Quality Assurance Project Plan

Methoprene Monitoring in Grant County Mosquito Control District #1

by
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May 2005

303(d) Listings Addressed in this Study: None

Waterbody Numbers:
Moses Lake - WA 41-9250
Potholes Lake - WA 41-9280
Crab Creek - WA 41-1010
Rocky Ford Creek - WA 41-2010
Winchester Wasteway - WA 41-1110
Frenchman Hills Wasteway - WA 41-1120
Lind Coulee - WA 41-3500

User Study ID: AJOH0047

Approvals

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Kelly McLain, WQP- PDS-TSU	Date
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Will Kendra, Section Manager, EAP-WES-TSU	Date
Approved by:	May 16, 2005
Stuart Magoon, Director, Manchester Environmental Laboratory	Date
Approved by:	May 19, 2005
Cliff Kirchmer, Ecology Quality Assurance Officer	Date

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Abstract

A Quality Assurance Project Plan is provided for monitoring the mosquito larvicide methoprene and selected breakdown products in Grant County Mosquito Control District #1 (MCD #1) during the 2005 spray season. MCD #1 is the largest user of methoprene in Washington State. Seventy-two water samples will be collected during May and August 2005; methoprene, methoprenic acid, citronellic acid, and citronellal will be analyzed at sub-parts per billion levels. The objectives of the study will be to determine the persistence of these chemicals, evaluate the environmental significance of concentrations detected, and provide data for renewal of the NPDES permit.

Background

Methoprene* (trade name Altosid) is permitted for use to control mosquitoes in Washington State. Methoprene mimics a juvenile growth hormone, preventing mosquito larvae from maturing into adults. Unable to metamorphose, the mosquitoes die in the pupal stage. Methoprene comes in liquid, granular, pellet or briquette form and is applied directly to the water where mosquito larvae are found.

Grant County Mosquito Control District #1 (MCD #1) began using methoprene in 1983 as a replacement for organophosphate insecticides. MCD #1 currently uses about 400 gallons of methoprene annually to control mosquitoes over a 1,000 square mile area (Figure 1.) The application season stretches from early April to mid-October. Most of the application is done by aerial spraying, often in combination with *Bacillus thuringiensis* (Bti), a naturally occurring bacterium which is also active against mosquito larvae. There is insignificant use of methoprene granules or pellets; briquettes are used in some catch basins. Grant County is the largest user of methoprene in Washington State and may be one of the largest users in the nation.

Methoprene degrades relatively quickly in water. It has low toxicity to humans, is practically non-toxic to birds, and has only slight toxicity to fish. Methoprene is, however, highly toxic to some freshwater invertebrates, although the effects are not permanent and populations recover. More detailed information on fate, effects, and potential environmental concerns can be found in a literature review in Appendix A.

The Washington State Department of Ecology (Ecology) Water Quality Program (WQP) has requested that the Environmental Assessment Program (EA Program) monitor methoprene concentrations in the surface waters of MCD #1 during the 2005 application season. The WQP wants to know what kind of concentrations of methoprene and its breakdown products occur when there is treatment on this scale and to verify that the concentrations are not a significant concern for aquatic life. This information is needed by November 2006 before the next issuance of the NPDES permit, scheduled for May 2007.

In commenting on the current NPDES permit for aquatic mosquito control, the Washington Department of Fish and Wildlife (WDFW) raised concerns about possible adverse impacts of methoprene to several wildlife species (Beach, 2003). One of these species was the northern leopard frog, which is found only in Grant County.

The northern leopard frog (*Rana pipiens*) is a Washington State endangered species. Leopard frogs were historically present at >18 locations in eastern Washington, with occupied areas primarily distributed along the Columbia River and its tributaries. Leopard frogs have declined for unknown reasons, with dams, non-native fish and bullfrogs, and agriculture (chemicals and land conversion) all potential factors. Surveys conducted in Washington since 1992 have documented northern leopard frogs at only two areas, both in the Crab Creek drainage in Grant

*2,4-Dodecadienoic acid, 11-methoxy-, 3,7,11-trimethyl-, 1-methylethyl ester; CAS No. 40596-69-8

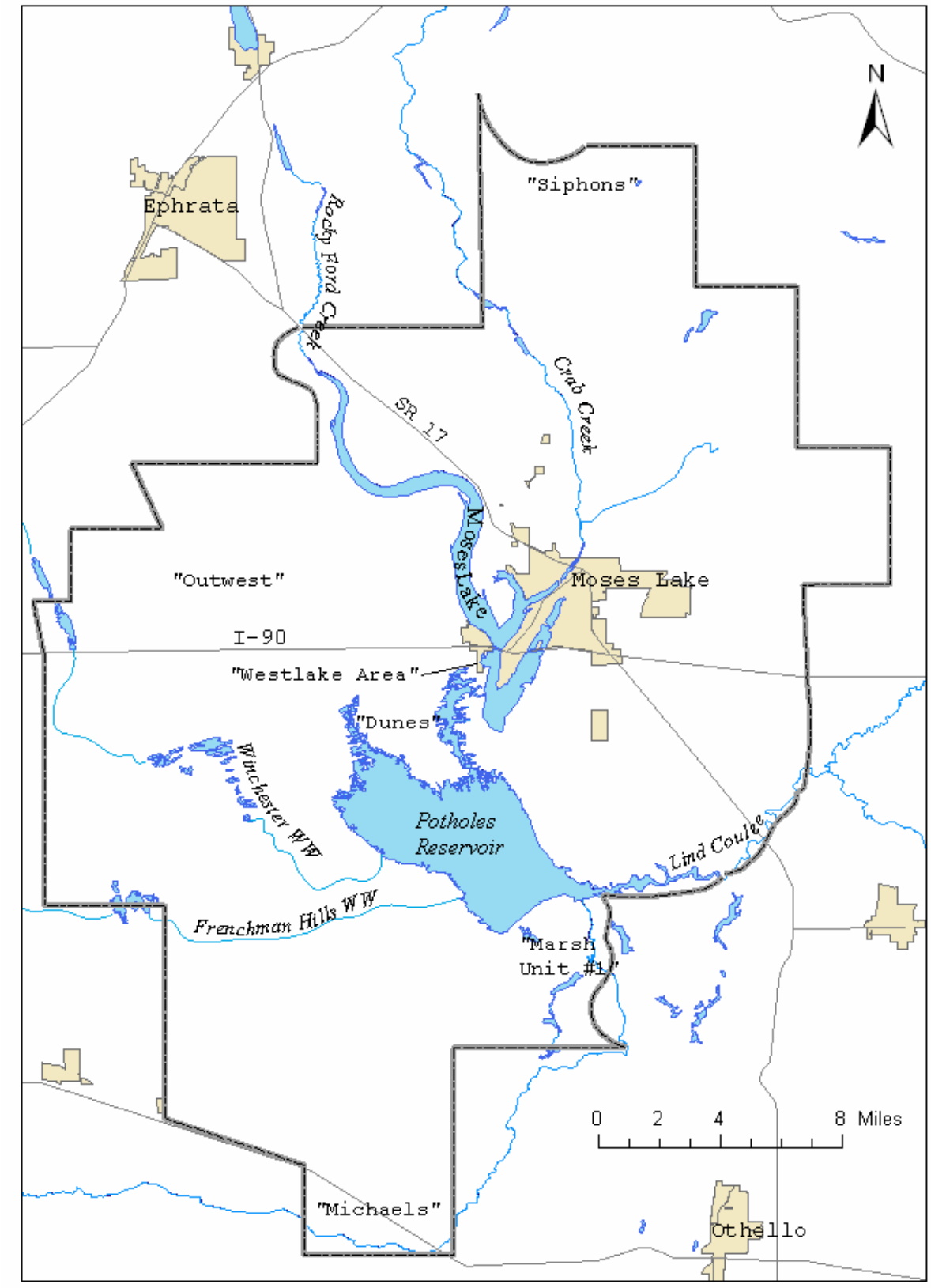

Figure 1. Approximate Boundary of Grant County Mosquito Control District #1

County (Leonard et al., 1999; McAllister et al., 1999). Both populations occur on land administered by WDFW: one at the Gloyd Seeps Wildlife Area (GWA) and one at the Columbia Basin Wildlife Area (CWA) at Potholes Reservoir, which is jointly administered with the U.S.D.I. Bureau of Reclamation. The CWA population is larger with more potentially suitable habitat and may, therefore, have greater likelihood for persistence. However, both populations are small, spatially restricted, and considered highly vulnerable to extinction. CWA is located in the Westlake area, to the west of Moses Lake and south of I-90 (see Figure 1). (Germaine, 2004)

WDFW has recommended that only Bti be used in northern leopard frog habitats (Beach, 2003). Section S1.4 of the NPDES permit states that: *Methoprene is restricted in areas designated by Washington Department of Fish and Wildlife except when a health threat exists in those areas as determined by the state and local health departments.* With the concurrence of the Washington Department of Health, Grant County Health District granted a temporary lifting of the methoprene restriction that has allowed Grant County to apply methoprene to ponds in the Westlake area.

Project Description

In response to WQP's request, the EA Program will monitor methoprene applications in MCD #1 during the 2005 spray season. Approximately 72 field samples will be collected between May and August. The objectives will be to determine the persistence of methoprene and selected breakdown products in MCD #1, evaluate the environmental significance of concentrations detected, and to provide data pertinent to renewal of the NPDES permit. The sampling effort will generally be focused on time periods and locations where maximum concentrations are expected.

Organization and Schedule

Roles and Responsibilities

Name	Organization	Phone No.	Role
Kelly McLain	Ecology WQP	360- 407-6938	Client
Art Johnson	Ecology EAP-WES-TSU	360-407-6766	Project lead
Kristin Kinney	Ecology EAP-WES-TSU	360-407-7168	Field assistance
Dale Norton	Ecology EAP-WES-TSU	360-407-6765	Unit supervisor
Jim Thompson	Grant County MCD #1	509-765-7731	Methoprene applicator
Steve Germaine	WDFW Wildlife Program	360-902-2499	Westlake Ponds sampler
Jo Wisniewksi	WDFW Wildlife Program	509-750-1718	Westlake Ponds sampler
Bob Carrell	Manchester Laboratory	360-871-8804	Methoprene analyst
Dean Momohara	Manchester Laboratory	360-871-8808	Supervisor
Stuart Magoon	Manchester Laboratory	360-871-8801	Laboratory director
Cliff Kirchmer	Ecology EAP	360-407-6455	Quality assurance officer
Carolyn Lee	Ecology EAP-WES-TSU	360-407-6430	EIM data entry

Schedule

Date	Task
May and August 2005	Field work conducted and samples submitted to laboratory.
October 2005	All laboratory analyses completed and data reported to project lead.
January 2006	Draft report completed.
March 2006	Final report completed
March 2006	EIM data entry completed.

Cost Estimate

The Ecology Manchester Environmental Laboratory estimates the cost of analyzing methoprene and selected degradation products at $150 per sample. The total laboratory cost for this project is estimated at $14,100 (50% discounted price at Manchester Laboratory; true cost is 2X).

Quality Objectives

Measurement Quality Objectives (MQOs) state how good the data must be in order to meet project objectives. The MQOs for the methoprene study are shown in Table 1. Recoveries of surrogate compounds (2,4,6-tribromophenol and 2,4-dichlorophenylacetic acid) are considered the most significant, bottom-line results for estimating the accuracy of methoprene determinations.

Table 1. Measurement Quality Objectives for Methoprene Study

Parameter	Laboratory Control Samples (% recovery)	Duplicate Samples (RPD)	Matrix Spikes (% recovery)	Matrix Spike Duplicates (RPD)	Surroate Standards (% recovery)	Lowest Cncentration of Interest
Methoprene	70-130	40	70-130	40	70-130	0.2 ug/L

As shown in Table 1, the lowest methoprene concentration of interest in this study is 0.2 ug/L. Only limited water quality criteria are currently available for methoprene. Results from the study will be compared to the Ontario Ministry of the Environment water quality values listed below as one means of assessing the significance of the findings (OMOE, 2004). The OMOE Draft Interim Water Quality Objective of 0.2 ug/L is the basis for setting the lowest concentration of interest.

Methoprene (ug/L)	OMOE Water Quality Value
0.2	Draft Interim Water Quality Objective
1.6	Numerical Benchmark for Amphibians
10	Numerical Benchmark for Invertebrates
80	Numerical Benchmark for Fish

Study Design

The weekly schedule that MCD #1 typically follows for methoprene applications is summarized below (see Figure 1):

Day	Locations
Monday	Winchester Wasteway (upper), Rocky Ford Creek, Moses Lake, "Outwest"
Tuesday	Frenchman Hills Wasteway, Winchester Wasteway (Lower), "Michaels," "Marsh Unit #1"
Wednesday	Potholes Reservoir Dunes, "Westlake Area"
Thursday	Crab Creek
Friday	Lind Coulee, "Deadman's Corner", "Siphons"

The decision on specific areas to be sprayed within the above locations is based on surveys (i.e., dip samples) to determine larval densities. MCD#1 decides where to spray based on the previous day's survey. Methoprene applications are normally completed by noon.

Depending on the time of year, water levels, and other conditions, certain watersheds are more heavily sprayed that others. In general, the periods when the most methoprene is applied are as follows:

Watershed	Maximum Application Period
Crab Creek	June – August
Moses Lake	All season
Rocky Ford Creek	June – August
Potholes Reservoir Dunes	April – May
Winchester Wasteway	April – May
Frenchman Hills Wasteway	April – May
Lind Coulee	June – August
Westlake Area	April – May

As described in Appendix A, methoprene degrades relatively rapidly. The manufacturer states that the maximum expected field concentration is 10 ug/L when the liquid formulation is properly applied. Most of the methoprene would be expected to degrade within 7-10 days. The Ontario Ministry of the Environment has done extensive methoprene monitoring and reports rarely detecting concentrations greater than 0.2 ug/L (Tim Fletcher, Personal Communication). Some degradation products may have longer persistence.

In light of methoprene's short persistence, the focus of the present monitoring effort will be to collect worst-case samples. The budget allocated allows 72 field samples to be analyzed, including QC samples. Therefore, it is proposed that three sets of samples be collected in May during the period when Potholes Reservoir, Winchester Wasteway, and Frenchman Hills Wasteway are most heavily treated and that three sets be collected in August during the period when Crab Creek, Rocky Ford, and Lind Coulee are most heavily treated. Some focused

sampling will also be done of Westlake area ponds that WDFW has identified as having northern leopard frogs and that are likely to receive methoprene applications this year.

The specific sites listed below are proposed for monitoring:

Waterbody	Sampling Site
Winchester Wasteway	Road C S.E.
Frenchman Hills Wasteway	Road C S.E.
Moses Lake	Outlet at Sand Dunes Road
Crab Creek	Road 7 S.E.
Rocky Ford Creek	State Route 17
Lind Coulee	State Route 17
West Lake Area Ponds	To be Determined

The first six sampling sites are at the downstream end of the watersheds of interest. Results for these sites will integrate the effects of upstream spraying. Except for Moses Lake outlet, where only a few discrete shoreline areas are treated, methoprene applications typically occur within close proximity to each of these sites. There is no easily accessible and representative sampling location for the Potholes Dunes applications, so no samples will be taken in that waterbody.

Samples for these six sites will be collected over a three week period in May and a three week period in August. The week-1 samples will be collected on Monday, the week-2 samples on Wednesday, and the week-3 samples on Friday. For sites where spraying is being done the same day, the samples will be collected several hours after the applications are completed. This sampling design is intended to give results that are broadly representative of methoprene and breakdown product concentrations in MCD #1 during the peak application periods. Under this schedule, the delay between methoprene application and sample collection for any given site will vary from several hours to several days. Forty-two samples will be collected for this part of the study.

Grant County has indicated they will spray some ponds in the Westlake area this year. The peak application period for this area is April through May, which overlaps the period when early-stage tadpoles should be present. Therefore, the Westlake Pond samples will be collected in May.

A total of thirty samples have been allocated to the Westlake Ponds. WDFW has expressed an interest in sampling three ponds. Assuming that each pond is sprayed weekly, the general approach will be to sample on day-0 (day of application), day-1, day-3, and day-6 or-7 (prior to second application) and then to repeat the same sampling schedule the following week. If a pond is not sprayed the second week, then additional sampling will continue through day-14 or a different pond will be sampled, at WDFW's discretion.

Table 2 summarizes the sampling design proposed for the methoprene study.

Table 2. Sampling Schedule for Methoprene Study (Number of Samples)

Agency	Locations	No. of Sites	May-05			Aug-05			Total Samples
			week 2	week 3	week 4	week 1	week 2	week 3	
Ecology	Creeks, Waste-ways, etc.	6	8*	6	7†	8*	6	7†	42
WDFW	Westlake Ponds	3	15**	15**					30

*includes one field blank and one replicate sample

†includes on replicate sample

**includes one field blank and two replicate samples

Sampling Procedures

Methoprene samples will be collected as simple grabs using 1-liter amber glass bottles provided by the Manchester Laboratory (Table 3). Use of amber glass is important to prevent degradation by UV light. The sampling procedure will be to shove the inverted bottle straight downward into the water and gain the remainder of the sample underwater. This will incorporate some of the surface layer concentration in the sample, a potentially important factor in quiescent waters such as the Westlake Ponds.

Each bottle will be filled to the shoulder and a label attached showing the site name, sample number, date, time, and name of sample collector. Each bottle will be wrapped separately in bubble wrap, put in a plastic bag, and placed on ice in a cooler immediately after collection. The temperature of each waterbody will be recorded at the time of sample collection. The latitude and longitude of each sampling site will be recorded from a GPS receiver.

Table 3. Sample Containers, Preservation, and Holding Time for Methoprene Study

Parameter	Matrix	Minimum Quantity Required	Container	Preservative	Holding Time
Methoprene	surface water	1 liter	1 liter amber w/ Teflon-lined lid	Cool to 4^{o}C	7 days

Detailed instructions for sample collection and shipment will be provided to WDFW. WDFW will coordinate with MCD #1, the project lead, and Manchester Laboratory before collecting any samples.

Measurement Procedures

Table 4 shows the anticipated number of samples, expected range of results, required reporting limits, and analytical methods. The samples will be analyzed at Manchester Laboratory. To the extent possible, Manchester will extract all samples on the day received.

Table 4. Measurement Methods for Methoprene Study

Parameter	Sample Matrix	Approximate Number of Samples	Expected Range of Results	Required Reporting Limit	Sample Preparation Method	Analytical Method
Methoprene	surface water	72	0 - 10 ug/L	0.1 ug/L	SPE	Zimmerman et al. (2001)-modified

Methoprene and three of its breakdown products--methoprenic acid, citronellic acid, and citronellal--will be analyzed by GC/MS using a modification of a method described in Zimmerman et al., (2001). In this method, a surrogate compound is added and a small volume of sample is removed from the bottle. Then hexane is added directly to the remaining sample in the bottle and mixed. The hexane extract is removed, spiked with an internal standard, and evaporated under nitrogen. The sample components are separated, identified, and measured by injecting an aliquot of the concentrated extract into a high-resolution, fused-silica capillary column of a GC/MS system under selected-ion mode (SIM). Compounds eluting from the GC column are identified by comparing their measured ions and retention times to reference ions and retention times obtained by the measurement of control samples under the same conditions used for the water samples. The concentration of each identified compound is measured by relating the MS response of the quantitation ion produced by that compound to the MS response of the quantitation ion produced by the surrogate standard.

Manchester's modifications to the above method will consist of: 1) using solid phase extraction (SPE) in place of liquid-liquid extraction, 2) using the internal standard (dibromooctafluorobiphenyl-d_{10}) rather than the surrogate to calculate methoprene recovery, and 3) extracting 1 liter rather than 247 mL.

Manchester has conducted an Initial Demonstration of Capability (IDC) and determined that the Practical Quantitation Limit (PQL) for methoprene and methoprinic acid will be 0.1 ug/L. At this PQL there will be some uncertainty in determining whether the previously mentioned OMOE Draft Interim Water Quality Objective of 0.2 ug/L has been met.

PQLs for citronellic acid and citronellal could not be established. The IDC showed citronellal recoveries were poor - in the 6% to 8% range - possibly due to its volatility. Citronellic acid's recoveries were quite variable. Thus, citronellic acid and citronellal concentrations will be qualified as estimates, if found.

Quality Control

Field Quality Control

Field quality control samples will consist of transfer blanks and replicate samples (Table 5).

Table 5. QC Samples for Methoprene Study

Parameter	Field		Laboratory			
	Blanks	Replicates	LCS	Method blanks	Analytical duplicates	MS/ MSD
Methoprene	4/project	8/project	1/batch	1/batch	1/batch	4/project

The potential for contamination arising from sampling procedures, sample containers, or sample handling will be assessed with transfer blanks. Transfer blanks will also assess the potential for atmospheric contamination of samples collected soon after spraying.

Transfer blanks will be prepared in the field by pouring organic-free water, obtained from Manchester Laboratory, from one sample bottle to another and the bottle re-sealed. Two transfer blanks will be prepared during the sampling conducted by Ecology, one each in May and August. Two transfer blanks will also be prepared by WDFW for the West Lake Area sampling, one each in the early and later stages of the field work.

Field replicates will provide estimates of the total variability in the data (field + laboratory). The replicates will consist of two separate samples collected one immediately after the other. Eight replicates' samples will be collected in all, four during the sampling done by Ecology and four during the sampling done by WDFW. Samples for replication will be chosen to reflect a range of high and low concentrations.

All field QC samples will be submitted blind to the laboratory.

Laboratory Quality Control

Table 5 also shows the laboratory quality control samples to be analyzed for this project. To reduce cost, matrix spikes and matrix spike duplicates (MS/MSD) will be limited to four, selected at the discretion of the analyst. MS/MSD results reflect the process of sample duplication (field), analytic degradation (holding time), matrix interaction (sample/standard (surrogate or internal)), extraction efficiency, and analyte recovery.

In addition to the above QC samples, Manchester will conduct a small study to verify that seven days is an appropriate holding time for methoprene (Table 2). Six sample containers (1-liter amber glass w/Teflon-lined lids) of organic-free water will be spiked with methoprene to achieve a concentration of 10 ug/L. The samples will be stored under the same conditions as field samples (4^{o}C in the dark). Two sample containers each will be extracted for methoprene and breakdown products at one, three, and seven days after spiking. The holding time study will be initiated by Manchester at their earliest possible opportunity.

Data Management Procedures

Field data and observations will be recorded in a bound notebook of waterproof paper.

The data package from the laboratory will include a case narrative discussing any problems with the analyses, corrective actions taken, changes to the referenced method, and an explanation of data qualifiers. The data package should also include all associated QC results. This information is needed to evaluate the accuracy of the data and to determine whether the MQOs were met. This should include results for all blanks, surrogate compounds, and check standards included in the sample batch, as well as results for analytical duplicates and matrix spikes.

All project data will be entered into Excel spreadsheets. All entries will be independently verified for accuracy by another individual on the project team.

All project data will be entered into Ecology's Environmental Information Management System (EIM). Data entered into EIM follow a formal Data Validation Review Procedure where data is reviewed by the project manager of the study, the person entering the data, and an independent reviewer.

Audits and Reports

The Manchester Environmental Laboratory participates in performance and system audits of their routine procedures. Results of these audits are available on request.

A draft report of project findings will be prepared. The tentative date for this report is January 2006. The report will include, but not necessarily be limited to the following:

1) Map of the study area showing sampling sites.
2) Description of sampling and measurement procedures.
3) Assessment of data quality.
4) Summary tables of all chemical data.
5) Evaluation of methoprene's persistence.
6) Comparison of monitoring results with OMOE water quality values.
7) Recommendations pertinent to NPDES permit renewal.
8) Recommendations for follow-up studies as warranted.

The tentative date for the final report is March 2006.

Data Verification and Validation

Manchester will conduct a review of all laboratory data and prepare case narratives for all reported results. Manchester will verify that methods and protocols specified in the Quality Assurance (QA) Project Plan were followed; that all calibrations, checks on quality control, and intermediate calculations were performed for all samples; and that the data are consistent, correct, and complete, with no errors or omissions. Evaluation criteria will include the acceptability of holding times, instrument calibration, procedural blanks, spike sample analyses, precision data, laboratory control sample analyses, and appropriateness of data qualifiers assigned. Manchester will prepare written data verification reports based on the results of their data review. A case summary can meet the requirements for a data verification report.

The MQOs are the same as the laboratory's QC limits for this project. All MQOs will be validated, but the emphasis will be placed on the surrogates and lowest concentration of interest. Results for surrogate recoveries will be compared to QC limits. To evaluate whether the targets for reporting limits have been met, the results will be examined for "non-detects," and to determine if any values exceed the lowest concentration of interest.

The project lead will review the laboratory data packages and Manchester's data verification reports and validate the data. Based on these assessments, the data will be either accepted, accepted with appropriate qualifications, or rejected and re-analysis considered.

Data Quality Assessment

Once the data have been verified and validated, the project lead will determine if the data can be used to make the calculations, determinations, and decisions for which the project was conducted. If the results are satisfactory, data analysis will proceed.

Data analysis will consist primarily of assessing the evidence for persistence of methoprene and its breakdown products, and comparing the methoprene concentrations to the OMOE water quality values. If there is evidence of persistence, scatter plots will be used to illustrate the rate of degradation. If exceedances of water quality values are found, dot density plots will be used to show their locations, magnitude, and timing.

References

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Appendix A

A Literature Review on the Fate and Effects of Methoprene

by Brandi Lubliner, EA Program
March 11, 2005

Overview

Recently the West Nile Virus, a new human health threat to residents of the Unites States, has raised the bar in efforts to control mosquito populations. The long standing and commonly used mosquito larvicide Methoprene is considered to be an effective tool to control mosquito populations and reduce human health risk of several mosquito-borne diseases. Methoprene, a juvenile hormone analog, and its breakdown products are believed to disrupt primary gene regulation at the onset of metamorphosis, thus preventing insects from metamorphosing into adults (Yerushalmi et al., date unknown; Peterson et al., 2001; Hershey et al., 1998; and Degitz et al., 2003). Methoprene is effective at killing mosquitoes and midges, the typical target species for insecticide treatment; however, researchers are increasingly concerned about adverse impacts methoprene may have on nontarget species.

The relatively sudden increase in limb and retinal frog deformities in the United States over the last 10 years has lead to a myriad of studies investigating the potential causes for the deformities. Three prime suspects have emerged as likely causes of frog deformities: UV, methoprene, and trematodes (Johnson and Blaustein, 2003). While there is mounting evidence that each culprit alone can act to cause frog deformities, these elements likely act in concert and with other unknown synergistic environmental effects. However, few studies have looked into quantifying the cumulative impacts.

UV exposure treatments (full-sunlight for three months) to northern leopard frogs caused malformation in hind leg development at the larval stage in 97% of the sample population (Ankley et al., 2002). The global significance of this is noteworthy as the ozone layer thins and overall UV exposure increases. However, most frogs avoid prolonged exposure and will seek cover in the wetland field environment.

Johnson et al. (2002) revealed the astounding relationship between parasites and frog limb deformities. The trematode, *Ribeiroia ondatrae*, and its snail host (genus *Planorbella*) were significantly associated with population-level malformations in more than 12,000 amphibians in 101 sites in the Western U.S. The trematode populations were significantly associated in wetlands of anthropogenic origin and higher than normal orthophosphate levels. No evidence, directly or indirectly relating pesticides or breakdown products, was found in these malformed amphibians. (Johnson et al., 2002). The population level dynamics (fecundity, richness, and diversity) of trematode infections are not yet understood and research is ongoing.

Methoprene Dosage Studies

The solubility of Methoprene is 1.4 to 2.0 mg/L and it rapidly breaks down in both the lab and field environment. No studies have shown that methoprene is toxic in the field environment under properly applied conditions (Brown et al., 2000; Celestial and KcKenney, 1994; Degitz et al., 2003; Henrick, et al., 2002; Johnson et al., 2002; Peterson et al., 2001; and Pinkney et al., 2000). In 1994, Ross et al. evaluated the environmental concentrations over time for the four widely available methoprene products: Altosid Briquets, XR Briquets and Pellets, and ALTOSID Liquid Larvicide (ALL) formulations for the American Mosquito Control Association, Inc. They found that, over a 35-day study in a man-made microcosm, the geometric mean of the observed concentrations was as follows:

(s)-Methoprene	Geometric Mean
ALTOSID Liquid Larvicide	0.32ug/L
ALTOSID Briquets	0.32ug/L
ALTOSID XR Briquets	0.14ug/L
ALTOSID Pellets	0.24ug/L

The widely quoted maximum expected field concentration for properly applied Altosid Liquid Larvicide formulation 5% (s)-methoprene: 0.43lb/gallon (51g/L) applied to the maximum label rate (4 fluid ounces/acre [293ml/ha] to water 6 in. (15cm) deep should yield 10ug/L. The manufacturer states that the "residual" should be expected to last for seven to ten days when liquid Altosid is used.

In concert with the above study, Ross et al., 1994, also examined the maximum toxicant concentration that could be sustained by the fathead minnow at the most sensitive life stage (egg to juvenile). No affect to hatchability, fry survival, or total survival was found at the evaluated field concentrations used. However, growth (length and weight) were adversely affected by the highest concentrations (84 and 160ug/L).

Margin of safety for fathead minnow early life stages at field concentrations observed above. Margin of safety= MATC/mean concentration.

ALTOSID Liquid Larvicide	0.32ug/L	196
ALTOSID Briquets	0.32ug/L	196
ALTOSID XR Briquets	0.14ug/L	466
ALTOSID Pellets	0.24ug/L	240

Note: MATC (Maximum Acceptable Toxicant Concentration) is the geometric mean of the NOEC (no-observed effect concentration) and the LOEC (lowest observed effect concentration).

Lethal doses in laboratory experiments are well documented for some nontarget species that can occur in mosquito habitats at the time of insecticide treatments. The required dosages to kill non-target species far exceed the recommended use dose in the field (Brown et al., 2000; Celestial and KcKenney, 1994; Degitz et al., 2003; La Clair et al., 1998; Johnson et al., 2002; Peterson et al., 2001; Pinkney et al., 2000; and Yerushalmi et al., date unknown). However, it is important to

note that each study listed below is only a mortality study. The researchers did not examine sub-lethal impacts to the nontarget species such as behavior, fecundity, and growth.

Brown et al. (2000)

- Crimson-spotted rainbowfish (*Melanotaenia duboulayi*) did not reach LC_{50} at 12.5 times greater than estimated environmental concentrations (Altosid Liquid Larvicide 20% s-methoprene recommended rate of 12g/ha).
- Shrimp (*Ca. indistincta*) reached LC_{95} at 550 times greater than the estimated field concentration. (Altosid Liquid Larvicide 20% s-methoprene recommended rate of 60g/ha).
- Mosquito (Cx. Annulirostris) reached LC_{95} at 6 times greater than the estimated field concentration. (Altosid Liquid Larvicide 20% s-methoprene recommended rate of 60g/ha).
- (*Ae. Vigilax*) has LC_{90} = 0.17ppb cited research.

Celestial and KcKenney (1994)

- Crustacean (*R. harrisii*) – LD 100% no larval survival at 1000ug/L.
- Crustacean (*R. harrisii*) – reduced survival at 100ug/L.
- Crustacean (*R.. harrisii*) – no effect on survival less than 100ug/L.
- Cited Grass shrimp (*Palaemonetes pugio*) LD 100% at 1000ug/L and no effect on survival at 100ug/L.
- Cited crustacean (*Daphnia magna*) – reduced survival at 100ug/L.
- Cited target mosquitoes had similar lethal concentrations (*Culex pipens* and *triteniorhynchus*).

Breakdown Product Studies

The breakdown products of methoprene have received further scrutiny in recent years and have been found to be more persistent and more bioactive than the parent compound.
Several of the breakdown products can persist for longer periods of time and are acutely toxic to target and nontarget species. Degitz et al., 2003, investigated several methoprene degradation products to the northern leopard frog (*Xenopus laevis*) under laboratory conditions. He found that several of the degradation products are somewhat toxic in the lab; the concentrations necessary to cause toxicity were unlikely to be found in the field (not exceeding 0.01 mg/L). Evaluated concentrations over time of methoprene epoxide, 7-methoxycitronellal and methoprenic acid declined to near the detection limits within 7 days. 7-methoxycitronellic acid was the only degradation product to persist at its 24 hour concentrations at the end of the 7 day experiment.

Degitz accounted for different loss rates of each degradation product when in the presence of the frogs. In all cases, there were no concentration-dependent effects on the stability of the chemical over the 24 hours. For some of the chemicals there was measurable difference in loss rate, higher with the frogs present, which could be due to metabolism and/or further degradation.

Degradation Product	Toxicity Level	Toxic Effect	Time of Persistence
Methoprene			4 days 78.1% loss with frog present
Methoprenic acid	≥ 1.25 mg/L	Craniofacial malformations and edema	Minor loss, 5.9% over 4 days, without the frog
7-methoxycitronellal	≥ 2.5 mg/L	Developmental delay	4 days 99.8% loss with frog present
Methoprene epoxide	≥ 5 mg/L	Developmental delay	4 days 33.6% loss with frog present
Combination (7-methoxycitronellal + Methoprene epoxide)	10 mg/L	100% mortality before completion of exposure	
7-methoxycitronellic acid	≤ 30 mg/L		No time or concentration loss over 7 days

Degitz et al., 2003

The 'real world' losses are underestimated in this study because surface adhesion to plants and soils, uptake by other organisms, and UV exposure were not accounted for. This work is in agreement with an earlier field study in Minnesota by Henrick et al., 2002, that investigated (s)-methoprene treated waterbodies and found no statistical difference in frog deformities existed between Altosid Liquid Larvicide treated counties and untreated counties.

Ecosystem Dynamics

In an effort to examine the long-term effects of insecticide treatments on the nontarget macroinvertebrates, Hershey (1998) found that impacts to food web dynamics, species richness, and diversity had a two to three year lag time and caused population change within nontarget insect species. In her study, the first year of methoprene and *Bacillus thuringiensis israilensis* (Bti) showed no difference from control sites. Under a three year Bti regime, the density of nematocerans and nondiptheran predators decreased 67 and 80%, respectively. Under a methoprene treatment regime the pattern was stronger, where the overall density of nematocerans and nondiptheran predators decreased 65 and 46%, respectively by year two and 77 and 64%, respectively, by year three. The methoprene treatments are believed to have both direct and indirect effects on the food web.

The populations of insects that survive the treatment have been show to develop a tolerance to that insecticide. A significant tolerance to methoprene has been shown in studies in less than twenty generations laboratory selected of *culex* mosquitoes, houseflies, and flour beetles (Cornel et al., 2002). In California, routinely treated populations of mosquitoes began to show signs of tolerance to (s)-methoprene (Altosid Liquid Larvicide) in 1999. A study of these mosquitoes by Cornel et al., 2002, found that a common pasture mosquito, *Ochlerotatus nigromaculis*,

developed considerable resistance (several thousand fold) to methoprene products including ALL, Altoside XR-G, and a lesser extent to Altosid pellets. The area had received over 20 years of treatment with each summer receiving 10 applications or more, depending on when the fields were flood irrigated. The tolerance was reduced by switching to Bti for six consecutive applications.

Ramifications to the ecosystem of prolonged insecticide treatments need further examination. Methoprene altered *Daphnia* population dynamics were studied by Peterson et al., 2001. Exposure to nominal concentrations (10 and 100ug/L) of methoprene increased the incidence of all-female broods of the water flea (*Daphnia pulex*) and decreased the incidence of all-male broods. The decrease of male *Daphnia* could lead to a reduction of sexual reproduction, genetic recombination, and even a decrease in the population. *Daphnia* are a major part component of fish and invertebrate diets. In addition *Daphnia* consume a large amount of algae and a therefore play a major role in the control of water quality (Peterson et al., 2001). Olmstead et al. (2000) found that methoprene (0.16 and 0.32uM) exposure to Daphnia can alter the secondary sex characteristics. Hershey (1998) cautioned that the 3-year lag effect in the invertebrate community may have misled other researchers of shorter studies to think that Bti and methoprene were environmentally safe. Further investigation of biologically important food web species, is imperative to better understand the potential impacts on longer lived vertebrates, such as birds and fish that rely on the richness and diversity of untreated wetland ecosystems.

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