result, if we assume that a whole water sample might have concentrations an order of magnitude greater, is compatible with our estimate of a potential maximum 75,000 – 150,000 ng/L initial interface concentration. In all, USGS tracked eight methoprene applications in 2002-2003, collecting 10 samples, with four detections (three of them from the same 2003 application, including the 9,000 ng/L detection) (Abbene et al. 2005). The only other study to track methoprene in salt marshes that we are aware of was conducted in California; there, eight samples were collected the same day as the application with no detections of methoprene (Siemering 2004). An extensive sampling program of a fresh water complex in Washington State resulted in six detections of methoprene out of 68 total samples; five detections were on the day of application, with only one later than that (although it was 6 days later) (Johnson and Kinney 2006). In 2002, the SBU analysis of a variety of fresh and salt water samples as part of the lobster die-off investigation and in conjunction with USGS tracking of Suffolk County mosquito control applications had only one detection of methoprene from 19 samples, at a concentration of 7.39 ng/L, in a marsh ditch one day after the application (Zulkowsky 2005). Since 2004, USGS has continued its Suffolk County monitoring program (most recent information at

455 www.sciencebase.gov/catalog/item/55cb887ce4b08400b1fddc94?community=USGS+New+Yor
456 k+Water+Science+Center), with results similar to those reported here (and in Abbene et al.
457 2005). So, other studies have found methoprene to be detectable in a number of samples taken
458 the day of application, at concentrations that tend to be but are not always less than 1,000 ng/L.

459 **4.2 Sediment sampling discussion**

460 Methoprene was found in all but one sediment samples taken within 9 d of applications,
461 at relatively consistent concentrations. Intertidal samples tended to be about twice the
462 concentration of subtidal samples. Samples analyzed more than 10 days after applications had no
463 detectable methoprene. Although there were repeated applications of methoprene at these
464 marshes over the summer, this did not appear to result in increasing concentrations in the
465 sediments (Figure 4, for JN sediment samples). Samples collected after 9 d at JN contained no
466 detectable methoprene, suggesting that pesticide degradation and dissolution of coatings
467 minimize its persistence. A coarse estimate of the partitioning of methoprene between water and
468 sediments (see Appendix H) suggests that subtidal and intertidal sediment samples contained at
469 most 1% to a little more than 10% of pesticide applied in the application (Figure 5).

470

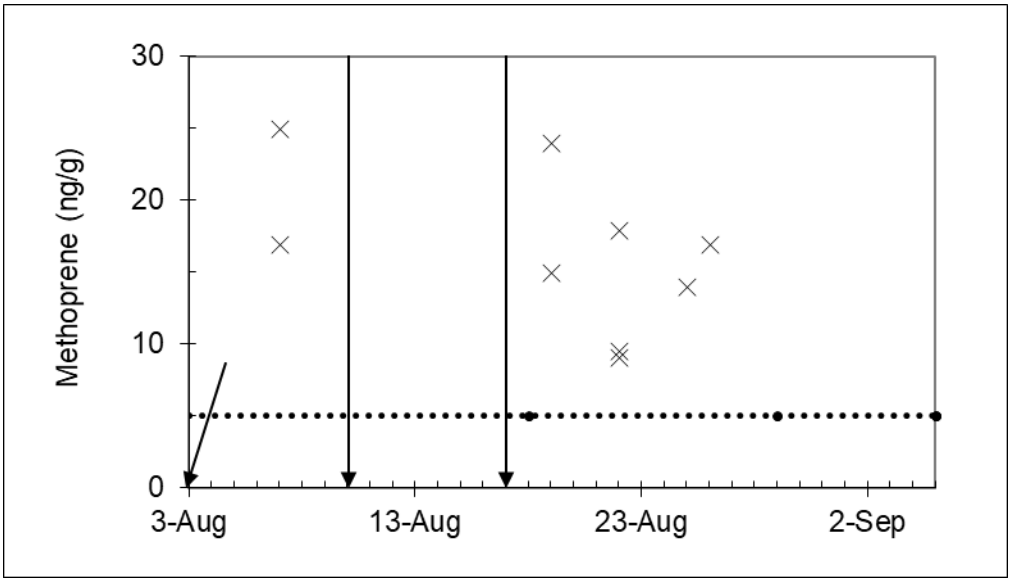


Figure 4. Time series of JN subsurface sediment concentrations (arrows indicate methoprene application dates; x = detections; dotted line = ND, with points indicating ND results)

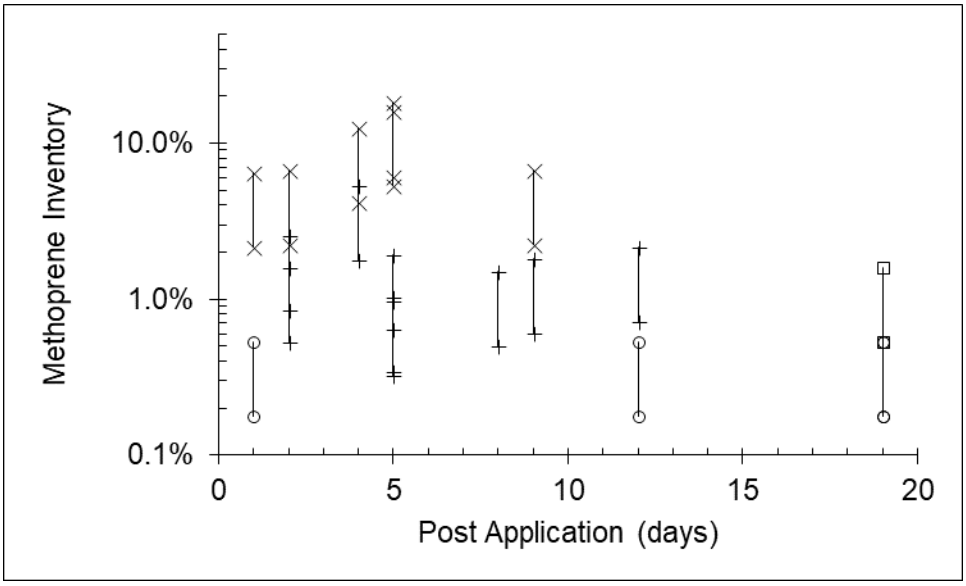


Figure 5. Subsurface and intertidal sediment sample concentration as a percent of applied methoprene (range indicates uncertainty regarding bulk density) (+ = subsurface detection; ○= subsurface non-detection; x = intertidal detection; □ = intertidal non-detection).

Reports from later USGS sampling of sediments in Suffolk County (most recent information at

www.sciencebase.gov/catalog/item/55cb887ce4b08400b1fddc94?community=USGS+New+York+Water+Science+Center) show that methoprene concentrations soon after applications are on the order of 100 ng/g or less. USGS has found that higher concentrations are associated with higher sediment organic carbon content; thus, sediment concentrations from similar application histories may vary from marsh to marsh. In 2004, organic carbon content data were not collected, and so cannot be related to this conclusion from the later USGS findings. The only other methoprene sediment sampling study (Siemering 2004) is difficult to interpret, as control and pre-application concentrations exceeded those associated with the post-application samples.

4.3 Marsh surface samples discussion

Aqueous and sediment samples taken on the marsh surface generally agreed with values obtained from the ditches (<100 ng/L for aqueous samples, ~50 ng/g for sediment samples). The two notable exceptions were from TP: a filtered interface panne aqueous sample of 2,000 ng/L from TP on August 3, and a September 5 algal mat sediment sample of 1,200 ng/g. Pannes are shallower water bodies than ditches; similar area application rates could result in greater concentrations due to a lack of dilution in the water column. Thus, the very high aqueous sample, since it was an interface sample, may be caused by relatively little mixing with a missing underlying water column. Pannes also are local low points on the marsh, and so may collect run-off, although water was only observed in them at high tide. Based on estimates of bulk density and the depth of the scraping, the higher sediment sample concentration appears to represent 125-200% of the theoretical maximum concentration that would result from a single application. Although there was no significant rain for 10 days before the application, tidal flows across the surface of the marsh may have collected in the panne, serving the same purpose.

Methoprene briquet sampling by others (Appendix C), especially in catch basins, has resulted in extremely infrequent instances of very high methoprene detections, much higher than were detected in our sampling effort. A discussion of sampling in dried-down marshes in Minnesota attributed some high environmental concentrations to proximity to dissolving briquets in a confined sampling space (Hershey et al. 1995), and something similar, such as unusual dissolution of the briquet coating in very shallow waters, may have released unanticipated amounts of pesticide in the catch basin settings. The higher results from briquet applications were not common, and there was no pattern to their occurrence. The sampling here was of liquid (micro-encapsulated) methoprene, and the sampled environments were very different in terms of water depth and water exchange. However, there were not enough samples taken in 2004 to rule out liquid methoprene as a potential source of similar very sporadic occurrences of very high concentrations from time to time. Marsh surface environments, in some ways, resemble catch basin settings in terms of a lack of exchange and varying water levels, and therefore may be reasonable locations for much more extensive sampling efforts to determine if any outlier concentrations can be produced with liquid methoprene in salt marsh settings.

4.4 Ecotoxicology discussion

None of the aqueous sample concentrations collected after two hours exceeded the lowest impact concentration of 100 ng/L, which had been determined after chronic exposures (28 days) for the fiddler crab *Uca pugnax* (Steuckle et al. 2008). A little more than a day after applications all water column detections were less than 10 ng/L. Thus, the data here show that organisms generally only had potential exposure to short periods of concentrations that were likely not high enough to elicit any toxic response, and which diminished rapidly. In addition, laboratory toxicity testing in conjunction with this field sampling exercise (but not yet published in the

scientific literature) on fish larvae (sheepshead minnow, *Cyprinodon variegatus*) and adult grass shrimp (*Paleomonetes pugio*) in water samples collected concurrently to those analyzed here in this study failed to demonstrate any acute mortality to either species in 96 hr static renewal toxicity tests (Barnes 2005). Concerns raised by some when open water fish kills occurred contemporaneously and in close conjunction with larvicide applications to salt marshes on Long Island appear to have little foundation, as concentrations in the mosquito control ditches were not great enough to appear capable of ecological effects. Limited sampling in open waters also provides some evidence that methoprene applications in salt marshes is unlikely to affect estuarine biota.

Measured sediment concentrations of methoprene were also much lower than levels likely to cause toxicity. Although toxicity data on sediment sorbed methoprene have not been reported to our knowledge, the partitioning of a non-ionic organic compound such as methoprene between pore water and sediments can be calculated knowing its octanol water partition coefficient (K_{ow}) and the fraction of organic matter in the sediment (f_{oc}) (DiToro et al. 1991). Using a K_{ow} of 5.5 and assuming f_{oc} is on the order of 0.05 (5%) (a conservative estimate for marsh peats), measured values of 100 to 1,000 ng/g of methoprene in creek sediments at equilibrium could result in ~10 to 100 ng/L of methoprene in pore waters. This calculation suggests that ditch sediment pore water concentrations would have been orders of magnitude below the lowest effect concentration reported for sediment organisms, 300 µg/L (Kikuchi et al. 1991) (although note the impact determinations were based on aqueous concentrations, not concentrations in sediment [Levy and Miller 1978; Reish et al. 1985; Kikuchi et al. 1992; Hoss and Weltje 2007]). This conclusion should be viewed with some caution, as there have been

some impacts (longer duration of the pre-molt stage, and greater weight loss then) measured for 100 ng/L chronic exposures in male fiddler crabs (Steuckle et al. 2008).

5.0 Conclusions

Ultra low detection levels of 0.5 ng/L in whole water samples achieved by the use of LC-TOF-MS provided a more comprehensive understanding of the analysis of loss of methoprene from water samples over time compared to more conventional analyses of contemporaneous filtered water samples by USGS. The greatest concentrations of methoprene were found for those samples taken from the air-water interface; samples taken from the subsurface were much lower. Filtration of water samples resulted in significantly lower concentrations, suggesting that filtration of water samples containing high K_{ow} contaminants such as methoprene may lead to underestimation of whole water concentrations. Although methoprene was detected in aqueous samples in ditches as long as 8 days after applications, the concentrations decreased rapidly from initial concentrations of sometimes more than 1,000 ng/L to approximately two magnitudes less within a day (over two tidal cycles). Very limited sampling away from application sites failed to detect methoprene, suggesting export from marshes may not be consequential. Analysis of sediment samples indicated the presence but not accumulation of methoprene despite repeated weekly applications. At the very most, 10% of applied material was deposited in surface sediments.

Our limited sampling for methoprene in salt marsh pannes and panne sediments suggested that higher concentrations may be found in these settings. This is operationally advantageous for vector control, as this is where salt marsh mosquitoes develop. However, not enough data were collected to test if marsh surface concentrations also decrease rapidly.

569 The ecology of invertebrates in ephemeral marsh surface environments like the pannes
570 we sampled and other puddled waters has only been partially characterized (Rochlin et al. 2011).
571 Thus, along with expansion of the limited sampling we conducted in ditches and associated
572 estuarine areas, and greater toxicity testing for estuarine and ditch sediment organisms, research
573 regarding the distribution and fate of methoprene on marsh surfaces following mosquito control
574 applications and the potential for impact to ephemeral invertebrate communities is clearly
575 warranted.
576

Appendices

Acknowledgements

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DVN entered into a consulting relationship with Central Garden and Pet (CG&P) of Walnut Creek, California on May 6, 2018. CG&P is the manufacturer of a line of methoprene-based mosquito control products. All work by DVN on the paper was conducted prior to this arrangement, as was the original submission of the paper for publication. To avoid any conflict

of interest or appearance thereof, DVN recused himself from later work on the paper, such as responses to reviewer comments. DVN has agreed not to share any pre-publication copies of the paper with CG&P until it is deemed appropriate by the journal.

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Tables

Table Captions

Table 1. Aqueous sampling (* = replicated sample; f = filtered, S = subsurface, I = interface; JN = Johns Neck, TP = Timber Point, HP = Havens Point, FP = Flax Pond, PC = Pattersquash Creek, DP = Davis Park) (note that sampling for the 8/17 application was in response to an aerial adulticide application 8/18)

Table 2. SBU sediment samples (TP = Timber Point, JN = Johns Neck, HP = Havens Point (control site), FP = Flax Pond (control site), S = subtidal, I = intertidal, PS = panne scraping)

Table 3. Combination of SBU methoprene results from filter material and filtered water concentrations on August 3, 2004

Table 4. SBU marsh surface sediment sampling results

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Applications	**Sampling**										
Date, Site, Time	**JN**				**TP**				**Other**		
	Date	**Time**	**Org.**	**Type**	**Date**	**Time**	**Org.**	**Type**	**Date**	**Location**	**Type**
8/3, TP, 7:05					8/3	7:35	SBU USGS	S, I, I_{panne} S, I	8/3	HP	S
						8:50	SBU USGS	S S		FP	S
						10:10	SBU	$I_{offsite}$			
					8/4	6:45	SBU USGS	S S			
					8/5	6:45	USGS	S			
					8/7	6:45	USGS	S			
8/3, JN, 11:30	8/3	11:15	SBU USGS	S S, I							
		12:30	SBU	I							
		12:50	SBU USGS	S S, I							
		14:25	USGS	S							
	8/4	12:15	SBU USGS	S S							
	8/5	12:15	USGS	S							
	8/7	12:15	USGS	S							
8/10, TP, 8:55					8/10	8:15	SBU	S	8/10	HP	S
						9:25	SBU	S		FP	S
						9:30	SBU	S			
					8/11	10:40	SBU	S			
8/10, JN, 12:55	8/10	12:00	SBU	S							
		13:25	SBU	S							
	8/11	12:00	SBU	S							
8/17, JN, 17:00	8/18	16:30	SBU USGS	S S, I					8/18	HP	S* S
		18:50	SBU	S*					8/18	PC	I*
		20:00	SBU USGS	S*, I* S, I							
		21:20	SBU	S							
		21:30	USGS	S							
		21:50	SBU	S							
	8/19	4:30	SBU USGS	S* S							
	8/20	20:15	USGS	S							
	8/22	20:15	USGS	S							
	8/25	17:45	SBU USGS	S*, $S_{channel}$ S, I							
		19:40	SBU USGS	S*, I, $S_{channel,}$ $I_{channel}$ S, I							
		21:10	SBU USGS	$S_{channel}$ S							
	8/26	4:30	SBU USGS	S*, $S_{channel}$ S							
	8/27	19:40	USGS	S							
	8/29	18:40	USGS	S							
9/1, TP, 10:00					9/2	15:30	SBU	S	9/14	DP	S, S, S
					9/4	12:00	SBU	S	9/15	DP	S, S, S

779 Table 1. Aqueous sampling (* = replicated sample; f = filtered, S = subsurface, I = interface; JN
780 = Johns Neck, TP = Timber Point, HP = Havens Point, FP = Flax Pond, PC = Pattersquash
781 Creek, DP = Davis Park) (note that sampling for the 8/17 application was in response to an aerial
782 adulticide application 8/18)
783

784

Date (relation to application)	Marsh	Samples taken
8/2 (6 d post, 1 d pre)	TP	S, $PS_{algal\ mat}$
	HP	I, S
	FP	I, S
8/7 (4 d post)	JN	I, S
(4 d post)	TP	I
8/11 (1 d post)	JN	I
8/18 (1 d post)	JN	I, S
	HP	I, S
8/19 (2 d post)	JN	I, S (2)
8/22 (5 d post)	JN	I (2), S (3)
8/25 (8 d post)	JN	S
8/26 (9 d post)	JN	I, S
8/29 (12 d post)	JN	S
9/5 (4 d post)	TP	S, PS, PS_{pond}, $PS_{sulfur\ panne}$
(19 d post)	JN	S (2), I, PS_{shore}, PS_{pond}

785

786 Table 2. SBU sediment samples (TP = Timber Point, JN = Johns Neck, HP = Havens Point
787 (control site), FP = Flax Pond (control site), S = subtidal, I = intertidal, PS = panne scraping)
788

789

790

Location	Time post-spray (min)	Sample type	Removed concentrations: filter results in ng/L equivalents	Aqueous concentration (ng/L)	Sum (ng/L)
TP	30	Interface	43,000	3,300	46,000
JN	60	Interface	680	23	700
TP	30	Subsurface	1,700	82*	1,800
JN	60	Subsurface	250	10	260

791 *USGS results

792 Table 3. Combination of SBU methoprene results from filter material and filtered water

793 concentrations on August 3, 2004

794

795

Date	Marsh	Relation to application	Sample type	Result (ng/g)
8/2	TP	(6 d post, 1 d pre)	algal mat	40
9/5	TP	4 d post	panne	1200
			marsh pond	58
			sulfur panne	64
	JN	19 d post	shore	13
			outer pond	<5

796 Table 4. SBU marsh surface sediment sampling results

797

Figures

Figure Captions

Figure 1. Location Map (TP = Timber Point, FP = Flax Pond, DP = Davis Park, JN = Johns Neck, PC = Pattersquash Creek, HP = Havens Point)

Figure 2. Aqueous sampling data from JN and TP marsh ditches, all events (+ = SBU whole water detections; ○= SBU filtered water detections; Δ = USGS filtered water detections; dotted line = USGS ND, with points indicating ND results; solid line = SBU ND, with slashes indicating ND results)

Figure 3. Sediment sampling results from JN and TP ditch sediments (x = intertidal detections; + = subtidal detections, dotted line = ND, with points indicating ND results)

Figure 4. Time series of JN subsurface sediment concentrations (arrows indicate methoprene application dates; x = detections; dotted line = ND, with points indicating ND results)

Figure 5. Subsurface and intertidal sediment sample concentration as a percent of applied methoprene (range indicates uncertainty regarding bulk density) (+ = subsurface detection; ○= subsurface non-detection; x = intertidal detection; □ = intertidal non-detection).

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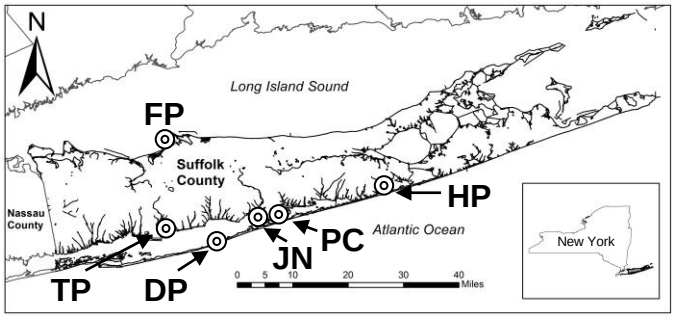

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816 Figure 1. Location Map (TP = Timber Point, FP = Flax Pond, DP = Davis Park, JN = Johns

817 Neck, PC = Pattersquash Creek, HP = Havens Point)

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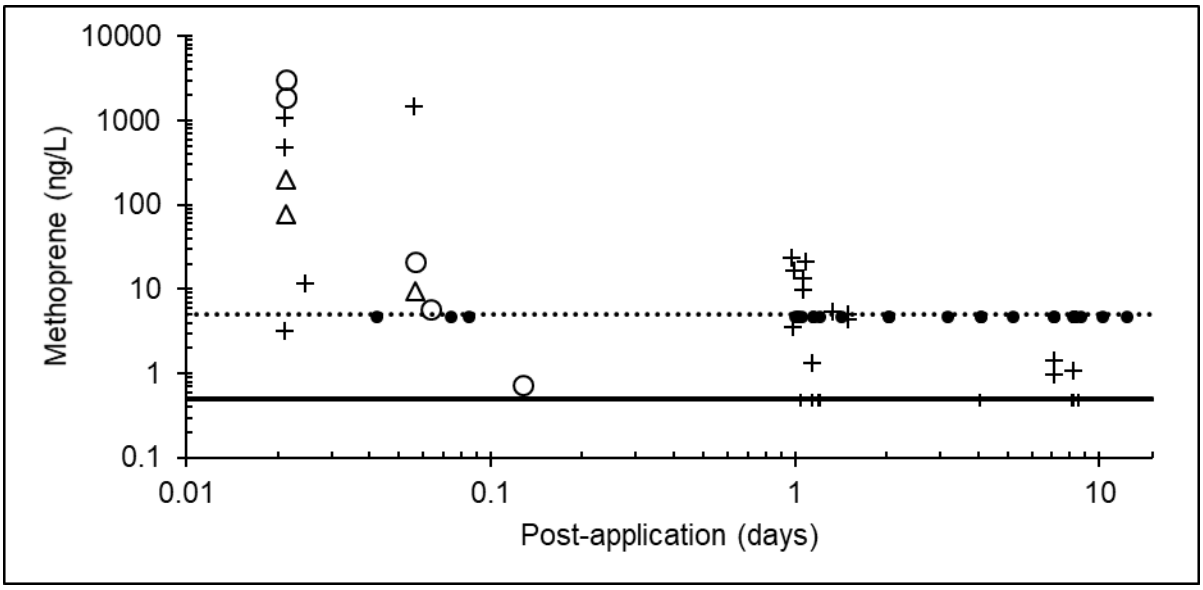

821

822 Figure 2. Aqueous sampling data from JN and TP marsh ditches, all events (+ = SBU whole
823 water detections; ○= SBU filtered water detections; Δ = USGS filtered water detections; dotted
824 line = USGS ND, with points indicating ND results; solid line = SBU ND, with slashes
825 indicating ND results)

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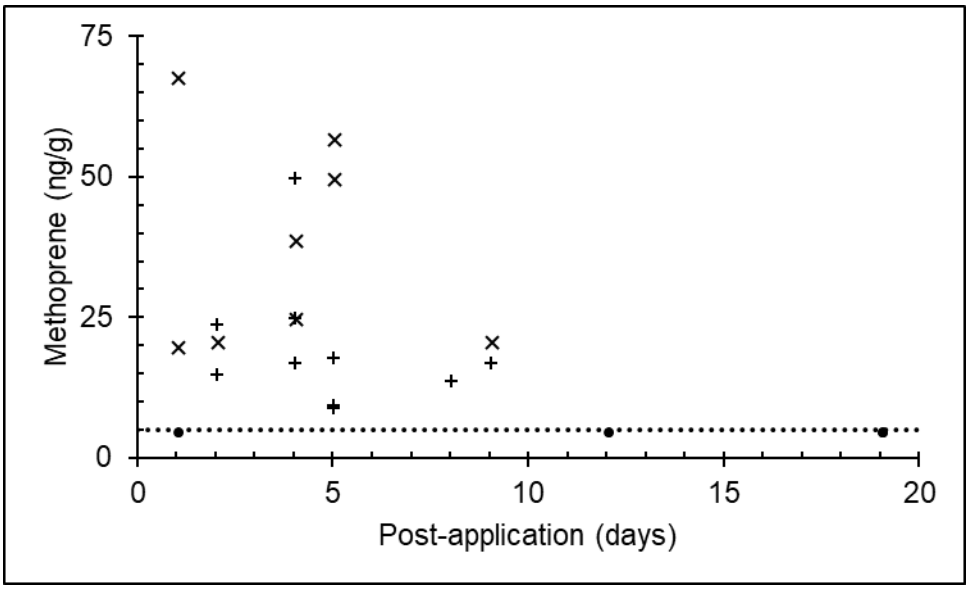

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830 Figure 3. Sediment sampling results from JN and TP ditch sediments (x = intertidal detections; +

831 = subtidal detections, dotted line = ND, with points indicating ND results)

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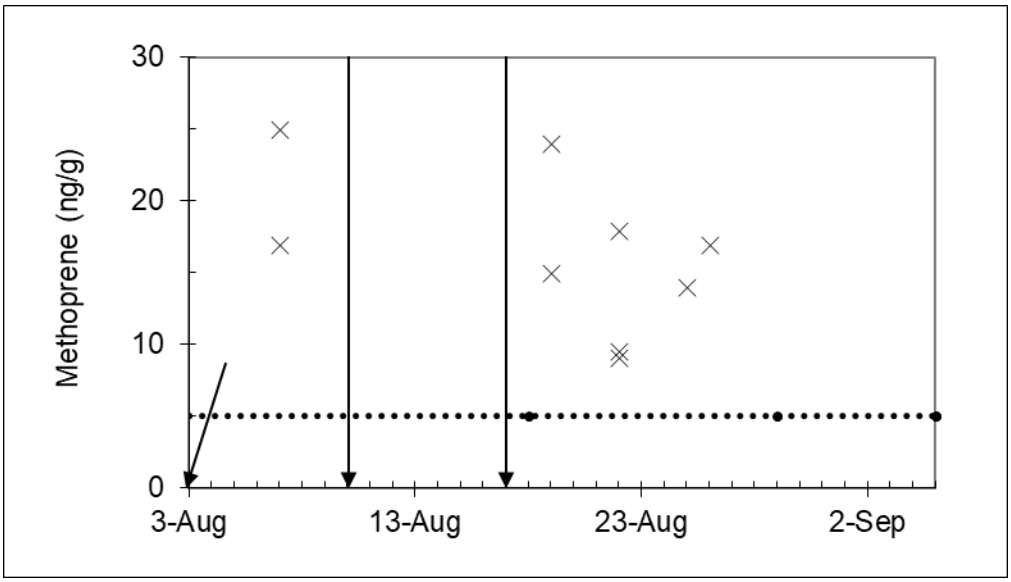

Figure 4. Time series of JN subsurface sediment concentrations (arrows indicate methoprene application dates; x = detections; dotted line = ND, with points indicating ND results)

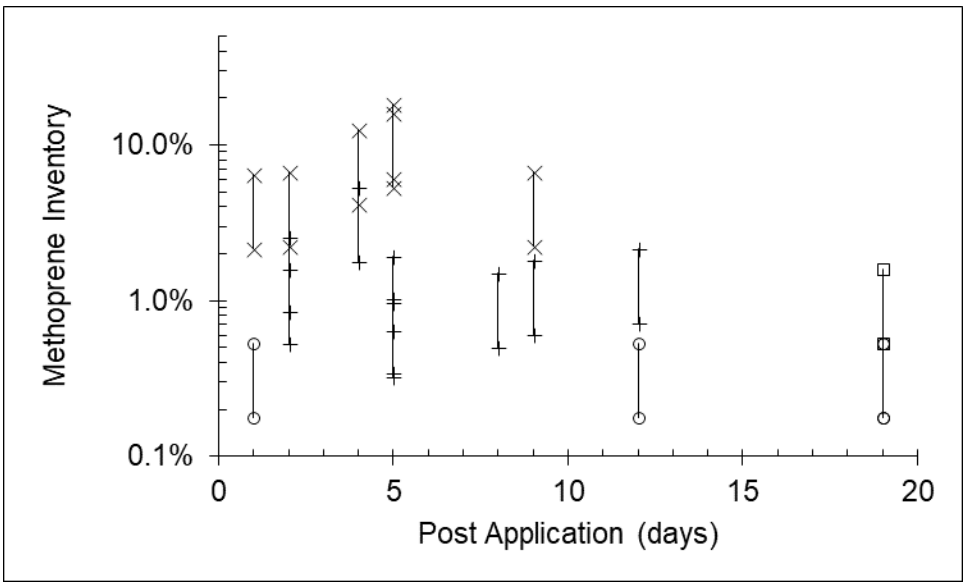

Figure 5. Subsurface and intertidal sediment sample concentration as a percent of applied methoprene (range indicates uncertainty regarding bulk density) (+ = subsurface detection; ○= subsurface non-detection; x = intertidal detection; □ = intertidal non-detection).

Appendices

Appendix A. Developmentally-related non-target organism impacts from methoprene

Organism	Effect	Concentration	Exposure	Reference
Fish	Gene Alteration	6 mg/L	24 hrs	[A1]
Zebrafish	Altered sex ratio (60% male)	5 ppm in diet	120 d	[A2]
Frogs	Developmental deformities	300 µg/L	96 hrs	[A3]
Tadpoles (Hyla versicor)	Tadpole survival with predator exposure, body length	659 µg/L[a]	10 d	[A4]
Mysid shrimp	Loss of fecundity	2 µg/L	18-21 d	[A5]
Mysid shrimp	Interference with embryonic development	100 µg/L	21 d	[A6]
American lobster	30% mortality, stage II larvae	1 µg/L	72 hrs	[A7]
American lobster	Concentration in eye stalks	50 µg/L	4 hrs	[A8]
American lobster	90% mortality, Stage IV larvae	50 µg/L	72 hrs	[A8]
American lobster	Changes in gene expression	50 µg/L	3-6 weeks	[A9]
American lobster	Hepatopancreas abnormalities	25 µg/L	24 hrs	[A10]
Uca pugnax (mud crab)	Weight gain through molting	100 ng/L	28-68 d	[A11]
Water fleas	Sex determination effects	489 µg/L (EC50)	7-10 d[b]	[A12]
Blue crab	Chitin synthesis inhibition (tissue cultures)	300 µg/L	6-20 hrs	[A13]
Eastern oyster	Number of larval swimmers	3.33 mg/L	96 hrs	[A14]
Eastern oyster	Reduced juvenile growth	625 µg/L	21 d	[A14]
Hard clam	Reduced juvenile growth	625 µg/L	21 d	[A14]
Daphnia magna	Reduced growth rate	4 µg/L	6 d	[A15]
Daphnia magna	Reduced feeding	400 µg/L	3 d	[A16]
Daphnia magna	Reduced fat content	725 µg/L	3 d	[A16]
Daphnia magna	Reduced molt frequency	1 µg/L, 60 ng/L[c]	6 d	[A15]
Daphnia magna	Inhibited molt	180 µg/L	3 d	[A16]
Daphnia magna	Increased age for reproductive maturity	10 µg/L	6 d	[A15]
Daphnia magna	Decreased fecundity	8 µg/L, ~60 ng/L[c]	6 d	[A15]
Daphnia magna	Enhancement of methyl farnesoate effects	310 µg/L	7-10 d	[A17]
Daphnia magna	Gene expression changes	39 µg/L	24 h	[A18]
Daphnia pulex	Suppression of methyl farnesoate effects	10 µg/L	6 d	[A19]
Pogonomyrmex	Intergenerational development impacts	5 g/L (topical application)	weekly (8 weeks)	[A20]

Osmia rufa	Termination of diapause	200 µg/bee		[A21]
Apis mellifera	Change in foraging (behaviorial development)	200 µg/bee		[A22]

[a] calculated as AI in solid application [b] effects must occur within a 12 hr. window [c] "double-segmented regression line"

849 **Appendix B. Theoretical estimates of environmental concentrations of liquid methoprene**
850

Citation	Concentration ng/L	Authors' Comments
[A13]	300,000-1,500,000	Concentrations likely to be seen in the environment
[A23]	80,000	Manufacturer salt marsh application rate into 6 in. deep water might be as high as this
[A24]	<50,000	No impacts to water fleas if environmental concentrations are less than 50 ppb, as is expected
[A14]	48,800	0.146 kg/AI/ha application rate, 30 cm deep pond
[A23]	40,000	Manufacturer salt marsh application rate into 12 in. deep water might be as high as this
[A25]	12,000	Effective field concentration (application rate for liquid Altosid into a 15 cm deep pool)
[A26]	11,000-20,000	Estimated field concentrations
[A27]	10,000	Typical field application rates
[A28] [A29].	8,000	Estimated environmental concentration (application rate for liquid Altosid into a 15 cm deep pool)
[A3]	4,400-6,000	Label application rate for a 0.25 m deep pond, no degradation or absorption
[A30]	≤ 4,000	Maximal rate of release (including briquets)
[A31]	1,500	Controlled release formulation, field test (no information on method)
[A32]	0.5	Modeled maximum concentration at any location in Long Island Sound, Sept. 1999, based on run-off of West Nile virus control events

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Appendix C. Selected data from papers reporting concentrations resulting from methoprene briquet use

Paper	Sample Type	DL (ng/L)	Detection Frequency	Max. (ng/L)	Notes
[S33]	Open fields	200	40%	8,300	Max.: 1 month after application
[S34]	Wetland ponds	400	50%	510,000	Each sample aggregated from 10 sub-samples
[S35]	Catch basins *Sedimented* *Half-full* *Empty* Outfalls	30	 *Most* *Few* *Rare*	 *15,000* *700* *150* 240	Max. immediately following application; daily sampling for 30 d
[S36]	Catch basins Outfalls Harbor Streams	~5	 3% 7%	4,350 1,520 100 650	Sampling of catch basins and outfalls not well defined
[S37]	Sh. catch basin Deep catch basin Sewer outfall	20-50		15,000 3,000 1,200	70% catch basin samples <300 ng/L
[S38]	Catch basins *Sedimented* *Cleaned*	5	 *<50%* *<20%*	 *845,000* *5,560*	One reference to a 2.2 mg/L (2,200,000 ng/L) exposure not explained
[S39]	Catch basin IC IC Outfalls PT Outfalls EOS	20-500	25% 17% ? 40% 0%	122,000 40 622,000 140	Max. sample not clearly explained
[S40]	Sh. catch basin Deep catch basin Outflow Pond	180-200	25% 0% 0% 0%	500	Trace amounts (50-120 ng/L) in catch basins and pond, but not reported: below DL
[S41]	Catch basins	140	7% (2006) 2/45 (2008)	13,000 11,400	18 μg/L (18,000 ng/L) detected pre-treatment; highest concentrations may be from pesticide fragment

DL = Detection limit; Max. = maximum concentration; IC = Inspection chamber; PT = post-treatment; EOS = end of season; Sh. = shallow; d = days

860 **Appendix D. Sample types**

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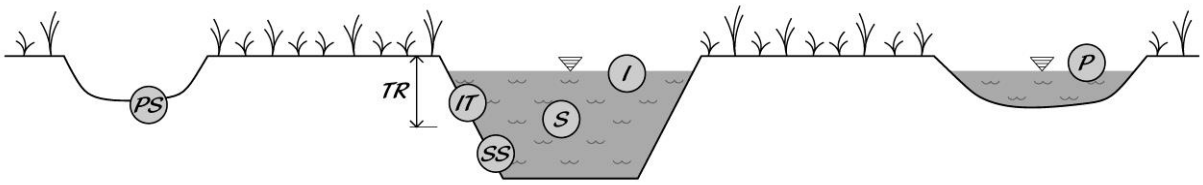

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863 Cut-away view. Aqueous samples: I = interface, S = subsurface, P = panne. Sediment samples:
864 TR = daily tidal range; IT = intertidal; SS = subtidal; PS = panne scraping.

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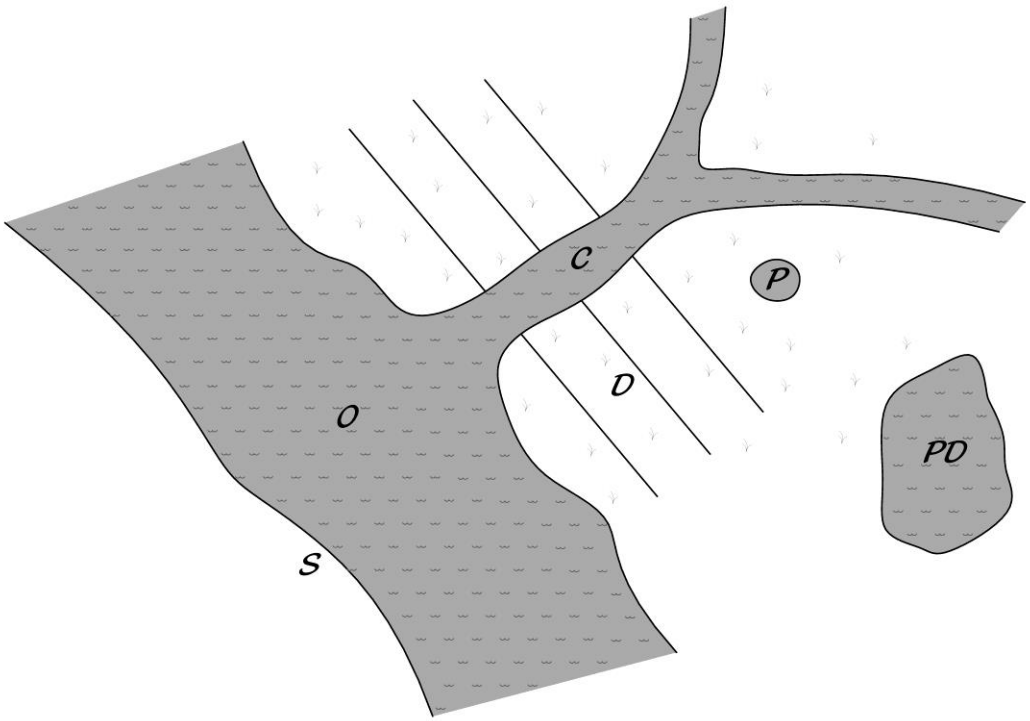

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867 Plan view: O = offshore (main river or channel); C = channel (inter-marsh); D = ditch (man-
868 made feature); P = panne (ephemeral surface water feature); PD = pond (permanent, larger
869 surface water feature); S = shore

Appendix E. SBU Sediment analysis procedures

For SBU sediment analyses, samples were freeze-dried and 2 g aliquots were extracted with a sonic probe in Teflon centrifuge tubes three times with 50:50 acetone:dichloromethane; *d*-6-malathion was spiked as a surrogate standard. Extracts were reconstituted in hexane and purified on Florisil SPE cartridges (Supelco, Bellefonte, PA) that had been preconditioned with ethyl acetate containing 1.5% H_2O, followed by hexane. After loading the sample to the cartridge, 6 mL of hexane was eluted and discarded and methoprene was eluted from the cartridge with 5 mL of hexane:ethyl ether:methanol (70:30:5). Solvent was evaporated to dryness, and reconstituted to 200 µL of hexane to which *d*-10-phenanthrene was added as an internal standard. GC-MS analysis was utilized for analysis of sediment extracts, as the greater amount of co-extracted organic matrix interfered with electrospray ionization. This was completed using an HP 5890 series II GC equipped with VG Quattro mass spectrometer with a RTX-5MS column (Restek, Bellefonte, PA): 30 meters, ID = 0.25 mm, and film thickness = 0.25 µm. GC parameters were inlet = 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 the column. The mass spectrometer was operated with electron impact ionization in selected ion monitoring mode. The quantitation ion for methoprene was 73 m/z with confirmation ions including 100 and 152. Identification of the detections as methoprene was supported by the shape and retention time of the MS 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), and the consistency of these with spiked matrix experiments.

Appendix F. Intercalibration Results

True Value (ng/l)	SBU Result (ng/l)	Percent Agreement	USGS Result (ng/l)	Percent Agreement
174	150	86.2	170	97.7
872	740	84.9	768	88.1

SBU and USGS analytical results compared to a known sample (organized by the Public and Environmental Health Laboratory, Suffolk County Department of Health Services, Hauppauge, NY)

Appendix G. Detection concentrations of liquid methoprene in the environment or in mesocosm experiments

Citation	Concentration ng/L	Comment
[A42]	9,030	25 min. after application, surface film, filtered
[A43]	6,000	Single highest measurement (mesocosm)
[A43]	2,200	Day 1 sample for liquid Altosid (average of 8 samples) (mesocosm)
[A43]	<1,000	85 percent of all samples, samples taken 1-35 days post application, all formulations (mesocosm)
[A42]	846	2 hr. after application, surface film, filtered
[A44]	640	Day 6, whole water, subsurface
[A42]	631	5 min. after application, surface film, filtered (1.71 ppb methoprenic acid)
[A43]	320	Geometric mean, samples taken over 35 days, liquid Altosid (ND results = 0.1 ppb) (mesocosm)
[A42]	216[a]	40 min. after application, surface film, filtered
[A44]	190	Day 1, replicate samples, whole water, subsurface
[A44]	140	Day 1, whole water, subsurface
[A44]	130-140	Day 1, replicate samples, whole water, subsurface
[A42]	82[a]	40 min. after application, subsurface, filtered
[A42]	39	1 hr. after application, surface film, filtered
[A42]	10[a]	30 min. after application, surface film, filtered
[A45]	7.39	Whole water, subsurface, one day after application (USGS filtered sample at the same time-place was <0.005) [A42]

[a] reported on here

Appendix H. Water column-sediment partitioning estimate procedure

We made a very rough estimate of the partitioning of methoprene between the water column and sediments. Using label application rates (280 ng/cm^2) and assuming relatively even distribution of the pesticide over the sampling depth:

$$C_s = AR/(BD \times d) \qquad \text{(Eq. 1)}$$

with

C_s = concentration in the sediment (ng/g)
AR = application rate (ng/cm^2)
BD = bulk density (g/cm^3) (see Table F-1)
d = depth of the sample (cm) (see Table F-1)

Sediment Type	Bulk Density Estimates (g/cm^3)	Depth of Sample (cm)
ST	0.1-0.3	1
IT	0.3-0.9	1
Other		
TP MS ps	1-1.5	0.3
JN MS pond	0.3-0.9	1
TP am	0.3-0.9	0.3
TP outer pond	0.1-0.3	1

Table F-1. Sediment pesticide calculations assumptions (ST = subtidal; IT = intertidal; TP = Timber Point; JN = Johns Neck; MS = marsh surface; am = algal mat; ps = panne scraping)

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