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Pelagic Organism Decline (POD): Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta 2008-2010

Progress Report

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1. Executive Summary

This report covers the project period of January 1– June 30, 2009. The study described here encompasses a sampling and toxicity monitoring program in the Sacramento-San Joaquin (SSJ) Delta, including several sites in Suisun Bay and the Napa River. Biweekly toxicity tests were performed using *Hyalella azteca*, an amphipod species resident in the Delta, and during March - May, additional ambient water from five sites (340, Cache-Lindsay, Hood, Light 55, and Suisun) was collected for larval delta smelt (*Hypomesus transpacificus*) toxicity testing. *In situ* monitoring was conducted at two DWR water quality monitoring stations (Rough & Ready Island on the San Joaquin River and Hood on the Sacramento River) using *H. transpacificus*, fathead minnows (*Pimephales promelas*), and *H. azteca*. A 7-d bioassay using low conductivity ambient waters from the lower Sacramento River watershed was conducted with the calanoid copepod, *Eurytemora affinis*. In addition, effect concentrations for pesticides, copper, and ammonia were determined for *H. transpacificus, P. promelas*, *H. azteca*, and *E. affinis*. Sensitivity testing with the water flea, *Ceriodaphnia dubia* is currently in progress.

<u>Water Quality at Field Sites</u>: Site-specific water quality parameters were routinely monitored in the field at the time of sampling. During this project period, temperature ranged from 7.3 (Cache-Ulatis) to 25.8^{o}C (Rough & Ready Island), dissolved oxygen from 5.5 (Suisun Slough at Rush Ranch) to 13.8 mg/L (Cache-Ulatis), specific conductivity from 116 (Hood) to 24,360 µS/cm (340), electrical conductivity from 98 (711) to 19,947 µS/cm (340), pH from 6.43 (915) to 8.61 (Cache-Ulatis) and turbidity from 2.4 (Rough & Ready Island) to 713.3 NTU (Napa). Total ammonia-N concentrations were highest at stations 405, 340 and Hood with maximum concentrations of 0.62, 0.59, and 0.56 mg/L, respectively. Un-ionized ammonia concentrations were highest at stations Cache-Lindsey and Light 55 with maximum concentrations of 0.021 and 0.017 mg/L, respectively.

<u>Field Monitoring with *H. azteca*</u>: The UC Davis Aquatic Toxicology Laboratory (UCD ATL) and California Department of Fish and Game (CDFG) collected water samples twice a month from 12 sites (340, 405, 508, 602, 609, 711, Light 55, Cache-Lindsey, Cache-Ulatis, 815, 902, and 915) by boat, and samples from four additional sites (Hood, Rough & Ready Island, Suisun, and Napa) via bank sampling. Samples exhibiting salinities greater than the *H. azteca* testing limit of 15 ppt were not collected. Waters were tested using a 10-day *H. azteca* water column bioassay with survival and growth as acute and chronic endpoints, respectively. Routine partial toxicity identification evaluation (TIE) tests were conducted on all water samples with piperonyl-butoxide (PBO), a chemical synergist/antagonist, to provide early evidence for the presence of two classes of toxic insecticides: pyrethroids and organophosphates. If toxicity (≥50% mortality within 7 days) was observed in a water sample, TIEs were initiated to identify the causative agent(s) and if a sample caused ≥50% mortality within 96 hours, follow-up samples were collected to investigate the source of toxicity. Water samples were submitted to the California Department of Fish and Game Water Pollution Control Laboratory (CDFG-WPCL) for chemical analysis when significant acute or chronic toxicity was detected.

<u>Acute Toxicity to *H. azteca*</u>: During this project period, one water sample collected from site 711 on 6/25/09 was acutely toxic, causing a significant reduction in amphipod survival within the 10 day test period. This sample reduced survival by 44%, but did not meet the re-sample or TIE triggers of ≥50% mortality within 96 hours or 7 days, respectively. An analytical sample was submitted to the CDFG-WPCL for analysis of a comprehensive suite of chemicals, and results are currently pending.

PBO Effect on 10-d Survival: The addition of PBO led to significant decreases in the survival of *H. azteca* in two ambient samples when compared to their non-PBO counterparts: site 340 collected 5/13/09 and Hood collected 6/23/09. The PBO-induced reduction in survival at site 340 was detected by the conservative Tukey's multiple comparison procedure and by USEPA standard statistical protocols, while the reduction in survival at Hood was only detected by the less conservative USEPA protocols. A sample collected at site 902 on 6/25/09 showed significantly reduced survival when treated with PBO compared to the PBO-treated control, but this reduction in survival was not significant when compared to survival in the untreated ambient sample water. Analytical samples from sites 340 and Hood were sent to the CDFG-WPCL for pyrethroid analysis. Pyrethroids were not detected in the sample collected from site 340 and results from Hood are currently pending.

Chronic Toxicity to *H. azteca*: Chronic toxicity (reduced growth compared to control) to *H. azteca* was not detected during this project period. In general, this endpoint was not a sensitive indicator of toxicity due to the variable size of the organisms, the variability of food content in Delta water samples, and the lack of food content in the control waters.

PBO Effect on 10-d Growth: The most common significant effects detected in *H. azteca* ambient sample tests were differences in growth resulting from the addition of PBO relative to the unmanipulated ambient samples. The conservative Tukey's test detected 5 significant reductions in growth (2.5% of samples tested) and 4 significant increases (2%), while the more sensitive USEPA protocol detected 18 reductions (9%) and 15 increases (7.5%). All samples resulting in a significant reduction or increase in growth detected by the Tukey's test were submitted for chemical analysis as were the majority of those detected by the more sensitive USEPA standard statistical protocols.

Toxicity Identification Evaluation (TIE): TIEs were not performed during this reporting period.

Analytical Chemistry Results: A total of 39 ambient water samples were analyzed for chemical contaminants during this reporting period, resulting in detections at 8 sites. Pyrethroid insecticides were detected in low concentrations from samples collected at Rough & Ready Island on 3/17/2009 (0.003 µg/L cyfluthrin) and Hood on 3/18/2009 (0.003 µg/L permethrin). The organophosphate insecticides chlorpyrifos, diazinon, and disulfoton were detected singularly or in combination at sites 508, 602, 815, 902, Cache-Ulatis, and Light 55. Although the majority of these detections were below the reporting limit of the analytical laboratory, the sample collected from Cache-Ulatis on 4/2/2009 resulted in the detection of 0.078 µg/L chlorpyrifos. Table 4-7 presents a detailed summary of samples submitted for chemical analysis, reason for submission, scan type, and results. In addition, beginning in February, water samples collected from sites 711, 902, Cache-Lindsey, Rough & Ready Island, and Suisun were submitted for routine metals analysis in order to obtain baseline metals data (Table 4-8). Results are pending.

Monitoring with Delta Smelt: During March – May, 2009, six delta smelt toxicity tests were conducted with samples collected from sites 340, Cache-Lindsey, Hood, Light 55, and Rough & Ready Island. At 96 hours, *H. transpacificus* survival was found to be significantly reduced relative to conductivity-specific and turbidity-specific controls in water from Cache-Lindsey collected on 4/15/09 and from Rough & Ready Island collected on 5/12/09. At 7 days, survival was reduced in Hood water collected on 4/28/09, Cache-Lindsey water collected on 4/30/09, and in Rough & Ready water collected on 5/12/09. Other instances of significantly reduced survival relative to conductivity-specific controls are difficult to interpret because of low turbidity in the sample waters, which is considered stressful to larval delta smelt. Survival was consistently high in samples collected at the high conductivity, high turbidity site at Suisun Slough at Rush Ranch, as was observed in tests performed in 2008. At site 340, where conductivity was higher and turbidity was lower than in Suisun Slough, survival was generally lower.

In Situ Monitoring: During the months of March - May, *in situ* monitoring was conducted at the DWR water quality monitoring stations located in Hood, CA (Sacramento River) and Rough & Ready Island in Stockton, CA (San Joaquin River). Six exposures using *H. transpacificus*, *P. promelas*, and *H. azteca* were conducted concurrently with ambient delta smelt toxicity testing in the laboratory. During this pilot project, no toxicity was detected in the Sacramento River at Hood or the San Joaquin River at Rough and Ready Island. *H. transpacificus* survival was generally higher in ambient water than in the control, potentially due to slightly higher water temperatures in the control system, *H. azteca* survival was consistently high in ambient water as well as controls throughout the *in situ* season. *P. promelas* survival was variable in both the control and ambient water. Poor *P. promelas* survival in controls was attributed to the addition of algal paste to optimize turbidity conditions for delta smelt larvae.

Copepod Testing: A 7-d bioassay using juvenile *E. affinis* was initiated on 5/1/09 with four low conductivity samples (711, Cache-Ulatis, Hood, and Light 55) and a series of low conductivity controls (100, 250, 500, 1000, 1900 µS/cm). The test method was modeled after the USEPA *Ceriodaphnia dubia* 7-d Survival and Reproduction Test (USEPA, 2002), chosen for its high survival, minimal water requirements, and ease of recording survival/mortality. In the control series, survival was highest in the 1900 µS/cm control (90% survival in 7-d) and decreased with decreasing conductivity. Survival was low in ambient samples with the exception of Cache-Ulatis (100% survival in 7-d) which may be due in part to the site's food content and/or higher turbidity. Survival was low in most of the ambient samples tested likely due to low conductivity, however, survival was always higher in ambient samples than in the corresponding conductivity controls.

Species Sensitivity Tests: Toxic effect concentrations for *H. transpacificus*, *P. promelas*, *H. azteca* and *E. affinis* were determined for a series of chemical contaminants present in the SSJ Delta, including pesticides, copper, and ammonia. Effect concentrations for *C. dubia* are currently being determined. *H. transpacificus* was found to be more sensitive than *P. promelas* to nearly all materials tested, while the relative sensitivities of the three invertebrate species varied depending on the material tested.

Sublethal Indicators of Contaminant Effects:

Three manuscripts are currently in preparation: *Beggel et al.* describe a study on the lethal and sublethal toxicity of commercial pesticide formulations and their active ingredients to larval fathead minnow (*P. promelas*), as the first part of an effort to link stress response at the molecular and the organism-level of biological organization. This study compared toxicity of two current-use insecticides, the pyrethroid bifenthrin, and the phenylpyrazole fipronil, to their commercial formulations, Talstar® and Termidor®. Commercial pesticide formulation contain a significant proportion (>90%) of so-called inert ingredients, which may alter the toxicity of the active ingredient(s). These insecticides are used for mosquito control, landscape treatment and structural pest control, and can be transported into surface water bodies via stormwater and irrigation runoff. The study presented here used fathead minnow larvae (*Pimephales promelas*), to determine effect thresholds for survival, growth and swimming performance after short-term (24 h) exposure to pure insecticides and insecticide formulations. Results demonstrate detrimental effects on swimming performance at 50% (fipronil) and 20% (bifenthrin) of the 24-h LC10. The LC10 was 0.92 $\mu g.L^{-1}$ for bifenthrin, and 305.57 $\mu g.L^{-1}$ for fipronil. Swimming performance was significantly impaired at 0.14 $\mu g.L^{-1}$ bifenthrin and 142 $\mu g.L^{-1}$ fipronil (measured). Detrimental effects on 7-d growth were observed following 24 h exposure to 53 $\mu g.L^{-1}$ (10% LC10) fipronil. Based on measured insecticide concentrations, both formulation products were more toxic than their pure active ingredients, suggesting that altered toxic effects due to inert ingredients should be considered in pesticide risk assessments and establishment of water quality criteria.

Connon et al. used a cDNA microarray with 8,448 Expressed Sequence Tags (ESTs) for delta smelt to study the effects of copper. Gene responses were measured in 60-day old juveniles exposed to 50$\mu g.L^{-1}$ copper chloride for 7 days. Responding genes were predominantly involved in digestion and metabolism, and neuromuscular activity with further effects on immune system, redox, and metal ion binding. Selected genes were assessed using q-PCR on 57-day old juveniles, exposed for 96 h to copper concentrations ranging from 2.0 to 32.0 $\mu g.L^{-1}$, concentrations which resulted in no mortality. Quantitative PCR expression analyses corroborated neuromuscular impairments. Our results support the use of molecular biomarkers such as amylase-3, myozenin, calpain, sarcoendoplasmic reticulum calcium ATPase (SER-Ca) and creatine kinase in delta smelt in the determination of digestive and neuromuscular responses to sublethal contaminant exposure.

In collaboration with Dr. D. Ostrach, tissue samples from juvenile striped bass exposed to SPMD extracts were analyzed for expression of four stress-responsive genes, vitellogenin, CYP1A, metallothionein and hsp70. Preliminary results are presented in Chapter 9.3.

Publications (published and in review) resulting from this project to date:

Geist J.P., Werner I., Eder K.J., Leutenegger C.M. 2007. Comparisons of tissue-specific transcription of stress response genes with whole animal endpoints of adverse effect in striped bass (*Morone saxatilis*) following treatment with copper and esfenvalerate. *Aquatic Toxicology* 85:28-39.

Brander Susanne M., Werner I., White J.W., Deanovic L.A. 2009. Toxicity of a dissolved pyrethroid mixture to *Hyalella azteca* at environmentally relevant concentrations. *Environmental Toxicology and Chemistry*: Vol. 28, No. 7 pp. 1493–1499.

Werner I., Deanovic L.A., Markiewicz D., Khamphanh J., Reece C.K., Stillway M., Reece C. In review. Monitoring water column toxicity in the Sacramento-San Joaquin Delta, California, USA, using the euryhaline amphipod, *Hyalella azteca*: 2006-2007. *Integrated Environmental Assessment and Monitoring.*

Connon, R.E., I. Werner. In review. Endocrine, neurological and behavioral responses to sublethal pyrethroid exposure in the endangered delta smelt, *Hypomesus transpacificus* (Fam. Osmeridae). *Marine Environmental Research.*

Connon R.E., Geist J., Pfeiff, J., Loguinov A.S., D'Abronzo L.S., Wintz, H., Vulpe C.D., I. Werner. In review. Linking mechanistic and behavioral responses to sublethal pyrethroid exposure in the endangered delta smelt, *Hypomesus transpacificus* (Fam. Osmeridae). *BMC Genomics*.

2. Background and Approach

In the last several years, abundance indices of numerous pelagic fish species residing in the Sacramento-San Joaquin Delta of California, USA, have shown marked declines and record lows for the endemic delta smelt *(Hypomesus transpacificus*), age-0 striped bass *(Morone saxatilis),* longfin smelt (*Spirinchus thaleichthys*) and threadfin shad (*Dorosoma petenense)*(Stevens and Miller, 1983; Stevens et al., 1985; Moyle et al., 1992; Moyle and Williams, 1990).While several of these species - including in particular longfin smelt and juvenile striped bass - have shown evidence of long-term declines, there appears to have been a precipitous "step-change" to very low abundance during the period 2002-2004 (Bryant and Souza, 2004; Hieb et al., 2005; Feyrer et al., 2007). It is presently unclear what might have caused this critical population decline, but toxic contaminants may be one of several factors acting individually or in concert to lower pelagic productivity.

The goal of this study is to assess the potential for contaminated water to contribute to the observed declines of pelagic species in the Delta. The 2008-2010 study design built on the results of our 2006-2007 Delta-wide monitoring project to investigate toxicity of Delta water samples to invertebrates and early life stages of fish species of concern. In 2006-2007, water samples for invertebrate toxicity testing were collected twice a month at 15 sites characterizing primary inflows to the Delta as well as geographic regions important to pelagic fish of interest (Werner et al., 2008). Test results in 2007 showed acute toxicity in the lower Sacramento and Suisun Bay, and the possible presence of pyrethroids (reduced survival after synergist addition) at sites 804 (Middle of Broad Slough, west end), Suisun Bay, off Chipps Island (508), and Suisun Bay, east of middle point (504). Chronic amphipod growth effects after synergist addition were repeatedly detected in the south-eastern Delta, the lower Sacramento and Suisun Bay indicating the presence of low concentrations of pyrethroid (negative growth effects after synergist addition) or – far less frequently - organophosphate (OP; positive growth effects after synergist addition) insecticides. Several samples contained detectable concentrations of pyrethroid insecticides, primarily lambda-cyhalothrin, cyfluthrin and permethrin. The OP diazinon was detected in one sample. Delta smelt survival was reduced in two water samples from the lower Sacramento River. The 2008-10 study intensified toxicity testing in some important areas (Cache Slough/lower Sacramento, Suisun Marsh and Bay) of the Delta where acute toxicity was detected in 2007, as well as the south-eastern Delta. If acute toxicity to the amphipod *Hyalella azteca* (≥50% mortality within 7 d) is detected, toxicity identification evaluations and chemical analysis are used to identify toxicant(s). If a sample causes ≥50% mortality within 96 h, follow-up samples are to be collected in an attempt to identify the sources of toxicity. Appropriate sites for follow-up sampling were determined early in 2008 using land use and point source information. In addition, laboratory toxicity tests with larval delta smelt were performed in late April-July on water samples from select locations of special concern such as Cache Slough, lower Sacramento and San Joaquin Rivers, and Suisun Marsh.

Single species toxicity tests are the traditional approach used for ambient toxicity testing and *in situ* tests to determine the presence of toxicity in water samples or a water body. Single species tests are valuable first tier assessments that can be used as screening tools to identify potentially toxic conditions in the environment. Results should be used as guidance for additional studies such as exposure characterizations to provide insight on possible causality or biological assessments to identify potential ecological impairment. Because of their limitations with regard to species sensitivity, exposure scenarios, and sublethal effects, these tests should not be used as the final quantitative indicator of absolute ecological impairment, but as one line of evidence or first tier investigation. Sources of uncertainty identified when extrapolating from single species tests to ecological effects include: variability in individual response to toxicant exposure; variation among species and different life-stages in sensitivity; effects of time varying and repeated exposures; the potential for sublethal effects difficult to quantify in standard toxicity tests, for example, endocrine disruption, immune system modulation, behavioral effects, and susceptibility to predation, and extrapolation from individual to population-level endpoints. This study begins to address two of the limitations listed above: exposure scenario and species sensitivity. *In situ* tests with fish (delta smelt and fathead minnows) and the invertebrate *H. azteca* will be conducted at suitable locations (Hood, Rough & Ready Island) to expose test species to water in the field and integrate potential water toxicity over time. With regard to species sensitivity, this study will generate effect data in the form of 96-h LC50, EC50, no observed effect level (NOEC), and lowest observed effect level (LOEC) in order to compare the sensitivity of Delta species with that of standard toxicity test species. Testing will include *Pseudodiaptomus forbesi*, *Eurytemora affinis*, *Ceriodaphnia dubia*, *H. azteca*, delta smelt, and fathead minnow for select chemicals.

Presently, the overwhelming lack of information on the toxic effects of contaminants on resident Delta species, among them delta smelt and two important prey species, *Pseudodiaptomus forbesi* and *Eurytemora affinis*, prevents an estimation of the risk of chemical contamination to pelagic organisms of concern. There is an urgent need for information on their sensitivity to toxic chemicals relative to standard test species. For standard test species, these tests will be performed using laboratory control water as well as Delta water to ensure environmental relevance of the test results. Delta smelt will only be tested in Delta (hatchery) water. Copepods will only be tested in laboratory control water. The chemicals were selected based on their known presence in the Delta, recent past or present, and are copper, ammonia, the organophosphate insecticides chlorpyrifos and diazinon, and the pyrethroid insecticides cyfluthrin, bifenthrin, and permethrin. Copper is used as a pesticide in various forms, is a common chemical in stormwater runoff, and is ubiquitous in the aquatic environment. Ammonia is released from wastewater treatment plants. Chlorpyrifos is one of the most heavily used agricultural insecticides, and has recently been shown to be present at toxic concentrations in Ulatis Creek (Werner & Kuivila, 2004, unpublished data) and agricultural drains (California Regional Water Quality Control Board Agricultural Waiver Program, 2007). Diazinon, cyfluthrin, bifenthrin and permethrin were detected in 2007 in water column samples from various sites in the Delta (Werner et al., 2008). Bifenthrin has also commonly been detected in sediment samples from the region (K. Larsen, CVRWQCB, personal communication).

Sublethal effects of aquatic contaminants are difficult to detect, quantify and interpret in an ecological context. Changes in the gene transcription of stress response genes in resident fish can be powerful biomarkers for the identification of sublethal impacts of environmental stressors on aquatic organisms, and can provide information on the causative agents. Molecular biomarkers have been developed for striped bass in 2006-07 (Geist et al., 2007), and are being used to detect and quantify stress responses in field-collected specimens from 2005-2009 (in collaboration with DFG and D. Ostrach, UC Davis) to detect sublethal toxic effects and help identify the causative chemical(s) or other stressors. Additional biomarkers for delta smelt have been selected and developed based on microarray studies with the immediate aim of selecting appropriate biomarkers for use in field and *in situ* studies, as well as in laboratory studies to determine cause and effect. A complementary study is focused on linking cellular biomarker responses detected in delta smelt and striped bass to ecologically relevant effects such as swimming ability, growth and survival using a model species (fathead minnow).

3. Toxicity Monitoring

3.1 Sampling Sites

Sampling occurred on a bi-weekly basis from the period of 6 January, 2009 through 25 June, 2009 (Tables 3-1, 3-2, Fig. 3-1).

Table 3-1. Site locations and sampling schedule for *H. azteca*

Station	Location	Latitude	Longitude	Collection day
340	Napa River, Historic 340 at the seawall	38-05’-51”N	122-15’-43.9”W	Wednesday
405	Carquinez Straight, just west of Benicia army dock	38-02’-22.9”N	122-09’-01.8”W	Wednesday
Suisun	Suisun Slough at Rush Ranch	38-12’-28.2”N	122-01’56.9”W	Tuesday
508	Suisun Bay, off Chipps Island, opposite Sac. North Ferry Slip	38-02’-43.8”N	121-55’-07.7”W	Wednesday
602	Grizzly Bay, northeast of Suisun Slough at Dolphin	38-06’-50.4”N	122-02’-46.3”W	Wednesday
609	Montezuma Slough at Nurse Slough	38-10’-01.9”N	121.56’-16.8”W	Wednesday
711	Sacramento River at the tip of Grand Island	38-10’-43.7”N	121-39’-55.1”W	Thursday PM
Light 55	Sacramento River Deep Water Channel at Light 55	38-16’-26.5”N	121-39’-13.6”W	Thursday AM
Hood	DWR water quality monitoring station	38-22’-03.6”N	121-31’-13.6”W	Tuesday
Cache-Lin	Confluence of Lindsey Slough/Cache Slough	38-14’-39.2”N	121-41’-19.5”W	Thursday AM
Cache-Ul	Upper Cache Slough, mouth of Ulatis Creek	38-17’-02.7”N	121-43’-04.3”W	Thursday AM
815	San Joaquin, Confluence of Potato Slough	38-05’-06.4”N	121-34’-20.4”W	Thursday PM
902	Old River at mouth of Holland Cut	38-01’-09.1”N	121-34’-55.9”W	Thursday PM
915	Old River, western arm at Railroad Bridge	37-56’-33”N	121-33’-48.6”W	Thursday PM
R&R	San Joaquin, Rough & Ready Island	37-57’45.4”N	121-21’55.9”W	Tuesday
Napa	Napa River in Napa City at end of River Park Blvd.	38-16’-39.7”N	122-16’-56.9”W	Tuesday

Table 3-2. Follow-up sampling sites

Station	Location	Follow-up Sampling
340	Napa River, Historic 340 at the seawall	Resample of 340
405	Carquinez Straight, just west of Benicia army dock	Resample of 405; Pacheco Creek
Suisun	Suisun Slough, downstream of Boynton Slough	Resample of Suisun; Upstream Boynton Slough, upstream Rush Ranch
508	Suisun Bay, off Chipps Island, opposite Sac. North Ferry Slip	Resample of 508; upstream Sac River, upstream San Joaquin River, 602
602	Grizzly Bay, northeast of Suisun Slough at Dolphin	Resample of 602; Suisun, 609, 508, 405
609	Montezuma Slough at Nurse Slough	Resample of 609; Nurse Slough, Mouth at Van Sickle Island
711	Sacramento River at the tip of Grand Island	Resample of 711; 704, Sac River near Locke, Gate from Moklumne
Light 55	Sacramento River Deep Water Channel at Light 55	Resample of Light 55
Hood	DWR water quality monitoring station	Resample of Hood
Cache-Lin	Confluence of Lindsey Slough/Cache Slough	Resample of Cache-Lin; Lindsey Slough, Cache-Ul
Cache-Ul	Upper Cache Slough, mouth of Ulatis Creek	Resample of Cache-Ul; upstream Ulatis Creek
815	San Joaquin, Confluence of Potato Slough	Resample of 815; Mokelumne Slough, Potato Slough, upstream San Joaquin River, San Joaquin River to Franks Tract Connector, 812
902	Old River at mouth of Holland Cut	Resample of 902; 815, 915, Connection Slough
915	Old River, western arm at Railroad Bridge	Resample of 915; North Woodward Island, 902, Rock Slough
R&R	San Joaquin, Rough & Ready Island	Resample of R&R; Calaveras, Port of Stockton, upstream San Joaquin River, French Camp
Napa	Napa River in Napa City at end of River Park Blvd.	Resample of Napa

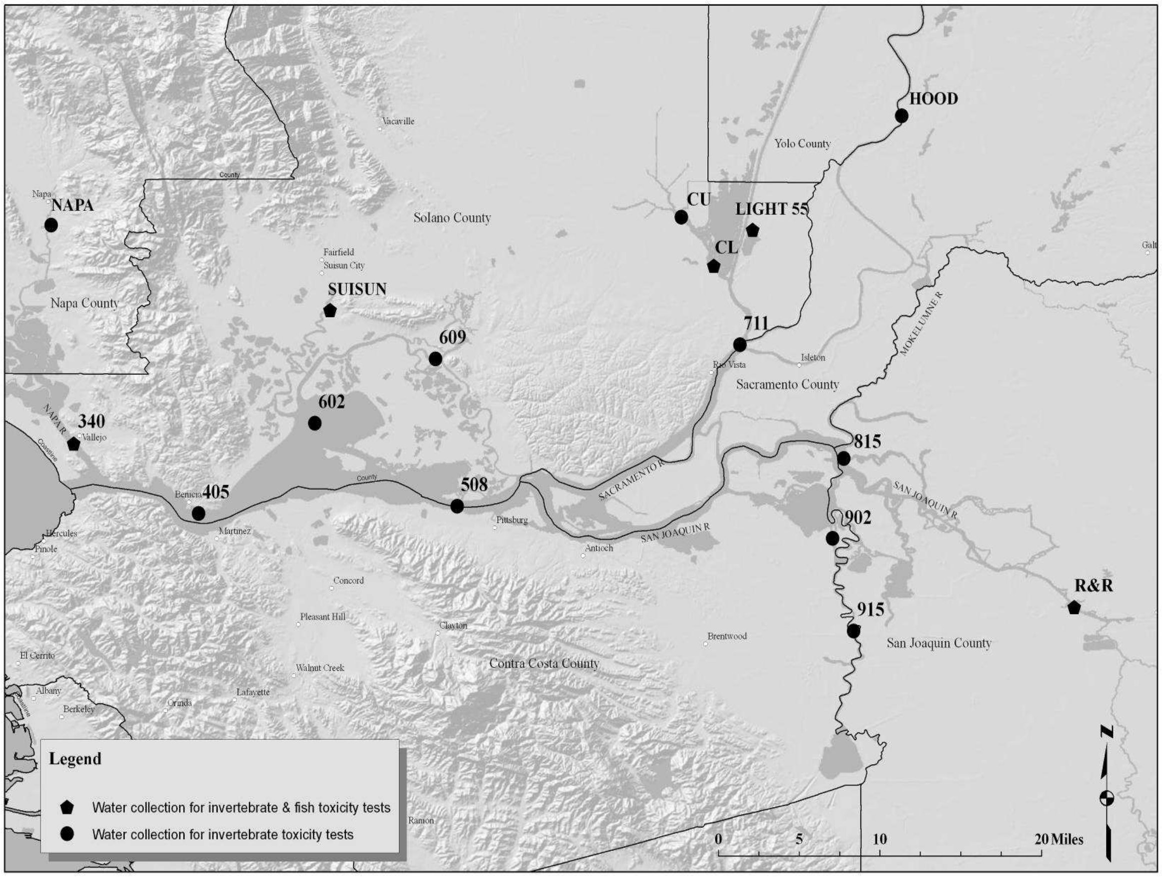

Figure 3-1. Water toxicity sampling locations based on IEP summer townet survey stations in 2008.

3.2 Collection of Water Samples

Staff from the UC Davis Aquatic Toxicology Laboratory (UCD ATL) and the California Department of Fish and Game (CDFG) collected water samples from 16 sites: 340, 405, 508, 602, 609, 711, Light 55, Cache-Lindsey, Cache-Ulatis, 815, 902, and 915 by boat and Hood, Rough & Ready, Suisun, and Napa via bank sampling. If the salinity exceeded the *H. azteca* testing limit of 15 ppt, samples were not collected for *H. azteca* toxicity tests. Subsurface grab samples were pumped from a depth of approximately 0.5 m using a standard water pump into clean, 1-gal amber LDPE cubitainers for invertebrate tests and 5-gal clear LDPE cubitainers for delta smelt tests. In addition, site water was also collected in 1-gal clear LDPE cubitainers and 1-L amber-glass bottles for analytical chemistry. Water samples were transported, stored and preserved following protocols outlined in the UCD ATL standard operating procedures (SOP), nos. 5-1 and 5-2 (UCD ATL, 2009). All cubitainers used for water collections were labeled with the site ID, collection date and time, and the initials of the sampler and then rinsed three times with ambient sample water prior to filling. Eight gallons of water were collected from each site for invertebrate testing along with two liters for analytical chemistry. During the Spring, an additional 35 gallons were collected for delta smelt toxicity testing. All samples were placed into an ice chest on wet ice for transport to the UCD ATL and ice was renewed as needed to keep the sample temperature at 0-6ºC (USEPA, 2002). Upon receipt at UCD ATL, water samples were stored in an environmental chamber at 0-6ºC.

3.3 Water Quality at Sampling Sites

Field measurements including pH, specific conductivity (SC), electrical conductivity (EC), dissolved oxygen (DO) and temperature were recorded for each site and sampling time. DO and SC were measured using YSI 85 meters, and pH was measured with a Beckman 240 pH meter. DO/SC and pH meters were calibrated according to the manufacturer's instructions at the start of each field day. Turbidity and ammonia nitrogen were measured within 24 hours of sample receipt at UCD ATL using a Hach 2100P Turbidimeter and a Hach AmVer Ammonia Test'N Tube Reagent Set, respectively. For ammonia measurements the "low range" test kit (0-2.5 mg/L N) was used first. If the maximum value was exceeded the "high range" test kit (0-50 mg/L N) was used. Unionized ammonia concentrations for all samples were calculated using measured total ammonia-N, field temperature, and field pH. General weather conditions and GPS coordinates were recorded for each site and sampling event. Tables 3-3 and 3-4 summarize minimum and maximum water quality data by site.

Table 3-3. Minimum and maximum water quality parameters measured at sites sampled during January - June 2009.

Sample	*N*	SC (uS/cm)		EC (uS/cm)		Temp (°C)		pH		DO (mg/L)	
		Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
340	9	9460	24360	6981	19947	10.0	18.6	6.58	7.88	8.7	13.0
405	10	4080	23650	3550	17370	10.2	19.7	7.14	7.84	8.9	13.0
508	13	358	12810	277	9121	8.3	20.5	6.54	7.87	8.8	11.7
602	13	425	19800	330	13306	8.6	22.1	6.75	8.00	8.9	13.4
609	12	2030	8000	1583	5568	9.5	22.0	6.66	7.70	7.5	11.9
711	13	120	417	98	299	7.9	23.2	6.61	7.68	8.1	12.1
815	13	176	572	156	406	7.8	22.7	6.58	7.87	8.2	12.0
902	13	204	830	193	571	7.6	23.6	6.58	7.90	8.0	11.8
915	13	217	745	209	511	7.6	24.1	6.43	7.80	7.7	12.6
Cache-Lindsey	13	183	674	155	543	7.9	22.1	6.80	7.86	8.4	12.1
Cache-Ulatis	13	207	674	187	543	7.3	21.2	6.88	8.61	8.3	13.8
Hood	13	116	303	99	216	8.2	23.2	6.55	7.55	7.5	12.0
Light 55	13	215	409	189	331	7.9	22.0	7.02	8.03	8.4	12.5
Napa	13	237	20870	176	16000	9.6	24.2	6.51	7.98	6.0	11.4
Rough and Ready	13	435	1107	442	797	8.2	25.8	7.08	7.94	6.0	11.3
Suisun Rush Ranch	13	2673	11780	2010	8317	8.5	20.4	6.51	7.53	5.5	11.9

Table 3-4. Minimum and maximum turbidity, ammonia, hardness and alkalinity measured at sites sampled during January - June 2009.

Sample	*N*	Turbidity (NTU)		Ammonia Nitrogen (mg/L)		Unionized Ammonia (mg/L)		Hardness (mg/L as CaCO3)		Alkalinity (mg/L as CaCO3)	
		Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
340	9	21.3	77.9	0.09	0.59	0.000	0.002	1040	2880	88	108
405	10	10.5	424.3	0.00	0.62	0.000	0.003	800	2400	80	104
508	13	9.1	40.4	0.00	0.31	0.000	0.004	92	1800	64	106
602	13	8.3	379.0	0.06	0.33	0.000	0.005	152	2280	68	106
609	12	24.4	137.7	0.00	0.34	0.000	0.005	292	880	78	100
711	13	4.3	146.3	0.04	0.47	0.000	0.012	48	100	46	134
815	13	4.0	16.7	0.00	0.30	0.000	0.013	56	124	52	94
902	13	3.8	12.3	0.00	0.15	0.000	0.005	56	140	58	92
915	13	3.1	9.2	0.00	0.15	0.000	0.006	72	140	58	98
Cache-Lindsey	13	6.4	132.7	0.07	0.47	0.000	0.021	64	114	62	118
Cache-Ulatis	13	9.1	151.3	0.00	0.23	0.000	0.007	68	226	70	204
Hood	13	5.4	43.9	0.02	0.56	0.000	0.004	44	80	50	92
Light 55	13	8.2	96.9	0.05	0.38	0.001	0.017	64	124	66	124
Napa	13	8.1	713.3	0.00	0.35	0.000	0.004	70	2360	54	284
Rough and Ready	13	2.4	13.3	0.02	0.43	0.000	0.006	96	212	68	240
Suisun Rush Ranch	13	20.5	395.3	0.08	0.46	0.000	0.004	380	1360	130	248

4. Monitoring with *Hyalella azteca*

4.1. Methods

4.1.1 Toxicity Testing

H. azteca purchased from Aquatic Research Organisms (Hampton, NH) were received at the UCD ATL 48 hours prior to test initiation and acclimated to laboratory conditions. Before initiating bioassays, the water samples were mixed rigorously in the original sampling containers, filtered through a 60-µm screen, brought to the test temperature of 23^{o}C, and aerated at a rate of 100 bubbles/min until the dissolved oxygen concentration was approximately 8.5 mg/L. Deionized water amended to US EPA moderately hard standards (DIEPAMHR) was used as the laboratory control water.

The 10-day tests consisted of four 250 ml replicate glass beakers, each containing 100 ml of sample, a one-square-inch piece of nitex screen for artificial substrate, and 10 organisms. Tests were initiated with 7 to 14 day old *H. azteca*. Animals in each replicate were fed 1000 µl of YCT (a mixture of yeast, organic alfalfa and trout chow) on test initiation and days 2, 4, 6, 8 following the renewal of 75% of the test waters. Each series of tests included a standard laboratory control, and if necessary, "high EC controls" and a "low EC control". "High EC" control water was reconstituted to EPA moderate hardness and the EC was adjusted to match the highest EC of the ambient water samples (typically found at the Napa River and site 405), with pre-filtered Pacific Ocean seawater obtained from Bodega Bay Marine Laboratory, Bodega Bay, CA. Multiple high EC controls were sometimes included when ambient waters showed a wide range of conductivities. "Low EC" control water was reconstituted to EPA moderate hardness and the EC was adjusted to match the lowest EC of the water samples (typically found at sites 711, Cache-Ulatis, Cache-Lindsey, and Hood) by diluting with deionized water.

All ambient samples were tested with and without the addition of piperonyl butoxide (PBO). PBO was added because of its synergistic and antagonistic action with pyrethroid and organophosphate insecticides, respectively. A five parts per million (5 ppm) PBO stock solution was prepared and added to 500 ml of sample water to yield the desired test concentration. Tests were conducted with 25 ppb of PBO, which did not affect survival or growth of *H. azteca* (Werner et al. 2008).

Tests were conducted at a temperature of 23 ± 2° C with a 16h:8h Light:Dark photoperiod. Mortality was recorded daily, and water was renewed on days 2, 4, 6 and 8. On day 10, the surviving *H. azteca* were dried and weighed to determine dry tissue weight per individual and relative growth.

4.1.2 Toxicity Identification Evaluations (TIEs)

If ≥50% mortality of test organisms occurred within 7 days in the survival and growth *H. azteca* bioassay, a TIE was initiated to characterize the cause of toxicity. TIEs involve procedures to either remove or inactivate specific classes of chemicals. After manipulation, the toxicity of a sample is tested and compared to the corresponding method blank. During this period, no TIEs were performed.

4.1.3 Statistical Analysis

Statistical analysis of *H. azteca* 10-day chronic toxicity data involved two endpoints: 10-day survival and 10-day weight, and was performed using JMP 5.0.1 (SAS 2003). We used one-way ANOVA and Tukey's multiple comparison procedure to evaluate all comparisons among waters not treated with PBO (one-tailed alpha = 0.05). Tukey's multiple comparison procedure is useful in this experimental design because it allows all possible pairwise comparisons between treatments to be examined while minimizing the chance of false positive results (experiment-wide alpha is maintained at 0.05 regardless of the number of comparisons examined). The USEPA protocol requires that data are tested for normality and homogeneity of variance before being tested using ANOVA. However, Zar (1996) reports that tests for homogeneity of variance perform poorly and are not recommended for testing the underlying assumptions of ANOVA, and reports that ANOVA is reliable for multisample testing among means even in cases of substantial heterogeneity of variances or considerable deviations from normality. Therefore, data were not tested for normality or homogeneity of variance before being tested with ANOVA and Tukey's procedure. In tests containing high or low conductivity samples (high EC > 10,000 uS/cm; low EC < 100 uS/cm), significant reductions in survival and weight were evaluated relative to the control with the most appropriate conductivity.

Comparisons involving PBO-treated waters and PBO effects were evaluated by full factorial two-way ANOVA (two-tailed alpha = 0.05). The three terms in the ANOVA were 1) the identity of test water, 2) the presence or absence of PBO and 3) an interaction term between test water and PBO presence. When there was a significant overall effect of PBO or interaction effect, a Tukey's multiple comparison procedure was performed to identify if a significant difference existed between any control or test water and its PBO treated counterpart, and to identify if any PBO-treated sample showed a significant decrease in survival or weight relative to the PBO-treated control of the most appropriate conductivity.

Since the statistical analyses used by ATL are very rigorous to minimize the occurrence of false positive results, we also examined the results of the *H. azteca* tests performed during this time period using the standard USEPA-recommended single-concentration statistical protocols in order to achieve the greatest possible statistical sensitivity (USEPA, 2002).

4.1.4 Analytical Chemistry

Water samples for analytical chemistry were collected at each sampling site during each sampling event using two acid-cleaned, 1-L amber-glass bottles. These samples were transported on ice and stored in an environmental chamber maintained at 4^oC upon receipt at the UCD ATL. 10 ml of dichloromethylene (DCM) was added to one 1-L sample to prevent possible degradation of insecticides during storage. If a sample noticeably affected survival or growth of *H. azteca*, it was then submitted to the California Department of Fish and Game – Water Pollution Control Laboratory, Rancho Cordova, CA, for chemical analysis on whole water samples. Samples submitted for total and/or dissolved metals analysis were sent to the California Department of Fish and Game – Moss Landing Marine Laboratory, Moss Landing, CA.

Water samples were typically sent in for pyrethroid or organophophate scans when a signal obtained with PBO indicated that one of these insecticide groups may be responsible for the observed toxic effect. When the possible cause of toxicity was less apparent, water samples were analyzed for a "comprehensive" suite of chemicals including metals (dissolved and total), PAHs, pyrethroids, organophosphates, carbamates, and fipronil and degredates. Appendix I gives analytes and their corresponding method detection and reporting limits for organophosphates, pyrethroids, carbamates, fipronil and metabolites, PAHs, and trace metals.

4.2 Results

A total of 200 water samples were collected and tested for toxicity with *H. azteca* during the reporting period of January 1 - June 30, 2009. Results of the toxicity tests are summarized below in Tables 4-1 through 4-5. Detailed results and water chemistry data are shown in Appendix B.

4.2.1 Acute Toxicity to *H. azteca* - Effects on 10-d Survival

During this project period, one water sample collected from site 711 on 6/25/09 was acutely toxic, causing a significant reduction in amphipod survival within the 10 day test period. This sample reduced amphipod survival by 44%, but did not meet the re-sample or TIE triggers of ≥50% mortality within 96 hours or 7 days, respectively. An analytical sample was submitted to the CDFG-WPCL for analysis of a comprehensive suite of chemicals, and results are currently pending.

PBO Effect on 10-d Survival: The addition of PBO led to significant decreases in the survival of *H. azteca* in two ambient samples when compared to their non-PBO counterparts: site 340 collected 5/13/09 and Hood collected 6/23/09. The PBO-induced reduction in survival at site 340 was detected by the conservative Tukey's multiple comparison procedure and by USEPA standard statistical protocols, while the reduction in survival at Hood was only detected by the less conservative USEPA protocols. A sample collected at site 902 on 6/25/09 showed significantly reduced survival when treated with PBO compared to the PBO-treated control, but was not significantly different from the ambient sample without PBO.

Table 4-1. 10-day Survival of H. azteca in treatments showing significant differences in survival compared to controls or with the addition of PBO, as detected by ANOVA and Tukey's multiple comparison procedure. Significant differences detected by USEPA standard statistical protocols are given in parenthesis.

Sample Type	Comparison	# Samples	Survival		Weight	
			Reduced	Increased	Reduced	Increased
Ambient	v EC-specific Control	200	1 (1)	-	0 (1)	-
PBO Treated	v EC-specific PBO Control	200	1 (4)	-	0 (0)	-
PBO Treated	Ambient	200	1 (2)	0 (0)	5 (18)	4 (15)

1. These numbers do not include quality assurance samples.

Table 4-2. Survival of *H. azteca* in treatments showing significant differences in survival compared to controls or with the addition of PBO as detected by Tukey's multiple comparison procedure.

Sample	Collection Date	Test Initiation Date	Survival (%)		Significance			Chem. Type	Chem. Result
			Non-PBO	PBO	v Non-PBO Control	v PBO Control	Non-PBO v PBO		
340	5/13/2009	5/16/2009	61	14	NS	S (19%)	S (23%)	P	ND
711	6/25/2009	6/26/2009	45	61	S (51%)	NS	NS	C	Pending

Chemical Analysis: P: Pyrethroid, O: Organophosphate, C: Comprehensive, Cb: Carbamate, M: Metal

Table 4-3. Survival of *H. azteca* in treatments showing significant differences in survival compared to controls or with the addition of PBO as detected by USEPA standard statistical protocols.

Sample	Collection Date	Test Initiation Date	Survival (%)		Significance			Chem. Type	Chem. Result
			Non-PBO	PBO	v Non-PBO Control	v PBO Control	Non-PBO v PBO		
340	5/13/2009	5/16/2009	61	14	NS	S (19%)	S (23%)	P	ND
Hood	6/23/2009	6/25/2009	87	66	NS	S (73%)	S (76%)	P	Pending
711	6/25/2009	6/26/2009	45	61	S (51%)	S (79%)	NS	C	Pending
902	6/25/2009	6/26/2009	90	85	NS	S (89%)	NS		

Chemical Analysis: P: Pyrethroid, O: Organophosphate, C: Comprehensive, Cb: Carbamate, M: Metal

4.2.2 Chronic Toxicity to *H. azteca* - Effects on 10-d Growth

Chronic Toxicity to *H. azteca*: Chronic toxicity (reduced growth compared to control) to *H. azteca* was not detected during this project period. In general, this endpoint was not a sensitive indicator of toxicity due to the variable size of the organisms, the variability of food content in Delta water samples, and the lack of food content in the control waters.

PBO Effect on 10-d Growth: The most common significant effects detected in *H. azteca* ambient sample tests were differences in growth resulting from the addition of PBO relative to the unmanipulated ambient samples. The conservative Tukey's test detected 5 significant reductions in growth (2.5% of samples tested) and 4 significant increases (2%), while the more sensitive USEPA protocol detected 18 reductions (9%) and 15 increases (7.5%). All samples resulting in a significant reduction or increase in growth detected by the Tukey's test were submitted for chemical analysis as were the majority of those detected by the more sensitive USEPA standard statistical protocols. Of the analytical results received to date, statistical differences detected by the more conservative Tukey's test were more likely to result in pesticide detections.

Table 4-4. Weight of *H. azteca* in treatments showing significant differences in weight compared to controls or with the addition of PBO as detected by Tukey's multiple comparison procedure.

Sample	Collection Date	Test Initiation Date	Weight (mg/individual)		Significance			Analytes	Chem. Result
			Non-PBO	PBO	v Non-PBO Control	v PBO Control	Non-PBO v PBO		
R&R	1/6/2009	1/8/2009	0.117	0.064	NS	NS	S (55%)	P	ND
CU	2/4/2009	2/5/2009	0.121	0.063	NS	NS	S (52%)	P	ND
902	2/4/2009	2/5/2009	0.119	0.044	NS	NS	S (37%)	P	ND
508	3/4/2009	3/5/2009	0.131	0.083	NS	NS	S (63%)	P	ND
815	3/18/2009	3/20/2009	0.046	0.087	NS	NS	S (189%)	O	Detect
508	4/1/2009	4/2/2009	0.087	0.130	NS	NS	S (149%)	O	Detect
CU	4/2/2009	4/3/2009	0.036	0.106	NS	NS	S (294%)	O	Detect
NAPA	6/9/2009	6/11/2009	0.053	0.040	NS	NS	S (75%)	P	Detect
R&R	6/23/2009	6/25/2009	0.075	0.133	NS	NS	S (177%)	O	Pending

Chemical Analysis: P: Pyrethroid, O: Organophosphate, C: Comprehensive, Cb: Carbamate, M: Metal

Table 4-5. Weight of *H. azteca* in treatments showing significant differences in weight compared to controls or with the addition of PBO as detected by USEPA standard statistical protocols.

Sample	Collection Date	Test Initiation Date	Weight (mg/individual)		Significance			Analytes	Chem. Result
			Non-PBO	PBO	v Non-PBO Control	v PBO Control	Non-PBO v PBO		
R&R	1/6/2009	1/8/2009	0.117	0.064	NS	NS	S (55%)	P	ND
508	1/21/2009	1/22/2009	0.045	0.073	NS	NS	S (162%)	O	ND
915	1/22/2009	1/23/2009	0.084	0.127	NS	NS	S (151%)	O	ND
902	1/22/2009	1/23/2009	0.127	0.075	NS	NS	S (59%)	P	ND
711	1/22/2009	1/23/2009	0.107	0.078	NS	NS	S (73%)	P	ND
CU	2/4/2009	2/5/2009	0.121	0.063	NS	NS	S (52%)	P	ND
902	2/4/2009	2/5/2009	0.119	0.044	NS	NS	S (37%)	P	ND
CL	2/4/2009	2/5/2009	0.105	0.060	NS	NS	S (57%)	P	ND
Light 55	2/4/2009	2/5/2009	0.079	0.050	NS	NS	S (63%)	P	ND
508	2/5/2009	2/6/2009	0.028	0.046	NS	NS	S (164%)		
Suisun	2/17/2009	2/19/2009	0.035	0.060	NS	NS	S (171%)	O	Pending
340	2/18/2009	2/19/2009	0.023	0.052	NS	NS	S (226%)	O	Pending
815	2/19/2009	2/20/2009	0.056	0.098	NS	NS	S (175%)	O	Pending
CU	2/19/2009	2/20/2009	0.074	0.042	NS	NS	S (57%)	P	Pending
508	3/4/2009	3/5/2009	0.131	0.083	NS	NS	S (63%)	P	ND
CU	3/5/2009	3/6/2009	0.073	0.040	NS	NS	S (55%)	P	ND
405	3/17/2009	3/19/2009	0.075	0.061	NS	NS	S (81%)		
340	3/17/2009	3/19/2009	0.073	0.057	NS	NS	S (78%)		
R&R	3/17/2009	3/19/2009	0.093	0.064	NS	NS	S (69%)	P	Detect
Light 55	3/18/2009	3/19/2009	0.072	0.097	NS	NS	S (135%)	O	Detect
915	3/18/2009	3/20/2009	0.093	0.069	NS	NS	S (74%)	P	ND
CU	4/2/2009	4/3/2009	0.036	0.106	NS	NS	S (294%)	O	Detect
902	4/2/2009	4/3/2009	0.090	0.124	NS	NS	S (138%)	O	Detect
405	4/14/2009	4/16/2009	0.030	0.044	S (65%)	NS	NS		
Suisun	4/15/2009	4/17/2009	0.050	0.090	NS	NS	S (180%)	O	ND
Suisun	4/28/2009	4/30/2009	0.090	0.119	NS	NS	S (132%)	O	ND
Hood	4/28/2009	4/30/2009	0.077	0.099	NS	NS	S (129%)	O	ND
602	4/29/2009	4/30/2009	0.054	0.081	NS	NS	S (150%)	O	ND
340	4/29/2009	4/30/2009	0.048	0.070	NS	NS	S (146%)		
609	5/27/2009	5/28/2009	0.090	0.075	NS	NS	S (83%)		
Light 55	6/11/2009	6/12/2009	0.086	0.064	NS	NS	S (74%)	P	Detect
902	6/11/2009	6/12/2009	0.081	0.060	NS	NS	S (74%)	P	Detect
CL	6/25/2009	6/26/2009	0.083	0.043	NS	NS	S (52%)	P	Pending
915	6/25/2009	6/26/2009	0.055	0.078	NS	NS	S (142%)	O	Pending

Chemical Analysis: P: Pyrethroid, O: Organophosphate, C: Comprehensive, Cb: Carbamate, M: Metal

4.2.3 Toxicity Identification Evaluation

TIEs were not performed during this reporting period.

4.2.4 Results of Analytical Chemistry

Whole water samples were submitted to CDFG-WPCL for chemical analysis after the detection of acute toxicity in either statistical method and samples exhibiting a reduction or increase in growth were evaluated for submission on a case by case basis. A total of 39 ambient water samples were submitted for analysis for chemical contaminants during this reporting period, resulting in detections in 8 out of 27 samples for which analysis has been completed. Apart from one detection in early January, all detections occurred from mid-March to early April.

Pyrethroids were not detected in the one sample analyzed to date that showed a significant reduction in survival, even though this reduction in survival was associated with PBO addition. Pyrethroids and organophosphates were, however, detected in some of the samples that showed reductions or increases in *H. azteca* weight, respectively. Pyrethroid insecticides were detected in low concentrations from samples collected at Rough & Ready Island on 3/17/2009 (0.003 µg/L cyfluthrin) and Hood on 3/18/2009 (0.003 µg/L permethrin). The organophosphate insecticides chlorpyrifos, diazinon, and disulfoton were detected singularly or in combination at sites 508, 602, 815, 902, Cache-Ulatis, and Light 55. Although the majority of these detections were below the reporting limit of the analytical laboratory, a sample collected from Cache-Ulatis on 4/2/2009 resulted in the detection of 0.078 µg/L chlorpyrifos. This sample was submitted to CDFG-WPCL following a significant increase in growth with the addition of PBO in the 10-d *H. azteca* bioassay. Although survival was not affected, this concentration of chlorpyrifos is greater than the 10-d control water LC50 of 67.2 pptr determined by UCD-ATL in January 2009. A sample collected from Light 55 on 3/19/09 caused a significant increase in growth when treated with PBO and resulted in the detection of 0.010 µg/L chlorpyrifos. Table 4-6 presents a detailed summary of samples submitted for chemical analysis, reason for submission, scan type, and results. In addition, beginning in February, water samples collected from sites 711, 902, Cache-Lindsey, Rough & Ready Island, and Suisun were submitted for routine metals analysis in order to obtain baseline metals data (Table 4-7). Results are pending.

Low levels of detected pesticides in samples showing survival or weight PBO effects may be due to the generally high pesticide sensitivity of *H. azteca*. Sensitivity studies show that effective concentrations of bifenthrin and cyfluthrin are close to the reporting and detection limits of the chemical analysis (Table 4-8). Analyte degradation may have further reduced our capability to detect the small amounts of pesticide capable of affecting *H. azteca*. Although samples destined for pyrethroid analysis were preserved with DCM within 12 hours of collection, the time interval from sample collection to observation of toxicity caused a latency of approximately two weeks from sample collection to delivery to the analytical laboratory.

Table 4-6. Results of chemical analysis of whole water samples during January – June 2009.

Site ID	Collection Date	*H. azteca* Performance Trigger	Scan Type	Results
Rough & Ready	1/6/2009	Weight reduced with PBO	pyrethroid	ND[2]
602	1/7/2009	Weight increased with PBO	organophosphate	8 ng/L disulfoton*
508	1/21/2009	Weight increased with PBO	organophosphate	ND
711	1/22/2009	Weight reduced with PBO	pyrethroid	ND
915	1/22/2009	Weight increased with PBO	organophosphate	ND
902	1/22/2009	Weight reduced with PBO	pyrethroid	ND
Hood	1/23/2009	Weight reduced with PBO	pyrethroid	ND
Cache-Ulatis	2/4/2009	Weight reduced with PBO	pyrethroid	ND
902	2/4/2009	Weight reduced with PBO	pyrethroid	ND
Cache-Lindsay	2/4/2009	Weight reduced with PBO	pyrethroid	ND
Light 55	2/4/2009	Weight reduced with PBO	pyrethroid	ND
Suisun	2/17/2009	Weight increased with PBO	organophosphate	pending
340	2/18/2009	Weight increased with PBO	organophosphate	pending
815	2/19/2009	Weight increased with PBO	organophosphate	pending
Cache-Ulatis	2/19/2009	Weight reduced with PBO	pyrethroid	pending
508	3/4/2009	Weight reduced with PBO	pyrethroid	ND
Cache-Ulatis	3/5/2009	Weight reduced with PBO	pyrethroid	ND
Rough & Ready	3/17/2009	Weight reduced with PBO	pyrethroid	0.003 µg/L cyfluthrin
815	3/18/2009	Weight increased with PBO	organophosphate	0.002 µg/L diazinon*, 0.003 µg/L chlorpyrifos*, 0.008 µg/L disulfoton*
Hood	3/18/2009	Weight reduced with PBO	pyrethroid	0.003 µg/L permethrin
915	3/18/2009	Weight reduced with PBO	pyrethroid	ND
Light 55	3/19/2009	Weight increased with PBO	organophosphate	0.010 µg/L chlorpyrifos
508	4/1/2009	Weight increased with PBO	organophosphate	0.002 µg/L chlorpyrifos*
902	4/2/2009	Weight increased with PBO	organophosphate	0.002 µg/L chlorpyrifos*, 0.008 µg/L disulfoton*
Cache-Ulatis	4/2/2009	Weight increased with PBO	organophosphate	0.078 µg/L chlorpyrifos, 0.017 µg/L disulfoton*
Suisun	4/15/2009	Weight increased with PBO	organophosphate	ND
Suisun	4/28/2009	Weight increased with PBO	organophosphate	ND
Hood	4/28/2009	Weight increased with PBO	organophosphate	ND
602	4/29/2009	Weight increased with PBO	organophosphate	ND
340	5/13/2009	Survival reduced with PBO	pyrethroid	ND
Napa	6/9/2009	Weight reduced with PBO	pyrethroid	0.009 µg/L esfenvalerate/fenvalerate
340	6/10/2009	Survival increased with PBO (NS)	organophosphate	ND
Light 55	6/11/2009	Weight reduced with PBO	pyrethroid	0.002 µg/L cypermethrin
902	6/11/2009	Weight reduced with PBO	pyrethroid	0.002 µg/L cypermethrin
Hood	6/23/2009	Survival reduced with PBO	pyrethroid	pending
Rough & Ready	6/23/2009	Weight increased with PBO (NS)	organophosphate	pending
711	6/25/2009	Survival. reduced v Control	comprehensive[1]	pending
Cache-Lindsay	6/25/2009	Weight reduced with PBO	pyrethroid	pending
915	6/25/2009	Weight increased with PBO	organophosphate	pending

[1] comprehensive chemical analysis includes PAH's, carbamates, pyrethroids, organophosphates, fipronyl and metabolites, and total and dissolved metals.
[2] no detection
* detection below reporting limit

Table 4-7. Samples submitted to the DFG-MLML for routine dissolved metals analysis.

Site	Sampling Date				
711	2/19/09	3/18/09	4/15/09	5/28/09	6/25/09
902	2/19/09	3/18/09	4/23/09	5/28/09	6/25/09
Cache-Lindsay	2/19/09	3/18/09	4/15/09	5/28/09	6/25/09
Rough & Ready Island	2/17/09	3/17/09	4/14/09	5/27/09	6/23/09
Suisun Slough & Rush Ranch	2/19/09	3/18/09	4/15/09	5/26/09	6/23/09

Table 4-8. Comparison of analytical detection limits and *H. azteca* sensitivities to organophosphate and pyrethroid pesticides. Toxicity values are averages calculated from dilution series using synthetic control water and delta water. LC50 / EC25 values were used preferentially, with LOEC substituted when necessary.

Pesticide	Analytical Chemistry		*H. azteca* Toxicity	
	Estimated Method Detection Limit (pptr)	Reporting Limit (pptr)	10-day Survival LC50 / LOEC (pptr)	10-day Weight EC25 / LOEC (pptr)
Chlorpyrifos	2.0	5.0	84.9	> 66
Diazinon	2.0	5.0	2900	2000
Bifenthrin	0.2	0.4	3.3	0.9
Cyfluthrin	0.4	0.8	2.7	1.5
Permethrin	0.6	1.0	59.0	> 80

5. Monitoring with Delta Smelt (*Hypomesus transpacificus*)

Test protocols followed those developed at UCD-ATL and described in detail by Werner et al. (2008) for toxicity testing with delta smelt larvae at different stages of development. A flow-through system was used for testing ambient waters and the methods used are summarized below.

5.1 Methods

5.1.1 Toxicity Testing

Test organisms and control water: Tests were performed using larval delta smelt ranging in age from 30-55 days old. Delta smelt were obtained from the UC Davis Fish Conservation and Culture Laboratory (UCD-FCCL) in Byron, CA. Hatchery water collected from the UCD-FCCL was used for all control treatments. Fish were transported to UCD-ATL following methods described by Werner et al. (2008).

Sampling sites: For flow-through tests, Delta water samples (35 gal/site) were collected from the DWR water quality monitoring stations at Hood (Sacramento River) and Rough & Ready Island (San Joaquin River), as well as from sites Light 55, Suisun Slough at Rush Ranch, Napa River at the Vallejo Seawall, and Cache Slough near the confluence with Lindsey Slough. Water collections for delta smelt toxicity testing occurred six times from 3/17/09 – 5/28/09.

Testing procedures: Upon arrival at UCD-ATL, the transport containers with fish were placed into a temperature-regulated water bath maintained at 16º C. 1-L beakers were used to collect the fish from the buckets, and fish were gently poured into a bread pan containing hatchery water at a depth of approximately 2 cm. The fish were carefully removed from the pan using 100 mL beakers and released into the replicate exposure tanks at random, submerging the beaker and allowing the fish to swim freely into the tanks. Twelve fish were placed into each of the tanks containing 7 L of water for a 48-h EC acclimation period. Hatchery water and EC-adjusted hatchery water was used as acclimation and control water. EC was adjusted with distilled water (Low EC Control) to match the lowest EC of ambient water samples. When the turbidity of the hatchery water was below 11 NTUs, Nanno 3600™, a concentrated *Nannochloropsis* algae solution (68 billion cells/ml; Reed Mariculture, Inc. Campbell, CA) was added to increase turbidity in control treatments. Turbidity in the Low Turbidity Control was matched to the lowest turbidity ambient sample on a daily basis. Antibiotics (Maracyn and Maracyn-2, Virbac AH Inc., Fort Worth TX) were added at the manufacturer's recommended dose throughout the acclimating and testing period. Final concentrations were 5.3 mg/L Maracyn (erythromycin) and 0.26 mg/L Maracyn-2 (minocycline). During acclimation and testing, fish were fed three times per day with 200 µL of *Artemia* and 300 µL of rotifers. At test initiation, the EC-adjusted control water was drawn down from 7 L to approximately 2 L to allow for an accurate count of living fish. Water quality parameters (EC, pH, temperature, DO, turbidity and ammonia) were measured daily. Dead fish were counted and removed daily. At test termination, surviving fish were counted, euthanized with MS-222, and preserved with liquid nitrogen for later molecular analysis.

5.1.2 Statistical Analysis

Data from exposures of delta smelt were analyzed using both USEPA standard single-concentration statistical protocols and by one-way ANOVA with Tukey's multiple comparison procedure (USEPA 2002). The USEPA method of data analysis showed the results of the tests according to the standardized statistical method used in aquatic toxicology monitoring and regulation throughout the United States. Each comparison of a sample to a control was treated as a separate statistical test, in accordance with USEPA 2002, Appendix H. The Tukey's procedure complemented the USEPA protocol by allowing comparisons other than each treatment paired with one control. Compared to the USEPA procedures, the Tukey's test provided a more conservative evaluation of significant differences between samples since it maintains the experiment-wide alpha at 0.05.

5.2 Results

5.2.1 Toxicity Tests

At 96 hours, *H. transpacificus* survival was found to be significantly reduced relative to conductivity-specific and turbidity-specific controls in Cache-Lindsey collected on 4/15/09 and in Rough & Ready Island collected on 5/12/09. At 7 days, survival was reduced in Hood collected on 4/28/09 and Cache-Lindsey collected on 4/30/09 and in Rough & Ready collected on 5/12/09. Other instances of significantly reduced survival relative to conductivity-specific controls are difficult to interpret because of low turbidity in the sample waters, which can affect delta smelt survival. Survival was consistently high in samples collected at the high conductivity, high turbidity site at Suisun Slough at Rush Ranch, as was observed in tests performed in 2008. At site 340, where conductivity was higher and turbidity was lower than in Suisun Slough, survival was generally lower, indicating that turbidity is an important factor influencing delta smelt survival.

Table 5-1. Survival in *H. transpacificus* tests examining the toxicity of water samples collected from sites in the Sacramento - San Joaquin delta. Results indicated in shaded boxes are significantly different from the most appropriate conductivity- and turbidity-specific control. Samples collected at Hood, Light 55, and Cache Sl. at Lindsey Sl. were compared to the Low EC Control. Those collected at the Rough and Ready DWR station were compared to the Mid EC Control. Those collected at Suisun Sl. at Rush Ranch were compared to the High EC Control. Those collected at the Napa River at Vallejo Seawall were compared to the High EC Control, and later to the Very High EC Control.

		Sampling Event	3/17/09 - 3/19/09	3/31/09 - 4/2/09	4/14/09 - 4/16/09	4/28/09 - 4/30/09	5/12/09 - 5/14/09	5/26/09 - 5/28/09
		Age of Delta Smelt	30 days	44 days	54 days	41 days	41 days	55 days
Endpoint	Treatment	Mean EC (uS/cm)	Survival					
96-hour Survival	Low EC Control	160	-	85.0	84.7^{N} / 65.0^{A}	79.2^{N} / 88.2^{A}	76.4	79.2
	Low EC Low Turbidity Control	186	-	66.8	46.7	92.5	68.8	87.5
	Low EC Low Turbidity Control with Tannins	174	-	31.8	-	-	-	-
	Hood	157	-	51.0*	67.0*	79.5	62.9	89.7
	Light 55	262	-	69.3	71.4	85.0	84.7	91.9
	Cache Lindsey	234	-	53.6*	55.3	82.5	94.7	91.3
	Mid EC Control	644	-	81.4	75.6	88.0	80.3	70.8
	Rough and Ready Island	593	-	43.0*	59.8	90.7	56.7	86.1
	High EC Control	3751	-	86.1	82.5	100.0	86.4	92.5
	Low Turbidity Control	3750	-	81.6	83.3	88.6	85.4	92.5
	Suisun	3672	-	97.7	94.7	97.5	80.4	89.2
	Very High EC Control	15776	-	-	-	-	72.1	70.8
	340	15078	-	88.6	62.2**	97.7	68.9	67.5
7-day Survival	Low EC Control	160	8.3	70.0	58.9^{N} / 65.0^{A}	69.4^{N} / 85.9^{A}	71.4	76.4
	Low EC Low Turbidity Control	186	2.8	43.0	27.4	85.2	59.7	75.0
	Low EC Low Turbidity Control with Tannins	174	-	2.5	-	-	-	-
	Hood	157	8.7	19.5*	30.1*	55.3	52.3	71.1
	Light 55	262	23.6	40.7*	55.8	80.2	85.5	86.9
	Cache Lindsey	234	2.8	25.0*	46.9	67.5	80.1	81.3
	Mid EC Control	644	15.3	69.5	67.5	76.4	71.9	62.8
	Rough and Ready Island	593	2.8	9.3*	42.2*	88.2	28.1	72.8
	High EC Control	3751	18.6	64.5	70.0	100.0	80.8	82.5
	Low Turbidity Control	3750	18.1	61.6	61.9	86.1	55.2	71.4
	Suisun	3672	95.0	95.5	92.2	93.1	85.7	86.4
	Very High EC Control	15776	-	-	-	-	62.5	68.1
	340	15078	88.8	74.8	62.2	88.2	63.9	62.5

*: These samples showed significantly lower survival compared to an EC-specific control, but not compared to an EC- and turbidity-specific control.

**: Significantly reduced survival was likely caused by extremely high conductivity.

A: Antibiotics added. Antibiotics were added to all treatments in tests initiated 4/30/09 and later.

N: No antibiotics added.

6. *In Situ* Monitoring on the Sacramento & San Joaquin Rivers

During the months of March - May, *in situ* monitoring was conducted at the DWR water quality monitoring stations located in Hood, CA (Sacramento River) and Rough & Ready Island in Stockton, CA (San Joaquin River). Six exposures using *H. transpacificus, P. promelas*, and *H. azteca* were conducted concurrently with ambient delta smelt toxicity testing in the laboratory. During this pilot project, no toxicity was detected in the Sacramento River at Hood or the San Joaquin River at Rough and Ready Island. *H. transpacificus* survival was generally higher in ambient water than in the control, potentially due to slightly higher water temperatures in the control system, *H. azteca* survival was consistently high in ambient water as well as controls throughout the *in situ* season. *P. promelas* survival was variable in both the control and ambient water. Poor *P. promelas* survival in controls was attributed to the addition of algal paste to optimize turbidity conditions for delta smelt larvae. Additional information including system design and exposure methods are provided below.

6.1 System Design

In situ devices were installed inside DWR water quality monitoring stations located directly above the Sacramento River in the town of Hood, CA and next to the San Joaquin River on Rough & Ready Island in Stockton, CA. Positioning the devices inside these small buildings had several advantages over placing the replicate cages inside the river itself, including improved temperature control, flow control, and ease of daily access. The device located at Rough & Ready Island was slightly different in layout than the device at Hood due to space restrictions, but overall function was the same. Ambient water was supplied from DWR's sampling station pump and delivered to the exposure chamber at 3.8 liters per minute (LPM). The apparatus consisted of three main parts: the ambient exposure chamber, the control exposure chamber, and the control sump. Plumbing that connected these three parts consisted primarily of common polyvinyl chloride (PVC) plumbing supplies. The function of each main part is described below.

The ambient exposure chamber consisted of a customized, white acrylic tank surrounded by an outer bath filled with flowing ambient water to maintain temperature. During the acclimation period for delta smelt, the chamber was filled with control water supplied from the control sump below, and at test initiation, control water was switched over to ambient water and the outer bath was drained. Held within the chamber were four replicate cages for each of the three test species (Figure 6-1). The largest cages, used for larval delta smelt, *H. transpacificus*, were made from one gallon high density polyethylene (HDPE) buckets. These buckets and lids were black to provide optimal lighting conditions (less than 1 ft-candle through a hole in the lid) for *H. transpacificus*. Cages used for *P. promelas* and *H. azteca* were constructed from two manufactured parts; a low density polyethelene pipe cap (Niagra, Erie, PA) and nylon tea strainer (The Republic of Tea, Navato, CA). The exposure chamber lid that covered these cages was constructed from clear acrylic in order to allow ambient light into the chamber (16:8 light:dark cycle).

The control exposure chamber, exposure cages and lids were identical to those in the ambient system. Control water was supplied from the control sump immediately below and the control exposure chamber was also surrounded by an outer ambient water bath in order to maintain the temperature within 1 °C of the ambient water at all times. Flow was set at 3.8 LPM.

The control sump consisted of an 11 gallon HDPE bath containing a 210 gallons per hour (GPH) pond pump, which supplied recirculating control water to the control exposure chamber at all times, and to the ambient exposure chamber during acclimation only. The control water consisted of hatchery water diluted with deionized water or salted up with Instant Ocean to the same specific conductance as its corresponding ambient water. Approximately half the control water was replaced daily to reduce an accumulation of total ammonia in the control system and the control sump was aerated to ensure that dissolved oxygen levels remained at or near saturation.

6.1.1 Methods

H. transpacificus obtained from the UCD FCCL were transported directly from the hatchery to each site. Upon arrival, the fish were loaded into replicate buckets containing SC adjusted hatchery water that matched their rearing conditions. The acclimation water also contained Nanno 3600 Instant Algae (ReedMariculture, Inc., Cambell, CA) to raise the turbidity to a minimum of 6 NTU. Over the course of the next 48 hours, the conductivity of the hatchery water was lowered slowly by adding deionized water or dilute hatchery water, until the conductivity matched that of the ambient water. At test initiation, organisms had been acclimated to an appropriate conductivity and temperature. Adult *H. azteca* were obtained from in house cultures and were acclimated in the lab for a minimum of 48 hours prior to the event. *P. promelas* were obtained from Aquatox, Inc. (Hot Springs, AR) and were acclimated a minimum of 24 hours prior to the event then deployed in the *in situ* exposure at 7 days old. A piece of dryed and leached leaf, measuring one cm squared, was placed into each *H. azteca* replicate cage prior to test initiation. All *in situ* species were fed once daily during the exposure period. *P. promelas* and *H. azteca* survival was recorded prior to test initiation and each day during the exposure. *H. transpacificus* survival was recorded at test initiation, on day 4, and at test termination due to the limited visibility in replicate buckets and the need to minimize disturbance.

Turbidity, temperature, total ammonia, pH, DO, SC, EC, hardness and alkalinity were measured in both the ambient and control exposure chambers daily. Once water was inside the exposure tanks, sediment did settle out to some degree causing an increase in sedimentation over the course of the experiment. Turbidity was also measured at the ambient water source to determine the turbidity going into the system. To the extent possible, SC, turbidity, and temperature were manipulated in the control to parallel the ambient exposure system. The SC and turbidity of the control water was adjusted daily immediately following a partial water exchange. Although we intended to adjust the turbidity of the control water to match the ambient water, we were unable to match the turbidity since the addition of too much alga confounds exposure results by increasing ammonia and producing more pathogens. Turbidity readings were consistently lower in the control water than the ambient water.

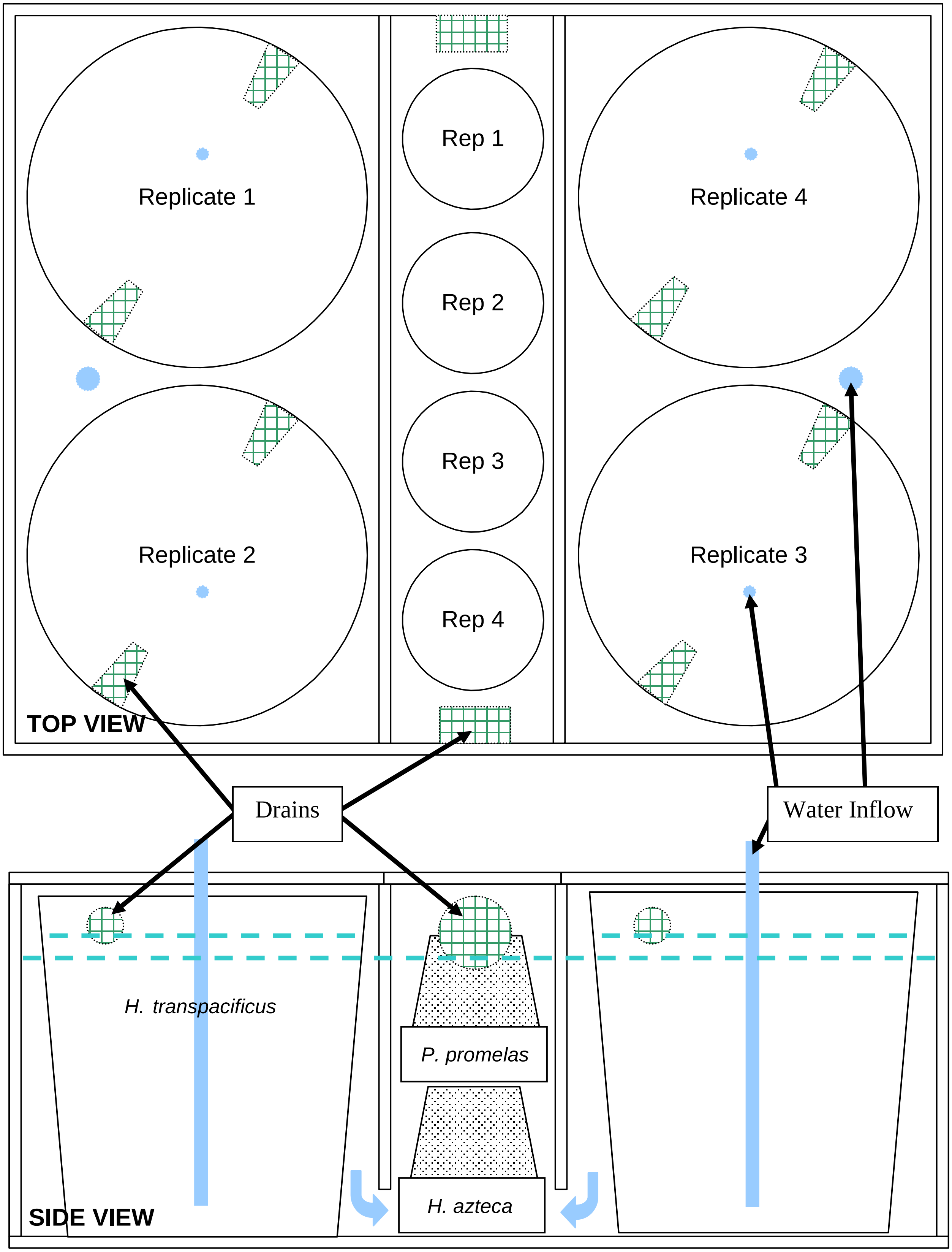

Figure 6-1. Top and side view of an exposure chamber for *in situ* devices.

6.1.2 Statistical Analysis

At each site during each sampling event, the performance of each species was compared between control and ambient treatments using USEPA standard single-concentration statistical protocols.

6.2 Results

Tables 6-1 and 6-2 show the survival of *H. transpacificus*, *P. promelas* and *H. azteca* at the Rough and Ready DWR Station at Stockton and the Hood DWR Station on the Sacramento River. No significant reductions in survival were detected at either site during any sampling event. *H. transpacificus* survival was generally higher in ambient waters than in the controls, *H. azteca* survival was consistently high throughout the *in situ* season, and *P. promelas* survival was variable in both the control and ambient waters.

Table 6-1. 96-hour and 7-day survival of animals examined in flow-through tests initiated at the Rough and Ready DWR Station, Stockton, CA.

Date	Treatment	*H. transpacificus*				*P. promelas*				*H. azteca*			
		96-hr Survival (%)		7-day Survival (%)		96-hr Survival (%)		7-day Survival (%)		96-hr Survival (%)		7-day Survival (%)	
		mean	se	mean	se	mean	se	mean	se	mean	se	mean	se
3/19/2009	Control	-	-	22	10.4	94	6.3	71	16.1	95	5.0	95	5.0
	Ambient	-	-	35	9.3	80	0.0	65	9.6	100	0.0	100	0.0
4/2/2009	Control	61	8.9	41	7.9	60	8.2	45	5.0	95	5.0	95	5.0
	Ambient	75	6.8	61	9.4	90	10.0	90	10.0	100	0.0	100	0.0
4/16/2009	Control	63	9.7	59	12.4	65	12.6	65	12.6	100	0.0	100	0.0
	Ambient	71	9.8	66	12.5	45	12.6	40	8.2	100	0.0	100	0.0
4/30/2009	Control	79	12.5	68	15.8	75	9.6	70	12.9	95	5.0	90	10.0
	Ambient	61	16.5	47	14.1	70	12.9	70	12.9	100	0.0	85	9.6
5/14/2009	Control	15	9.6	0	0.0	95	5.0	95	5.0	100	0.0	95	5.0
	Ambient	15	8.6	15	8.6	100	0.0	100	0.0	100	0.0	100	0.0
5/28/2009	Control	-	-	-	-	100	0.0	100	0.0	100	0.0	100	0.0
	Ambient	-	-	-	-	100	0.0	100	0.0	100	0.0	95	5.0

Table 6-2. 96-hour and 7-day survival of animals examined in flow-through tests initiated at the DWR Station on the Sacramento River at Hood.

Date	Treatment	*H. transpacificus*				*P. promelas*				*H. azteca*			
		96-hr Survival (%)		7-day Survival (%)		96-hr Survival (%)		7-day Survival (%)		96-hr Survival (%)		7-day Survival (%)	
		mean	se	mean	se	mean	se	mean	se	mean	se	mean	se
3/19/2009	Control	-	-	21	5.5	100	0.0	85	15.0	100	0.0	95	5.0
	Ambient	-	-	46	8.4	85	9.6	75	15.0	95	5.0	95	5.0
4/2/2009	Control	75	4.8	62	8.8	85	9.6	30	5.8	95	5.0	80	8.2
	Ambient	84	10.3	77	7.0	90	5.8	85	5.0	85	9.6	80	8.2
4/16/2009	Control	59	5.0	29	5.1	95	5.0	95	5.0	95	5.0	90	5.8
	Ambient	74	10.5	64	13.8	90	5.8	85	9.6	95	5.0	85	9.6
4/30/2009	Control	47	10.9	43	13.3	95	5.0	95	5.0	100	0.0	100	0.0
	Ambient	43	6.5	40	6.9	100	0.0	95	5.0	100	0.0	100	0.0
5/14/2009	Control	56	18.8	44	15.7	95	5.0	95	5.0	100	0.0	100	0.0
	Ambient	69	12.0	50	10.2	100	0.0	100	0.0	100	0.0	100	0.0
5/28/2009	Control	13	8.0	4	4.2	85	5.0	85	5.0	95	5.0	95	5.0
	Ambient	34	7.9	27	8.4	95	5.0	85	15.0	100	0.0	90	5.8

6.2.1 Discussion

One of the greatest advantages to the *in situ* exposure is that the organisms experience the fluctuations of toxicant concentrations for the same length of time that stationary organisms in the river would experience them. In contrast, organisms that are exposed in a laboratory setting to a one-time grab sample experience the same water sample for a defined test period. A one-time grab sample can be collected when the concentrations of a chemical are at its peak, fall well below the peak concentration or miss a chemical pulse entirely. Laboratory static renewal tests utilizing one-time sub surface grab samples can therefore overestimate or underestimate toxicity depending on when a sample is collected relative to a toxic pulse moving through the system. The in situ devices renew water continuously with approximately 95% of the water renewed every half hour. The constant flow to the system is representative of the river conditions throughout the exposure period.

No toxicity was detected in the Sacramento River at Hood or the San Joaquin River at Rough and Ready Island suggesting that any toxicant(s) that may have traveled through the system were not at high enough concentrations for enough time to cause reduced survival to the test species. *H. transpacificus* survival was generally higher in ambient water than in the control, which decreased our ability to detect a toxic event with the species. A number of variables, including natural food supply, temperature, and turbidity may have contributed to higher delta smelt survival in ambient water compared to the controls. *H. azteca* survival was consistently high in ambient water and control water while *P. promelas* survival was variable in both, possibly due to the promotion of bacterial growth following the addition of *Nannochloropsis*.

Despite our efforts to slowly acclimate the *H. transpacificus* to the conductivity and temperature conditions of river water at *in situ* sites, survival of delta smelt remained low. Our recommendations are to use a test species that is more tolerant of transport, salinity and temperature stresses. *P. promelas* and *O. mykiss* appear to be far more tolerant of such stressors. *O. mykiss* might be a suitable species to use during the cold months and a warmwater species might be more suitable during the warmer months.

7. *E. affinis* 7-d Toxicity Testing

7.1 Methods

7.1.1 Toxicity Testing

A 7-d bioassay using juvenile *E. affinis* (starter culture obtained from S. Teh, UC Davis) was developed and a test initiated on 5/1/09 with four samples collected from sites 711, Cache-Ulatis, Hood, and Light 55, all of which are sites with low conductivity water. A series of low conductivity controls at 100, 250, 500, 1000, and 1900 µS/cm were included to evaluate the effects of conductivity on copepod survival. The organisms were cultured at 1900 µS/cm (1 ppt). Test methods were modeled after the USEPA *Ceriodaphnia dubia* Survival and Reproduction Test (USEPA, 2002), chosen for its high likelihood of copepod survival (methods test conducted in April, 2009), minimal water requirements, and ease of recording survival. Each experimental treatment consisted of ten replicate vials, each containing 15 ml of water and one organism. Tests were conducted at 16ºC. Eighty percent of test water was renewed daily, and copepods were fed 15 µl of diluted Shellfish Diet (Reed Mariculture, Campbell, CA) which consists of four microalgae, daily. Diet was prepared by adding 30 ml of concentrated Shellfish Diet (approximately 2 billion cells per ml) to 300 ml of culture water. Moderately hard synthetic water was used for culturing and control treatments. Survival was recorded daily. Initial and final water quality measurements including SC, EC, temperature, pH, and DO were taken on Day 0 and Day 1. Ammonia and turbidity were measured for all ambient water samples.

7.1.2 Statistical Analysis

Data from this exposure was analyzed using USEPA standard single-concentration statistical protocols (USEPA 2002). The USEPA method of data analysis showed the results of the tests according to the standardized statistical method used in aquatic toxicology monitoring and regulation throughout the United States. Each comparison of a sample to a control was treated as a separate statistical test, in accordance with USEPA 2002, Appendix H.

7.2 Results

In the control series, survival was best in the highest conductivity treatment of 1900 µS/cm (90% survival after 7 d) and decreased with decreasing conductivity. Survival was generally low in ambient samples with the exception of Cache-Ulatis (100% survival after 7 d). This sample had the highest turbidity (45.9 NTU) and specific conductance (329 µS/cm) of all four sites which may have contributed to better animal performance, despite the low survival encountered in the corresponding conductivity control (Table 7-1). Survival in all ambient samples was higher than survival in the corresponding control water, however it is apparent that conductivity was the most important factor determining copepod survival in all samples tested.

Table 7-1. Results of a *E. affinis* 7-d test initiated 5/1/09 evaluating the toxicity of samples collected on 4/28/09 and 4/30/09.

Treatment	Measured Specific Conductivity (uS/cm)	Survival (%)[1]	
		Mean	SE
L16 Media @ 1 ppt	1930	90	10.0
L16 Media @ 1000 µS/cm	1003	50	16.7
L16 Media @ 500 µS/cm	517	30	15.3
L16 Media @ 250 µS/cm	282	20	13.3
L16 Media @ 100 µS/cm	129	0	0.0
Sacramento R. Deep Water Channel, Light 55	271	50	22.4
Sacramento River at tip of Grand Island (711)	136	20	13.3
Upper Cache Slough at mouth of Ulatis Creek	329	100	0.0
Sacramento River at Hood DWR Station	142	20	13.3

1. Highlighted cells indicate statistically significant reductions in survival compared to the L16 media @ 1 ppt. Ambient samples showed no significant decreases in survival compared to the most appropriate conductivity control waters. Data were analyzed using USEPA standard statistical protocols.

8. Species Sensitivity Studies

Effect concentrations for pesticides, copper, and ammonia were determined for *H. transpacificus*, *P. promelas*, *H. azteca*, and *E. affinis*. Although *C. dubia* sensitivity testing is currently in progress, LC50 values obtained from published literature are presented for comparison. Results obtained from sensitivity testing in 2008 are also included.

Samples from each pesticide concentration as well as a control were submitted to CDFG-WPCL to verify nominal chemical concentrations. In tests evaluating toxicity in both control and hatchery waters, only samples of hatchery water were submitted for chemical analysis. Total ammonia measurements for the ammonia chloride tests were measured at the UCD ATL. Sensitivity testing methods for each species are described below.

8.1 Methods

8.1.1 *H. transpacificus* Sensitivity Tests

Larval delta smelt ranging in age from 45 to 47 days post hatch (DPH) were obtained from the UCD FCCL in Byron, CA. The organisms were acclimated a minimum of 24 hours with hatchery water adjusted to a specific conductance (SC) of 900 µS/cm using Instant Ocean and a pH of 7.9 using HCl. *H. transpacificus* were fed *Artemia* nauplii three times daily during acclimation and exposures. After the acclimation period, ten organisms were randomly loaded into each of the four replicate buckets using a 50 ml beaker. Mortality was recorded daily using a small flashlight. On Day 2 of the exposures, 80% of test solutions were renewed during which dead fish, excess artemia, and other detritus were removed. At the end of each 96-h exposure, surviving organisms were euthanized with MS-222 and preserved with liquid nitrogen for subsequent molecular studies.

8.1.2 *P. promelas* Sensitivity Tests

Larval fathead minnows were obtained from Aquatox, Inc. (Hot Springs, AR). Organisms used in sensitivity tests were <48 hours old and were acclimated to laboratory conditions 24 hours prior to test initiation.7-d LC50 test methods followed those outlined in the Fathead Minnow Larval Survival and Growth Test (USEPA, 2002). These tests were performed in deionized water amended to US EPA moderately hard standards (DIEPAMH) as well as hatchery water filtered through a 1 micron filter. Water was adjusted to an SC of 900 µS/cm using Instant Ocean and a pH of 7.9 using HCl. Stock solutions were prepared by dissolving pesticides in methanol and ammonia and copper in glass distilled water. Chemicals were spiked into test solutions on Days 0, 2, 4, and 6. Where methanol was used as a solvent, solvent control treatments containing 0.05% methanol (equal to the highest concentration added to insecticide treatments) were added. These methanol treatments were aerated after recognition of dissolved oxygen problems associated with the addition of methanol, likely due to bacterial growth and associated respiration. Mortality was recorded daily, and at test termination, a portion of organisms were preserved using liquid nitrogen for subsequent molecular studies while the rest were dried to a constant weight for the biomass endpoint. If ten surviving fish were present in a replicate at test termination, five were preserved with liquid nitrogen and five were dried; if nine surviving fish were present in a replicate, 4 were preserved with liquid nitrogen and five were dried; if eight surviving fish were present in a replicate, four were preserved with liquid nitrogen and four were dried. If there were seven or less surviving fish in a replicate, all were dried to calculate biomass and average weight per individual.

8.1.3 *H. azteca* Sensitivity Tests

H. azteca purchased from Aquatic Research Organisms were received at the UCD ATL 48 hours prior to test initiation and acclimated to laboratory conditions. The 10-day sensitivity tests were conducted in both DIEPAMHR and water collected from the UCD FCCL. Waters were adjusted to a SC of 900 µS/cm using Instant Ocean and a pH of 7.9 using HCl. Prior to initiating bioassays, the water samples were brought to the test temperature of 23º C and aerated at a rate of 100 bubbles/min until the dissolved oxygen concentration was approximately 8.5 mg/L.

Sensitivity tests consisted of four 250 ml replicate glass beakers, each containing 100 ml of sample, a one-square-inch piece of nitex screen and 10 organisms. Tests were initiated with 7-14 day-old *H. azteca*. Animals in each replicate were fed 1000 µl of YCT on test initiation and on days 2, 4, 6 and 8, following the renewal of 75% of the test waters. Each series of sensitivity tests included a standard laboratory control, hatchery water control and any applicable method blanks.

Tests were conducted at a temperature of 23 ± 2º C with a 16h:8h, light:dark photoperiod. Mortality was recorded daily and waters were renewed on days 2, 4, 6 and 8. On day 10, the surviving *H. azteca* were dried and weighed to determine dry tissue weight per individual and relative growth. Effect data such as NOEC, LOEC, LC_{10}, LC_{50} and EC_{25} were calculated on both the 96-h and 10-d endpoints.

8.1.4 *E. affinis* Sensitivity Tests

Please refer to Appendix A for *E. affinis* sensitivity testing methods.

8.1.5 Statistical Analysis

Lethal and sublethal effective concentrations were calculated using CETIS v. 1.1.2 (Tidepool Scientific Software, McKinleyville, CA, USA, 2006). NOEC and LOEC were calculated using USEPA standard statistical protocols (USEPA 2002). LC50s and EC50s were calculated using linear regression, non-linear regression, or linear interpolation methods. For each endpoint, toxicity is defined as a statistically significant difference ($p < 0.05$) to the laboratory control. Percentage minimum significant differences (PMSD) of Dunnett's multiple comparison procedure were calculated for all multiple concentration statistical tests.

8.2 Results

8.2.1 *H. transpacificus* Sensitivity Tests

Delta smelt sensitivities to ammonia/ium, the organophosphate insecticide chlorpyrifos, and the pyrethroid insecticides esfenvalerate and permethrin, were tested at the 96-hour survival endpoint during July, 2009 (Table 8-1). Effect concentrations obtained from tests conducted in 2008 are also presented (Table 8-2). Among pesticides tested in both 2008 and 2009, delta smelt were most sensitive to bifenthrin, followed in order of decreasing sensitivity by esfenvalerate, cyfluthrin, chlorpyrifos, and permethrin.

Table 8-1. Measured 96-h effect concentrations for ammonia/ium and nominal 96-h effect concentrations for pesticides in *H. transpacificus* tests conducted in July, 2009.

Age (days post hatch)	Analyte	NOEC	LOEC	96-hour LC10		96-hour LC50	
				Estimate	95% C.I.	Estimate	95% C.I.
47	Total Ammonia Nitrogen (mg/L)	14.4	29.0	5.38	< 1.9 - 9.38	11.81	8.09 - 18.47
	Un-ionized Ammonia (mg/L)	0.191	0.333	0.084	< 0.002 - 0.127	0.164	0.119 - 0.239
47	Chlorpyrifos (µg/L)	200	> 200	12.89	< 12.5 - 14.6	18.62	< 12.5 - 23.3
45	Esfenvalerate (µg/L)	0.188	0.375	< 0.094	< 0.094 – 0.319	0.239	0.051 – 0.282
45	Permethrin (µg/L)	5.0	> 5.0	-	-	-	-
45	Chlorpyrifos (µg/L)	< 18.75	18.75	NA[1]	NA[1]	10.7	1.5 - 31.1

[1] The LOEC was the lowest concentration tested thus LC10 estimate is not considered reliable.

Table 8-2. Measured 96-h effect concentrations for ammonia/ium, copper, and pesticides in *H. transpacificus* tests conducted in April - May, 2008.

Age (days post hatch)	Analyte	NOEC	LOEC	96-hour LC10		96-hour LC50	
				Estimate	95% C.I.	Estimate	95% C.I.
51	Total Ammonia Nitrogen (mg/L)	5	9	4.2	NA	12.0	NA
	Un-ionized Ammonia (mg/L)	0.066	0.105	0.055	NA	0.147	NA
49	Copper, Total (µg/L)	40.4	78.2	50.4	NA	88.1	NA
49	Copper, Dissolved (µg/L)	41.4	76.2	50	NA	87	NA
49	Bifenthrin (µg/L)	0.120	0.260	0.095	0.061 – 0.117	0.143	0.116 – 0.169
49	Cyfluthrin (µg/L)	0.407	0.890	0.260	0.067 – 0.357	0.420	0.261 – 0.558

8.2.2 *P. promelas* Sensitivity Tests

Cyfluthrin and permethrin sensitivities of *P. promelas* were examined at 96-h survival, 7-d survival, and 7-d biomass endpoints (Tables 8-3 and 8-4) in July, 2009. Effect concentrations obtained from sensitivity tests conducted in 2008 are also presented (Tables 8-5 through 8-9). Analytical data for 2009 tests are pending, therefore nominal effect concentrations are presented here. These tests compared performance in hatchery water obtained from the UCD FCCL to performance in conductivity and pH-adjusted DIEPAMH control water. Performance when exposed to cyfluthrin did not differ between hatchery water and control water. The permethrin sensitivity test showed no differences in sensitivity as measured by the survival endpoints, but a decline in biomass was seen at a lower permethrin concentration in fish exposed in hatchery water, compared to those exposed in DIEPAMH control water. *P. promelas* showed greater sensitivity to cyfluthrin than to permethrin.

Table 8-3. Nominal 96-h and 7-day effect concentrations of cyfluthrin in a *P. promelas* test initiated on 7/07/09.

Endpoint	Matrix	Cyfluthrin (µg/L)					
		NOEC	LOEC	LC10 / EC10		LC50 / EC25	
				Estimate	95% C.I.	Estimate	95% C.I.
96-hr Survival	DIEPAMH @ 900 uS/cm	1.000	2.000	1.056	0.997 – 1.091	1.414	1.371 – 1.483
	Hatchery Water @ 900 uS/cm	1.000	2.000	1.036	0.978 – 1.093	1.388	1.345 – 1.430
7-day Survival	DIEPAMH @ 900 uS/cm	1.000	2.000	0.919	0.590 – 1.136	1.353	1.269 – 1.431
	Hatchery Water @ 900 uS/cm	1.000	2.000	1.049	0.992 – 1.085	1.398	1.355 – 1.424
7-day Biomass	DIEPAMH @ 900 uS/cm	1.000	2.000	1.026	0.312 – 1.099	1.147	1.008 – 1.215
	Hatchery Water @ 900 uS/cm	1.000	2.000	1.072	0.948 – 1.072	1.189	1.081 – 1.189

Table 8-4. Nominal 96-h and 7-day effect concentrations of permethrin in a *P. promelas* test initiated on 7/07/09.

Endpoint	Matrix	Permethrin (µg/L)					
				LC10 / EC10		LC50 / EC25	
		NOEC	LOEC	Estimate	95% C.I.	Estimate	95% C.I.
96-hr Survival	DIEPAMH @ 900 uS/cm	4.0	8.0	5.2	4.5 - 7.3	10.0	8.2 - 11.2
	Hatchery Water @ 900 uS/cm	8.0	16.0	8.2	4.2 - 8.8	11.1	10.3 - 11.5
7-day Survival	DIEPAMH @ 900 uS/cm	4.0	8.0	4.8	4.1 - 5.8	9.3	6.0 - 10.9
	Hatchery Water @ 900 uS/cm	8.0	16.0	8.0	4.3 - 8.7	10.9	10.3 - 11.5
7-day Biomass	DIEPAMH @ 900 uS/cm	8.0	16.0	8.6	5.3 - 8.6	11.4	10.4 - 11.4
	Hatchery Water @ 900 uS/cm	8.0	16.0	8.6	0.6 - 8.6	9.6	8.5 - 9.6

Table 8-5. Measured 96-h and 7-day effect concentrations for ammonia/ium (mg/L) in a larval fathead minnow test initiated on 9/17/08. D900 = DIEPAMH adjusted to 900 µS/cm. HW = Hatchery water from the Fish Conservation and Culture Laboratory of the University of California Department of Animal Sciences in Byron, CA.

			Estimate (mg/L)	95% C.I.	NOEC	LOEC	PMSD
Total Ammonia Nitrogen	D900 Ammonia - 96-h Survival	LC_{10}	17.1	16 – 21	15	30.8	16.51%
		LC_{50}	29.9	26 – 34			
	D900 Ammonia - 7-day Survival	LC_{10}	17.1	16 – 21	15	30.8	16.51%
		LC_{50}	29.9	26 – 34			
	D900 Ammonia - 7-day Biomass	EC_{25}	20.6	17 – 25	15	30.8	22.82%
Un-ionized Ammonia	D900 Ammonia - 96-h Survival	LC_{10}	0.597	0.56 - 0.73	0.518	1.004	16.51%
		LC_{50}	1.000	0.89 - 1.12			
	D900 Ammonia - 7-day Survival	LC_{10}	0.597	0.56 - 0.73	0.518	1.004	16.51%
		LC_{50}	1.000	0.89 - 1.12			
	D900 Ammonia - 7-day Biomass	EC_{25}	0.713	0.61 - 0.86	0.518	1.004	22.82%
Total Ammonia Nitrogen	HW Ammonia - 96-h Survival	LC_{10}	16.0	15 – 16	15.2	29.8	4.96%
		LC_{50}	20.9	20 – 21			
	HW Ammonia - 7-day Survival	LC_{10}	16.0	15 – 16	15.2	29.8	4.96%
		LC_{50}	20.9	20 – 21			
	HW Ammonia - 7-day Biomass	EC_{25}	17.1	15 – 18	15.2	29.8	17.85%
Un-ionized Ammonia	HW Ammonia - 96-h Survival	LC_{10}	0.662	0.63 - 0.68	0.629	1.121	4.96%
		LC_{50}	0.827	0.80 - 0.85			
	HW Ammonia - 7-day Survival	LC_{10}	0.662	0.63 - 0.68	0.629	1.121	4.96%
		LC_{50}	0.827	0.80 - 0.85			
	HW Ammonia - 7-day Biomass	EC_{25}	0.703	0.64 - 0.74	0.629	1.121	17.85%

Table 8-6. Measured 96-h and 7-day effect concentrations for copper (µg/L) in a larval fathead minnow test initiated on 8/7/08. D900 = DIEPAMH adjusted to 900 µS/cm. HW = Hatchery water from the Fish Conservation and Culture Laboratory of the University of California Department of Animal Sciences in Byron, CA.

			Estimate (µg/L)	95% CI	NOEC	LOEC	PMSD
Nominal	D900 Copper - 96-h Survival	LC10	47	43 - 66	31.3	62.5	8.06%
		LC50	99	87 - 113			
	D900 Copper - 7-day Survival	LC10	38.9	35 - 48	31.3	62.5	12.31%
		LC50	80.08	70 - 91			
	D900 Copper - 7-day Biomass	EC25	>125	NA	125	>125	64.57%
Total	HW Copper - 96-h Survival	LC10	132	81 - 150	132	260	6.37%
		LC50	216	188 - 248			
	HW Copper - 7-day Survival	LC10	90	79 - 117	69.2	132	7.92%
		LC50	162	146 - 180			
	HW Copper - 7-day Biomass	EC25	132	65 - 163	69.2	132	18.61%
Dissolved	HW Copper - 96-h Survival	LC10	125	74 - 141	125	238	6.37%
		LC50	200	175 - 228			
	HW Copper - 7-day Survival	LC10	82	72 - 109	62.3	125	7.92%
		LC50	151	136 - 168			
	HW Copper - 7-day Biomass	EC25	125	57 - 154	62.3	125	18.61%

Table 8-7. Nominal and measured 96-h and 7-day effect concentrations for bifenthrin (µg/L) in a larval fathead minnow test initiated on 9/24/08. D900 = DIEPAMH adjusted to 900 µS/cm. HW = Hatchery water from the Fish Conservation and Culture Laboratory of the University of California Department of Animal Sciences in Byron, CA.

			Estimate (µg/L)	95% C.I.	NOEC	LOEC	PMSD
Nominal	D900 - Bifenthrin - 96-hr Survival	LC_{10}	0.125	0.098 – 0.147	0.125	0.250	14.31%
		LC_{50}	0.214	0.188 – 0.244			
	D900 - Bifenthrin - 7-day Survival	LC_{10}	0.101	0.079 – 0.117	0.125	0.250	13.35%
		LC_{50}	0.166	0.146 – 0.188			
	D900 - Bifenthrin - 7-day Biomass	EC_{25}	0.138	0.118 – 0.157	0.125	0.250	24.84%
Measured	HW - Bifenthrin - 96-hr Survival	LC_{10}	0.026	0.023 – 0.034	0.024	0.038	29.91%
		LC_{50}	0.057	0.048 – 0.067			
	HW - Bifenthrin - 7-day Survival	LC_{10}	0.024	0.018 – 0.029	0.024	0.038	19.34%
		LC_{50}	0.045	0.038 – 0.053			
	HW - Bifenthrin - 7-day Biomass	EC_{25}	0.040	0.021 – 0.054	0.038	0.096	32.42%

Table 8-8. Nominal and measured 96-h and 7-day effect concentrations for esfenvalerate (µg/L) in a larval fathead minnow test initiated on 8/19/08. D900 = DIEPAMH adjusted to 900 µS/cm. HW = Hatchery water from the Fish Conservation and Culture Laboratory of the University of California Department of Animal Sciences in Byron, CA.

			Estimate (µg/L)	95% C.I.	NOEC	LOEC	PMSD
Nominal	D900 Esfenvalerate - 96-h Survival	LC_{10}	0.541	0.522 – 0.553	0.500	1.000	5.12%
		LC_{50}	0.779	0.721 – 0.842			
	D900 Esfenvalerate - 7-day Survival	LC_{10}	0.536	0.518 – 0.542	0.500	1.000	6.56%
		LC_{50}	0.719	0.700 – 0.739			
	D900 Esfenvalerate - 7-day Biomass	EC_{25}	0.607	0.575 – 0.635	0.500	1.000	17.43%
Measured	HW Esfenvalerate - 96-h Survival	LC_{10}	0.516	0.490 – 0.537	0.500	0.920	7.10%
		LC_{50}	0.668	0.649 – 0.682			
	HW Esfenvalerate - 7-day Survival	LC_{10}	0.518	0.492 – 0.534	0.500	0.920	7.38%
		LC_{50}	0.669	0.650 – 0.680			
	HW Esfenvalerate - 7-day Biomass	EC_{25}	0.582	0.527 – 0.582	0.500	0.920	23.60%

Table 8-9. Nominal and measured 96-h and 7-day effect concentrations for chlorpyrifos (µg/L) in a larval fathead minnow test initiated on 8/19/08. D900 = DIEPAMH adjusted to 900 µS/cm. HW = Hatchery water from the Fish Conservation and Culture Laboratory of the University of California Department of Animal Sciences in Byron, CA.

			Estimate (ug/L)	95% C.I.	NOEC	LOEC	PMSD
Nominal	D900 Chlorpyrifos - 96-h Survival	LC_{10}	233	180 - 272	200	400	5.38%
		LC_{50}	>400	NA			
	D900 Chlorpyrifos - 7-day Survival	LC_{10}	202	113 - 230	200	400	11.07%
		LC_{50}	332.6	228 - 384			
	D900 Chlorpyrifos - 7-day Biomass	EC_{25}	79.1	41 - 131	25	50	15.10%
Measured	HW Chlorpyrifos - 96-h Survival	LC_{10}	171	128 - 203	144	311	13.64%
		LC_{50}	> 311	NA			
	HW Chlorpyrifos - 7-day Survival	LC_{10}	145	88 - 167	144	311	18.91%
		LC_{50}	252.7	NA			
	HW Chlorpyrifos - 7-day Biomass	EC_{25}	60.6	10 - 171	43.2	82.4	24.39%

8.2.3 *H. azteca* Sensitivity Tests

Effect concentrations of pesticides, ammonia and copper are presented for 96-hour survival, 10-day survival, and 10-day weight endpoints (Tables 8-10 through 8-16). We have calculated effect concentrations for bifenthrin, chloropyrifos and permethrin based on measured concentrations. Effect concentrations for ammonia were derived for nominal ammonium concentrations, measured total ammonia nitrogen and un-ionized ammonia calculated from measured ammonia nitrogen, and the mean pH and temperature during the test. Analytical data for cyfluthrin, diazinon and copper are pending and therefore nominal effect concentrations are presented here.

All *H. azteca* sensitivity studies included a comparison of sensitivities in hatchery water collected from the UCD FCCL with sensitivities in DIEPAMHR control water. Both hatchery water and control water was adjusted to an SC of 900 µS/cm (855-945) and pH 7.9 ± 0.1.

Effect concentrations in hatchery water did not differ detectably from those in DIEPAMHR for most of the toxicants and endpoints tested. *H. azteca* tended to be more sensitive to cyfluthrin in hatchery water, and also to bifenthrin in hatchery water for the 10-day survival and weight endpoints, though differences were not significant. Sensitivity to permethrin and copper was detectably higher in DIEPAMHR for the 10-day survival endpoint. Sensitivity to total ammonia/um and un-ionized ammonia was significantly higher in DIEPAMHR as measured by ammonia nitrogen and un-ionized ammonia concentrations.

Most of the sensitivity studies showed a decrease in *H. azteca* weight with increasing toxicant concentration, but this effect was not observed in tests with chlorpyrifos, permethrin, and cyfluthrin (DIEPAMHR only).

Table 8-10. Nominal 96-h and 10-day effect concentrations of cyfluthrin in a *H. azteca* test initiated on 12/12/08.

Endpoint	Matrix	Cyfluthrin (ng/L)					
		NOEC	LOEC	LC10 / EC10		LC50 / EC25	
				Estimate	95% C.I.	Estimate	95% C.I.
96-hour Survival	DIEPAMHR @ 900 uS/cm	1.95	3.91	2.12	2.04 - 2.21	3.04	2.75 - 3.54
	Hatchery Water	0.98	1.95	1.30	1.01 - 1.83	2.70	2.25 - 3.17
10-day Survival	DIEPAMHR @ 900 uS/cm	1.95	3.91	2.12	2.05 - 2.20	2.97	2.73 - 3.57
	Hatchery Water	0.98	1.95	1.22	0.99 - 1.58	2.39	1.95 - 2.83
Weight	DIEPAMHR @ 900 uS/cm	1.95	> 1.95	-	-	-	-
	Hatchery Water	< 0.98	0.98	0.29	0.16 - 0.66	0.88	0.45 - >3.9

Table 8-11. Nominal 96-h and 10-day effect concentrations of diazinon in a *H. azteca* test initiated on 12/30/08.

Endpoint	Matrix	Diazinon (ng/L)					
		NOEC	LOEC	LC10 / EC10		LC50 / EC25	
				Estimate	95% C.I.	Estimate	95% C.I.
96-hour Survival	DIEPAMHR @ 900 uS/cm	2000	4000	2210	1410 - 2690	4440	3300 - 5470
	Hatchery Water	2000	4000	2410	2000 - 3480	4900	2790 - 5810
10-day Survival	DIEPAMHR @ 900 uS/cm	2000	4000	1340	1150 - 2350	2670	2190 - 3080
	Hatchery Water	2000	4000	2110	1950 - 2240	3120	3000 - 3270
Weight	DIEPAMHR @ 900 uS/cm	1000	2000	930	< 500 - 1390	1270	0.000 - 1780
	Hatchery Water	2000	> 2000	1050	550 - 2020	> 2000	-

Table 8-12. Measured 96-h and 10-day effect concentrations of bifenthrin in a *H. azteca* test initiated on 1/14/09.

Endpoint	Matrix	Bifenthrin (ng/L)					
		NOEC	LOEC	LC10 / EC10		LC50 / EC25	
				Estimate	95% C.I.	Estimate	95% C.I.
96-hour Survival	DIEPAMHR @ 900 uS/cm	2	8	2.4	2.3 - 2.4	4.4	4.0 - 5.0
	Hatchery Water	3	6	2.9	1.4 - 3.4	4.3	4.0 - 4.9
10-day Survival	DIEPAMHR @ 900 uS/cm	2	8	2.3	2.3 - 2.3	4.2	4.2 - 4.2
	Hatchery Water	1	3	1.2	1.0 - 1.6	2.3	1.6 - 4.5
Weight	DIEPAMHR @ 900 uS/cm	0.6	2	0.5	< 0.6 - 1.2	1.3	< 0.6 - 2.3
	Hatchery Water	< 1	1	0.2	0.1 - 0.2	0.5	0.4 - 0.7

Table 8-13. Measured 96-h and 10-day effect concentrations of chlorpyrifos in a *H. azteca* test initiated on 1/15/09.

Endpoint	Matrix	Chlorpyrifos (ng/L)					
		NOEC	LOEC	LC10 / EC10		LC50 / EC25	
				Estimate	95% C.I.	Estimate	95% C.I.
96-hour Survival	DIEPAMHR @ 900 uS/cm	14	128	28	15.9 - 82.3	186.1	31.0 - 259.2
	Hatchery Water	66	133	78.3	75.4 - 83.4	146.6	131.4 - 161.8
10-day Survival	DIEPAMHR @ 900 uS/cm	14	128	18.1	13.6 - 22.5	67.2	32.6 - 164.5
	Hatchery Water	66	133	72.1	70.5 - 73.8	102.6	91.2 - 114.7
Weight	DIEPAMHR @ 900 uS/cm	14	> 14	-	-	-	-
	Hatchery Water	66	> 66	-	-	-	-

Table 8-14. Measured 96-h and 10-day effect concentrations of permethrin in a *H. azteca* test initiated on 1/21/09.

Endpoint	Matrix	Permethrin (ng/L)					
		NOEC	LOEC	LC10 / EC10		LC50 / EC25	
				Estimate	95% C.I.	Estimate	95% C.I.
96-hour Survival	DIEPAMHR @ 900 uS/cm	19	90	25.3	21.9 - 36.2	78.3	33.9 - > 90
	Hatchery Water	69	> 69	> 69	-	> 69	-
10-day Survival	DIEPAMHR @ 900 uS/cm	19	90	22.9	22.2 - 23.4	47.8	40.8 - 52.5
	Hatchery Water	40	69	44.1	1.6 - 56.0	> 69	-
Weight	DIEPAMHR @ 900 uS/cm	90	> 90	-	-	-	-
	Hatchery Water	69	> 69	-	-	-	-

Table 8-15. Measured 96-h and 10-day effect concentrations of ammonia/ium in a *H. azteca* test initiated on 2/26/09.

Analyte	Endpoint	Matrix	NOEC	LOEC	LC10 / EC10		LC50 / EC25	
					Estimate	95% C.I.	Estimate	95% C.I.
Total Ammonia Nitrogen (mg/L)	96-hour Survival	D. @ 900 uS/cm	37.0	78.0	39.4	27.3 - 49.8	102.2	84 - 133
		Hatchery Water	76.0	156.8	53.9	40.0 - 68.9	149.3	115 - 234
	10-day Survival	D. @ 900 uS/cm	37.0	78.0	42.8	29.5 - 52.3	72.9	62 - 84
		Hatchery Water	19.4	39.2	32.3	23.6 - 39.8	72.9	62 - 88
	Weight	D. @ 900 uS/cm	19.0	37.0	6.3	2.9 - 15.7	20.2	< 4.85 - 28.8
		Hatchery Water	156.8	> 156.8	40.5	< 4.85 - 50.6	52.5	18 - 67
Un-ionized Ammonia (mg/L)	96-hour Survival	D. @ 900 uS/cm	1.010	1.512	1.025	0.823 - 1.168	1.714	1.542 - 1.976
		Hatchery Water	1.702	2.500	1.513	1.231 - 1.697	2.406	2.138 - 2.99
	10-day Survival	D. @ 900 uS/cm	1.010	1.512	1.113	0.904 - 1.238	1.454	1.331 - 1.564
		Hatchery Water	0.793	1.378	1.151	0.947 - 1.291	1.731	1.591 - 1.904
	Weight	D. @ 900 uS/cm	0.658	1.01	0.292	0.180 - 0.587	0.688	0.107 - 0.876
		Hatchery Water	2.500	> 2.500	1.392	< 0.279 - 1.501	1.516	0.954 - 1.64

Table 8-16. Nominal 96-h and 10-day effect concentrations of copper in a *H. azteca* test initiated on 4/10/09.

Endpoint	Matrix	Copper (mg/L)					
		NOEC	LOEC	LC10 / EC10		LC50 / EC25	
				Estimate	95% C.I.	Estimate	95% C.I.
96-hour Survival	DIEPAMHR @ 900 uS/cm	0.25	0.5	0.291	0.224 - 0.343	0.484	0.422 - 0.553
	Hatchery Water	0.5	1	0.352	0.274 - 0.412	0.570	0.500 - 0.650
10-day Survival	DIEPAMHR @ 900 uS/cm	0.125	0.25	0.125	0.036 - 0.140	0.174	0.165 - 0.183
	Hatchery Water	0.25	0.5	0.207	0.153 - 0.295	0.318	0.293 - 0.344
Weight	DIEPAMHR @ 900 uS/cm	< 0.125	0.125	0.018	0.015 - 0.024	0.045	0.038 - 0.062
	Hatchery Water	< 0.125	0.125	0.024	0.013 - 0.057	0.060	0.032 - 0.147

8.2.4 *Eurtytemora affinis* Sensitivity Tests

E. affinis: 96-h LC10 and LC50 values were determined for ammonia, copper, the organophosphate insecticide chlorpyrifos, and the pyrethroid insecticides bifenthrin, cyfluthrin, and permethrin by Dr. Teh (UC Davis, CA). Data generated from these tests show *E. affinis* are highly sensitive to copper and ammonia (See Appendix A for full results).

8.2.5 Interspecies Comparison of Sensitivity to Select Toxicants

A comparison of *H. azteca*, *E. affinis* and *C. dubia* shows markedly differing sensitivities to ammonia/um, copper and pesticides (Table 8-17). Effect concentrations for *H. azteca* and *E. affinis* were calculated from test results presented in this report, while *C. dubia* sensitivity values were obtained from the USEPA ECOTOX Database (http://cfpub.epa.gov/ecotox/). Tests to obtain effect concentrations under comparable water quality conditions are currently in process at UCD-ATL. Conductivity, pH and temperature were different in *E. affinis* exposures than in *H. azteca* exposures. *E. affinis* was the most sensitive to ammonia/um and copper, while *H. azteca* was much less sensitive to these materials. The copper sensitivities of *C. dubia* are intermediate, but are more similar to *H. azteca*. *C. dubia* was most sensitive to chlorpyrifos, and was more sensitive than *H. azteca* to diazinon. *H. azteca* was the most sensitive to all pyrethroid insecticides tested (bifenthrin, cyfluthrin and permethrin). *C. dubia* showed the least sensitivity to these materials, while the sensitivity of *E. affinis* was intermediate.

Table 8-17. Comparison of sensitivities for the invertebrates *H. azteca*, *E. affinis*, and *C. dubia* to ammonia, copper, chlorpyrifos, diazinon, bifenthrin, cyfluthrin, and permethrin. * indicates measured concentrations.

Chemical	*H. azteca* 96-h LC50[1]		*E. affinis* 96-h LC50[2]		*C.dubia* 96-h LC50[3]
	Estimate	95% C.I.	Estimate	95% C.I.	
Ammonia Nitrogen*	102.2 mg/L*	84-133 mg/L	7.56 mg/L*	4.07 – 8.95 mg/L	-
Un-ionized Ammonia	1.714 mg/L*	1.542 - 1.976 mg/L	0.12 mg/L*	0.06 – 0.14 mg/L	-
Copper	484 µg/L	422 - 553 µg/L	3.48 µg/L	2.85 – 4.15 µg/L	302 µg/L
Chlorpyrifos	186.1 ng/L*	31.0 - 259.2 ng/L	803.2 ng/L	640.2 – 926.4 ng/L	60 ng/L
Diazinon	4440 ng/L	3300 - 5470 ng/L	-	-	270-570 ng/L
Bifenthrin	4.4 ng/L*	4.0 - 5.0 ng/L	11.37 ng/L	8.04 – 14.80 ng/L	37-281 ng/L
Cyfluthrin	3.04 ng/L	2.75 - 3.54 ng/L	12.72 ng/L	8.05 – 55.55 ng/L	-
Permethrin	78.3 ng/L*	33.9 - >90 ng/L	158.1 ng/L	125.6 – 176.0 ng/L	570-1090 ng/L

[1] Experimental conditions: SC= 900 µS/cm, pH 7.9, T= 23.0ºC
[2] Data obtained from S. Teh (UC Davis); Experimental conditions: EC= 3000 µS/cm, pH 8.0, T= 20.0ºC
[3] Data from public databases; experimental conditions varied

A comparison of *H. transpacificus* and *P. promelas* sensitivities to copper, ammonia and pesticides shows higher sensitivity of *H. transpacificus* to all materials with the exception of bifenthrin and permethrin (Table 8-18). *P. promelas* was more sensitive to bifenthrin.

Table 8-18. Comparison of 96-h sensitivities of 39 – 51 day old delta smelt and <48 h old fathead minnows in hatchery water to selected chemicals. * indicates measured concentrations.

	Delta Smelt			Fathead Minnow		
	LOEC	LC10	LC50	LOEC	LC10	LC50
Copper (total)	78.2 µg/L*	50.4 µg/L*	88.1 µg/L*	260 µg/L*	132 µg/L*	216 µ/L*
Copper (dissolved)	76.2 µg/L*	49.8 µg/L*	86.5 µg/L*	238 µg/L*	125 µg/L*	200 µg/L*
Ammonia Nitrogen	9.0 mg/L*	4.2 mg/L*	12.0 mg/L*	29.8 mg/L*	16.0 mg/L*	20.9 mg/L*
Un-ionized Ammonia	0.105 mg/L*	0.055 mg/L*	0.147 mg/L*	1.121 mg/L*	0.662 mg/L*	0.827 mg/L*
Chlorpyrifos	>200 µg/L	12.89 µg/L	18.62 µg/L	311 µg/L*	171 µg/L*	>311 µg/L*
Bifenthrin	0.260 µg/L*	0.095 µg/L*	0.143 µg/L*	0.038 µg/L*	0.026 µg/L*	0.057 µg/L*
Cyfluthrin	0.890 µg/L*	0.260 µg/L*	0.420 µg/L*	2.000 µg/L	1.036 µg/L	1.388 µg/L
Permethrin	>5 µg/L	-	-	16.0 µg/L	8.2 µg/L	11.1 µg/L
Esfenvalerate	0.375 µg/L	-	0.239 µg/L	0.920 µg/L*	0.516 µg/L*	0.668 µg/L*

9. Sublethal Indicators of Contaminant Effects

9.1

TOXICITY OF COMMERCIAL INSECTICIDE FORMULATIONS AND THEIR ACTIVE INGREDIENTS TO LARVAL FATHEAD MINNOW (PIMEPHALES PROMELAS)

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ABSTRACT

In addition to the active ingredient(s), commercial pesticide formulation contain a significant proportion (>90%) of so-called inert ingredients, which may alter the toxicity of the active ingredient(s). Toxic effect concentrations are, however, generally determined using only the pure active ingredient. This study compares the aquatic toxicity of two current-use insecticides, the pyrethroid bifenthrin, and the phenylpyrazole fipronil, to their commercial formulations, Talstar® and Termidor®. Both are used for mosquito control, landscape treatment and structural pest control, and can be transported into surface water bodies via storm water and irrigation run-off. We used larval fathead minnow (Pimephales promelas), to determine effect thresholds for survival, growth and swimming performance after short-term (24h) exposure to pure insecticides or the respective formulations. The LC50 and LC10 for bifenthrin were 1.9 $\mu g.L^{-1}$ and 0.92 $\mu g.L^{-1}$, respectively, and for fipronil 398.29 $\mu g.L^{-1}$ and 305.57 $\mu g.L^{-1}$ (nominal). Detrimental effects on growth were observed at 10% of the LC10 or 53 $\mu g.L^{-1}$ (nominal) fipronil. Swimming performance was significantly impaired at 20% of the LC10 or 0.14 $\mu g.L^{-1}$ bifenthrin, and at 20% of the LC10 or 142 $\mu g.L^{-1}$ fipronil (measured). Both formulations were more toxic than the pure active ingredient, suggesting that altered toxic effects due to inert ingredients should be considered in pesticide risk assessments and establishment of water quality criteria.

INTRODUCTION

The effects of pesticides on non-target aquatic species have been a major concern for many years [1-4]. Pesticide residues have been frequently detected at toxic concentrations in surface waters and sediments of the Central Valley in California, USA, an area of intensive agriculture. It is, however, a general misconception that attributes pesticide use to agricultural activities alone, as they are also heavily used in urban areas where application by homeowners and professionals for mosquito control, landscape treatment and structural pest control results in an extensive source of pesticide contamination (REFS). Irrigation run-off during dry seasons and storm water run-off contribute pesticide residues to urban streams and waterways at concentrations potentially hazardous to aquatic ecosystems. The pyrethroid bifenthrin is one of the most frequently found contaminants in waters and stream sediments from areas with urban and agricultural land use [5, 6]. Another insecticide widely used in urban areas is the phenylpyrazole fipronil [7]. Lin et al. [8] and Sprague et al. [9] found fipronil to be present in run-off from metropolitan areas throughout (?) the United States. These pesticides are commercially available in a large number of formulated products, generally containing <10% of the active ingredient. Inert ingredients generally comprise more than 90% in volume of commercially available insecticide formulations, and need not be identified on the product label, unless classified as highly toxic [27], [28].

Reported toxicity thresholds such as LC/EC_{50} for fish and other aquatic species are generally determined using the pure active ingredient of commercial pesticide products [17], whereas a significant proportion of available insecticide products consist of so called “inert” or “other” ingredients. These ingredients serve several functions, acting as adjuvants, solvents, emulsifiers, surfactants and/or preservatives, and may therefore alter the toxicity of the active ingredient. Over 90% of the volume comprising “inert” ingredients need not to be identified on the product label as they are considered to have non-toxic characteristics, but several studies have shown that the toxicity of commercial formulations may be different from that of the active ingredient [18], [13], [19].

The two insecticides selected for this study differ in their structure and mechanism of action. Bifenthrin [[1α3α(2)]-(±)(2-methyl[1,1’-biphenyl]-3-yl)methyl 3-(2-chloro-3,3,3, trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate] (CAS number 82657-04-3) is a fourth generation synthetic pyrethroid [13]. Like all pyrethroids, bifenthrin is highly toxic to aquatic organisms. The main mode of action is the interference with Na^+ channel gating in the nerve cell endings. This leads to continuous neurotransmission, causing hyperexcitability, tremors, convulsions and ultimately death [14], [15]. LC_{50} values of bifenthrin for fish have been reported for Sheephead minnow (17.5 $\mu g.L^{-1}$ 96h LC_{50}), Bluegill sunfish (0.35 $\mu g.L^{-1}$, 144h LC_{50}) and Rainbow Trout (0.15 $\mu g.L^{-1}$, 96h LC_{50}) [16]. Fipronil (5-amino-1 [2,6-dichloro-4-(triflouromethyl) phenyl]-4 [(triflouromethyl) sulfinyl]- 1H-pyrazole-3-carbonitrile) (CAS number 120068-37-3) is a “new generation” insecticide in that its mode of action differs from other substance classes like organophosphates and pyrethroids, to which numerous insects have developed resistance. It interferes with the function of γ-aminobutyric acid (GABA)–gated Cl^- channels. GABA is a major inhibitory neurotransmitter in the vertebrate central nervous system. In insects and mammals, the behavioral effects of GABA antagonists include hyperactivity, hyperexcitability, and convulsions, which are correlated with increased spontaneous nerve activity [10]. Fish LC_{50} values have been reported for Sheephead Minnow (130 $\mu g.L^{-1}$), Bluegill Sunfish (54 $\mu g.L^{-1}$) and Rainbow Trout (250 $\mu g.L^{-1}$) [10], [11]. No data on direct run-off studies were reported at the time this study was undertaken, but recent monitoring work confirms that fipronil and its degradation products are present in water and sediments of urban creeks supplying the Sacramento and San Joaquin rivers of California in low concentrations (4.0 - 8.0 $ng.L^{-1}$) [10], [8]. Furthermore, Schlenk et al. [12] reported fipronil concentrations as high as 9 $\mu g.L^{-1}$ for surface waters downstream of fipronil treated rice fields. The bifenthrin formulation; Talstar®, contains 7.9% of the active ingredient contained in so called microcapsules (Product information,[20]). The insecticide itself is thereby enclosed in a coat of “inert” ingredients, to ensure a slow release of the active ingredient and stabilization against environmental degradation [21]. Termidor®, the fipronil formulation, contains 9.1% active ingredient forming a liquid suspension [22].

In this study, we tested the hypothesis that the toxicity of the pure active ingredients, bifenthrin and fipronil, differs from the toxicity of their respective insecticide formulation, Talstar® and Termidor®, using mortality, swimming performance and growth as toxicological endpoints in larval fathead minnow (Pimephales promelas Rafinesque). We used a short exposure period of 24 h, reflective of somewhat realistic exposure scenarios where pesticides are transported off agricultural areas [25] [26]. The fathead minnow is a well-known model for evaluating toxicity to fish, and can be obtained year-round at specific developmental stages.

MATERIAL AND METHODS

Fish source and acclimation

Fathead minnow larvae were obtained from Aquatox Inc. (Hot Springs, AR, USA) at 7 d post-hatch on the day of arrival. The fish were allowed a minimum acclimation period of four hours in control water at a temperature of 25˚C. Almost no mortality occurred during acclimation, and the fish fed and swam normally.

Pesticide exposure

Acute Toxicity

Pure chemicals bifenthrin and fipronil were obtained by ChemService, West Chester, PA, USA. Commercial insecticide formulations Talstar® Select (US EPA Reg.No. 279-3155) and Termidor® (US EPA Reg.No. 7969-210) were purchased online from http:\\www.doyourownpestcontrol.com. All pesticide exposure experiments were conducted at the University of California Davis, Aquatic Toxicology Laboratory, School of Veterinary Medicine. To determine acute toxicity, 7-day old larval fish were exposed to the following nominal concentrations: 0.75, 1.0, 1.5, 2.0, 3.0 and 4.0 $\mu g.L^{-1}$ bifenthrin, 3.0, 4.0, 4.5, 5.0 and 6.0 $\mu g.L^{-1}$ of the bifenthrin formulation Talstar®, 150, 200, 350 and 400 $\mu g.L^{-1}$ of fipronil and 150, 200, 350, 400 and 450 $\mu g.L^{-1}$ of fipronil formulation Termidor® in a 24h acute toxicity assay (Table 1). Method controls consisted of deionized well water, modified with salts to meet US EPA specifications (electric conductivity (EC): 265 - 293 µmhos; hardness: 80-100 as mg $CaCO_3.L^{-1}$; alkalinity: 57-64 as mg $CaCO_3.L^{-1}$, [23]). For the pure substances we used 1 $ml.L^{-1}$ methanol (MeOH) as the solvent carrier and one treatment group containing the same MeOH concentration was added as a solvent control. No solvent carrier was required for the formulations as they are designed to mix with water. The exposure concentrations used for acute toxicity testing refer only to concentrations of active ingredient in the respective formulation to ensure direct comparability. Talstar ® contains 7.9% bifenthrin per volume and Termidor® contains 9.1% of fipronil.

Sublethal Toxicity

Sublethal exposure concentrations used for the swimming performance and growth test series were calculated as percentages of the LC_{10}-values derived from acute toxicity tests and were: 10%, 20%, 33% and 50% of LC_{10}, plus method control and solvent control as described above (Table 2). Four replicate 600ml Pyrex beakers were used per concentration, each replicate containing 250 mL treatment solution and 10 fish. At test initiation the larvae were randomly distributed into beakers and exposed for 24 h at a water temperature of 25˚C and a 16:8 light-dark ratio. Fish were not fed during the exposure period.

Sub-samples of each test solution (1 L) were submitted for chemical analysis to the California Department of Fish and Game Water Pollution Laboratory (Rancho Cordova, CA, USA). Talstar® samples were filtered through 0.45µm glass fiber filter prior to analysis to separate microcapsules from the water phase, and determine "particulate" and dissolved bifenthrin concentrations. Measured insecticide concentrations are listed in Table 2.

7-d Growth

Subsequent to the 24 h pesticide exposure, fish were transferred to method control water and maintained for 6 days at 25˚C and a 16:8 light:dark photoperiod. Each of six treatments, per substance, consisted of four replicate beakers containing 10 fish. For transfer, fish were gently rinsed using a fine-meshed sieve and released into vessels containing control water. On days 2-7, approximately 80% of the water was exchanged daily, the number of surviving fish was recorded, and physicochemical parameters were measured for each treatment before and after the water exchange and at test termination. After each water renewal the beakers were distributed randomly. Fish were fed ad libitum twice a day with newly hatched Artemia nauplii (30 – 50 Artemia on average, every eight hours). At test termination, surviving fish were euthanized with MS-222 (Tricaine Methanesulfonate, Sigma, St. Louis, MO, USA), then transferred to pre-weighed aluminium weigh boats and dried for 24 hours at 100˚C. Dry weight per fish (± 0.001 mg) was calculated by measuring whole dry weight divided by the number of fish remaining per replicate.

Swimming performance ("one minute racetrack")

A subsample of fish (n=7/replicate) exposed to pesticides for 24 h in three replicate beakers containing 10 fish were used to determine swimming performance. Swimming-performance was tested at three different time points: (1) Immediately after the 24 h pesticide exposure; (2) after a total of 48 h (24 h recovery in control water), and (3) after a total of 7 d (6 d recovery in control water), using a circular "racetrack" following a method developed by Heath et al. [17, 18]. This racetrack consisted of a 13 cm diameter Petri dish with an upside-down 8 cm diameter Petri dish centrally placed, divided into 8 sectors by radiating lines drawn on the bottom of the testing dish, and filled with control water to a depth of 1 cm. Fish from randomly chosen beakers were transferred individually into the testing device and allowed to acclimatize for 1 minute. A plastic rod was then used to trigger the fish's escape response by repeatedly touching it at the tail fin. The number of lines or sectors crossed by the fish within 1 minute was recorded and used as a measurement of swimming performance. Water in the testing device was renewed after testing 7 fish from each replicate beaker.

Statistical analysis

We used the Comprehensive Environmental Toxicity Information System (CETIS) by Tidepool Scientific Software (McKinleyville, CA, USA) to calculate the statistics for 24h survival data (NOEC and LC_{50}) of the nominal concentrations of active ingredients. The Shapiro–Wilk normality test was used to evaluate whether quantitative data met the assumptions of the parametric ANOVA. For multiple comparisons the JMP 7.0 Software by SAS Institute Inc. was used. To evaluate differences between treatments in swimming performance and growth data we used one-way ANOVA and Tukey's multiple comparison test post hoc. Additionally, Dunett's multiple comparison test was used to compare formulation treatments to controls, and pure active ingredients to solvent controls. Data from the growth and swimming tests did not always meet the assumptions of normality and homogeneity of variances at the highest concentrations, but due to the strong signals, the ANOVA is considered to be robust [24].

RESULTS

Water chemistry

Physicochemical parameters measured at the start and end of the 24 h exposure period were the same for all treatments and within the acceptable range for the test organism. The measured mean values (± standard deviation) were pH: 7.51 (± 0.19), dissolved oxygen 7.17 (± 0.52) $mg.L^{-1}$, temperature: 23.06 (± 0.32) ˚C, and EC: 278.71 (± 6.05) $\mu S.cm^{-1}$.

Acute toxicity

Acute toxicity concentrations derived from fathead minnow exposures to both pure compounds and respective formulations are summarized in table 1. The pyrethroid bifenthrin and its formulation Talstar® were both highly toxic to 7-d old fathead minnows. The nominal 24-h LOEC and LC_{50} for Talstar® were 3.00 $\mu g.L^{-1}$ and 4.85 $\mu g.L^{-1}$, while the 24-h LOEC and LC_{50} for pure bifenthrin were 1.00 $\mu g.L^{-1}$ of 1.90 $\mu g.L^{-1}$, respectively. Fipronil was less toxic than bifenthrin. The nominal 24-h LOEC for pure fipronil and its formulation Termidor® was equally 350.00 $\mu g.L^{-1}$. The LC_{50} of the formulation was 379.47 $\mu g.L^{-1}$ and therefore slightly lower than that of the pure fipronil with an LC_{50} of 398.29 $\mu g.L^{-1}$.

Swimming performance

Nominal and measured pesticide concentrations are shown in Table 2.

Bifenthrin: Immediately following the 24h exposure to pure bifenthrin, the swimming performance of fish from the lowest concentration treatment (0.07$\mu g.L^{-1}$ or 10 % LC_{10}) showed no statistical difference to control or solvent control treatments (Figure 1). Swimming performance of fish exposed to concentrations ≥0.14 $\mu g.L^{-1}$ (20% LC_{10}) was significantly decreased compared to solvent controls ($p<0.001$). In comparison, exposure to the commercial formulation Talstar® led to decreased swimming performance at ≥0.03 $\mu g.L^{-1}$ dissolved bifenthrin (10% LC_{10}, $p<0.001$).

After transfer to control water for and maintenance for an additional 24 h, swimming performance of pesticide-exposed fish improved in most treatments. Fish exposed to 0.07 – 0.14 $\mu g.L^{-1}$ pure bifenthrin, and 0.03 – 0.05 $\mu g.L^{-1}$ Talstar® recovered completely (Figure 1). After a recovery period of six days, no statistically significant differences between treatments were observed. When comparing dissolved bifenthrin concentrations between pure bifentrhin and Talstar®, the formulation was more toxic than the pure active ingredient.

Fipronil: Swimming performance after 24 h was significantly decreased in fish exposed to concentrations ≥142 $\mu g.L^{-1}$ pure fipronil (20% LC_{10}, $p=0.0005$) and ≥148 $\mu g.L^{-1}$ Termidor® (33% LC_{10}, $p=0.0036$). Although the measured concentrations at this time point are in a similar range, the formulation showed a stronger impact on swimming at higher concentrations. Fish exposed to 192 $\mu g.L^{-1}$ Termidor® (50% LC_{10}) exhibited statistically significant lower swimming activity than fish exposed to 333 $\mu g.L^{-1}$ fipronil treatment (33% LC_{10}).

After 24h recovery in control water no significant differences in swimming performance were observed in fish exposed to pure fipronil, although in the highest concentration treatment values were slightly lower (365 μg.L^{-1}, p=0.0534) compared to the solvent control. After the 6-d recovery period, there was a statistically significant effect (p=0.0076) in this treatment. In contrast to the pure fipronil treatments, swimming performance of fish exposed to 192 μg.L^{-1} Termidor® (50% LC_{10}) remained suppressed after the 24 h recovery period. This effect persisted throughout the test, and no recovery of swimming performance was observed after 6 d (Figure 2).

7-d Growth and development

Bifenthrin: Exposure to pure bifenthrin at concentrations ≤0.35μg.L^{-1} bifenthrin (50% $LC_{10,}$) and Talstar® did not result in a reduction of 7-d growth.

Fipronil: Fish exposed to pure fipronil at all concentrations tested grew significantly more than fish exposed to the solvent alone (53 μg.L^{-1}:10% LC_{10}:, p=0.0165; 333 μg.L^{-1}: 33% LC_{10}, p=0.0067; 365 μg.L^{-1}: 50% LC_{10}, p=0.0035, Figure 3) Exposure to Termidor® did not result in negative or positive effects on growth.

Fish exposed to pure fipronil and Termidor® showed deformities of the spine, namely scoliosis and in some cases both scoliosis and lordosis (Figure). Spinal deformations were visible four to five days after the 24h pesticide exposure. At test termination 7% of the fish exposed to 365 μg.L^{-1} and 2% of the fish exposed to 333 μg.L^{-1} pure fipronil had developmental abnormalities. The same effect was visible for 6% of the fish exposed to 192 μg.L^{-1} and 2% of the fish exposed to 148 μg.L^{-1} Termidor®.

DISCUSSION

This study provides new information on the sublethal toxicity of two technical grade insecticides and two of their commercial formulations to larval fathead minnow after brief, 24 h exposures. Commercial pesticide formulations applied as sprayable solution, wettable powder or granules are of special concern with respect to aquatic environments, if the active ingredient becomes more susceptible to run-off or leaching through properties provided by inert ingredients, or if inert ingredients are toxic or synergize toxicity of the active ingredient. For example, Armbrust et al. [29] reported that the concentration of the insecticide imidacloprid was higher in run-off from turf that was treated with granules compared to application of a wettable powder. The physical properties of microencapsulated pesticide formulations like Talstar® and suspension liquids like Termidor® may also facilitate their environmental transport, and therefore increase the availability to non-target species.

In addition to increasing the likelihood of exposure, inert ingredients can also enhance the toxicity of the active ingredient. We found significant differences in toxicity between formulations and pure A.I. Both formulated products were more toxic than the respective A.I. alone, when compared based on measured dissolved insecticide concentrations. Talstar® impaired fathead minnow swimming performance at 0.03 μg.L^{-1} (10% LC_{10}) while pure bifenthrin was approximately 5 times less toxic (LOEC ≥0.14 μg.L^{-1}; 20% LC_{10}). For instance, emulsifiable concentrations of pyrethroids were found to be 2.2 to 8.5 times more lethal than the pure substance [14].

The observed differences in toxicity were most likely due to the inert ingredients rather than the enantiomeric or chiral composition of the active ingredient. Pure fipronil is a 50:50 racemic mixture, just like its formulation product. Bifenthrin consists of 97% cis-isomer both in the pure compound and the formulated product. Talstar® is formulated as a so called microencapsulation of bifenthrin, resulting in µm-sized particles, where the active pesticide forms a core that is coated by an outer wall consisting of "inert' ingredients [21], [30]. The toxicity of this formulation is therefore dependent on how fast and how much of the active ingredient is released through the capsule. As this formulation is designed to be more persistent at the site of application, the release is probably slow and could therefore explain why measured concentrations of dissolved bifenthrin were lower in the Talstar® experiment than in the exposures to pure bifenthrin (Table 2). However, microcapsules may have been ingested by the larval fish, thus adding a dietary exposure route to the aqueous exposure to dissolved bifenthrin. In the case of Termidor®, effects on swimming performance were initially measured at similar concentrations as the pure A.I., fipronil., but impairment was more persistent. In addition, spinal deformities were observed upon exposure to Termidor®, but were less pronounced than those observed following A.I. treatment. Stehr et al.[31] reported notochord degeneration and shortening along the rostral-caudal body axis in zebrafish (Danio rerio) embryos continuously exposed to fipronil at nominal concentrations at or above 0.7mM (333 $mg.L^{-1}$). They also reported ineffective tail flips and uncoordinated muscle contractions in response to touch. Although the concentrations used in our study were below that range, similar behavioral abnormalities were observed and resulted in a measurable decrease of swimming performance. Termidor® is a water-based suspension concentrate liquid containing 9.1% active ingredient. We do not have any information on the chemical composition of the inert ingredients of this formulation, therefore cannot provide a mechanistic explanation for our observations.

Seven-day growth of larval fathead minnows was not the most sensitive endpoint in our study. Although other pyrethroids have been shown to cause a reduction in growth of fathead minnow and other fish species [14]. [37], bifenthrin and Talstar (?) exposure did not significantly affect final fish weight. This may be due to the low concentrations used in our experiments (≤50% of the LC10). We did not rigorously quantify food uptake in this study, but during daily water renewal, remaining food quantity was observed to be greater in treatments with decreased swimming performance than in control treatments and at lower exposure concentrations. Growth of fathead minnows was enhanced after exposure to fipronil., while its formulation product, Termidor® did not have any impact on growth. Enhanced growth following exposure to fipronil has not been previously reported and causative factors should be investigated in more detail, but were beyond the scope of this investigation. A limited number of studies found fipronil to be altering normal thyroid function and thyroid hormone levels in rats [32], [33], [34] and chicken [35]. As thyroid hormones also play a role in larval and juvenile development of fish [36] the observed growth abnormalities may be related to this effect.

Swimming performance is a highly suitable endpoint for estimating individual level effects of environmental contaminants as it integrates biochemical and physiological processes [37], [38] [39], [40]. Our study demonstrated that short term (24 h) exposures to sublethal concentrations of bifenthrin and fipronil and two of their commercial formulations significantly impaired swimming performance of larval fathead minnows at concentrations far below the LC_{10} values for each of the tested substances. We used a simple and easy to perform test to assess swimming behavior.. It simulates predatory chase and integrates both neural and metabolic aspects of fish, since swimming involves nerve cell transmissions and muscle activity [41] which is particularly affected by pesticides with a neurotoxic mode of action. This is of crucial importance during early life stages where fish are highly vulnerable to predation. Inability to swim properly after a brief exposure to pesticides therefore has critical influence on individual fitness and survival, and potential population level consequences. As demonstrated in this study, fish can recover if given the chance, but in a field situation; not being able to feed or evade predators for a certain period of time, will likely lead to negative impacts on population dynamics.

In summary, our study has demonstrated that toxicity of commercial insecticide formulations is different from that of the pure ingredients. This information needs to be incorporated into environmental risk assessments of pesticides, possibly by increasing safety factors. The use of sublethal endpoints like swimming behavior offers a more environmentally relevant evaluation of the effects of pesticides on aquatic organisms than lethality or growth.

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Tables and Figures

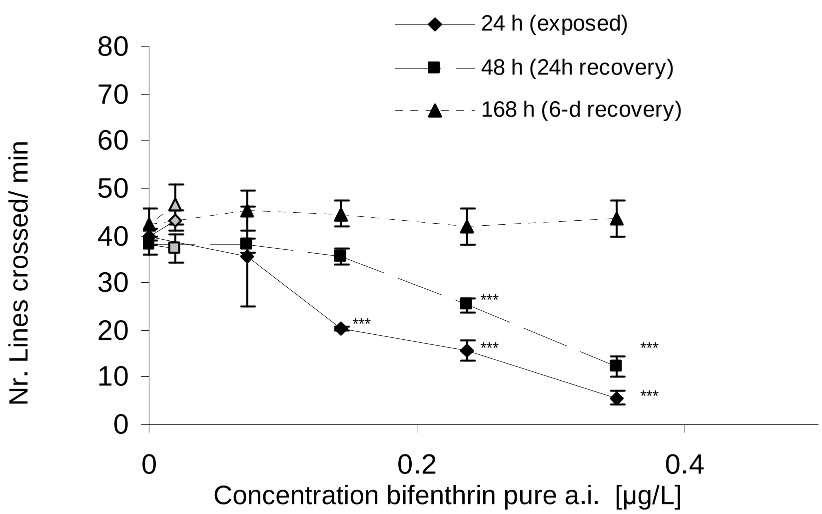
Table 1: Acute nominal effect concentrations for 7d old fathead minnow after 24h exposure to bifenthrin, fipronil and their formulations Talstar ® and Termidor ®. Effective Levels, LC_{50} and LC_{10} (with 95% confidence limits).

Substance	NOEL [µg/L]	LOEL [µg/L]	24h LC50 [µg/L]	24h LC10 [µg/L]
fipronil pure	300	350	398.29 (376.27 - 438.79)	305.57 (275.56 - 324.12)
fipronil formulation	200	350	379.47 (355.13 - 405.48)	233.01 (201.99 - 307.94)
bifenthrin pure	0.5	1	1.9 (1.69 - 2.12)	0.92 (0.72 - 1.09)
bifenthrin formulation	< 3	3	4.85 (4.47 - 5.34)	2.99 (2.36 -3.39)

Table 2: Nominal and measured concentrations for 24h exposure of 7d old fathead minnow to bifenthrin, Talstar®, fipronil and Termidor®. Treatment concentrations used for swimming performance and growth tests. Calculated as percentages of the LC_{10}-value (10%, 20%, 33% and 50% LC_{10}).

Substance	Concentration [µg/L]	10% LC10	20% LC10	33% LC10	50% LC10
bifenthrin pure	measured	0.07	0.14	0.24	0.35
	nominal	0.09	0.18	0.31	0.46
Talstar®	measured - dissolved	0.03	0.05	0.08	0.16
	measured - particulate	0.19	0.39	0.57	0.81
	nominal	0.29	0.59	0.99	1.49
fipronil pure	measured	53	142	333	365
	nominal	31	61	102	153
Termidor®	measured	28	128	148	192
	nominal	23	47	78	117

A



B

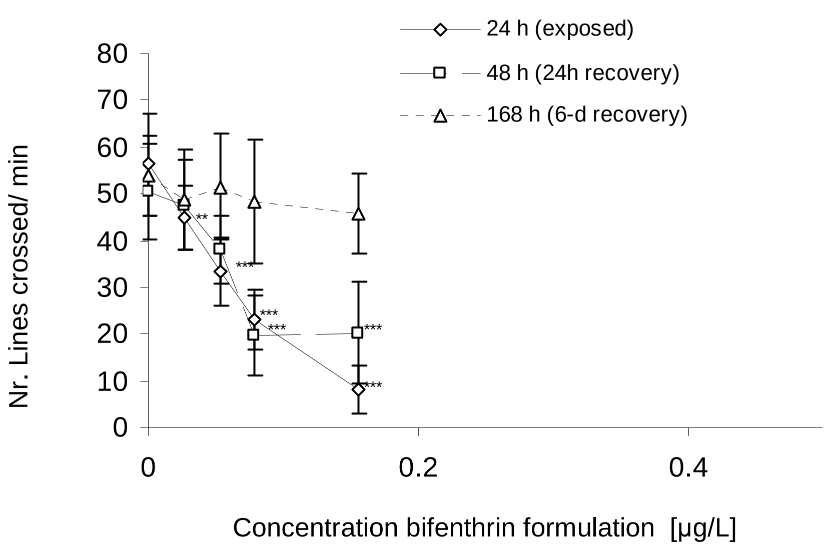
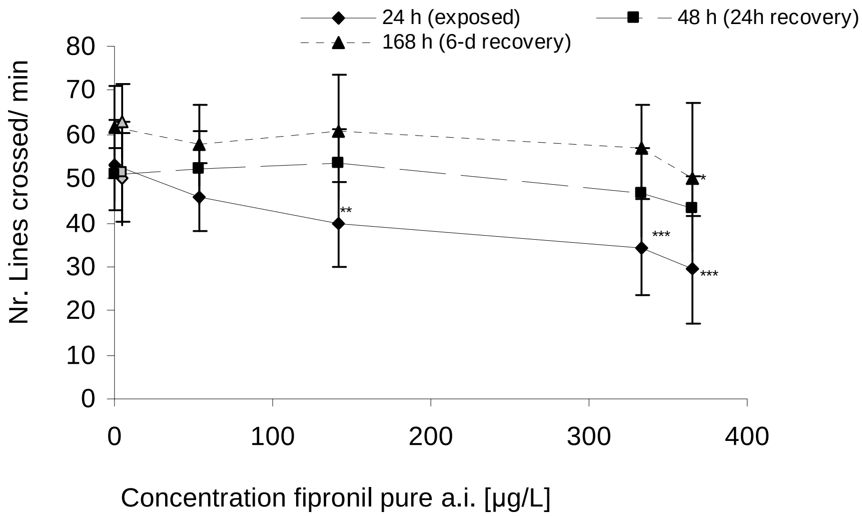


Figure 1: Swimming performance in 7-day old fathead minnow (Pimephales promelas) larvae after 24h exposure bifenthrin and Talstar®, 24h recovery and 6d recovery. Asterisks indicate significant differences in treatments compared to control/solvent control (*: p<0.05. **: p<0.01. ***: p<0.001). Data shown as arithmetic mean ±SD; n=7. A: pure bifenthrin, control group shifted to x=0.02 for visibility (grey); B: Talstar® .

A



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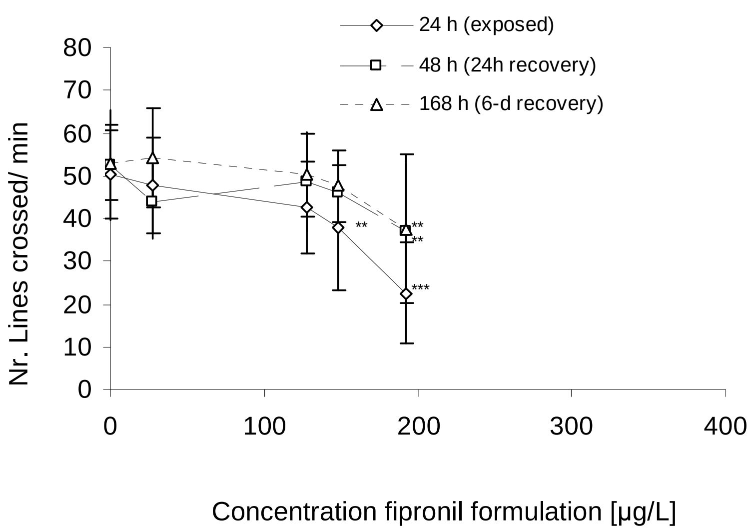

Figure 2: Swimming performance after 24h exposure, 24h recovery and 6d recovery. Asterisks indicate significant differences in treatments compared to control/ solvent control (*: p<0.05. **: p<0.01. ***: p<0.001). Data shown as arithmetic mean ±SD; n=7. A: pure fipronil, control group shifted to x=5 for visibility (grey); right: Termidor®.

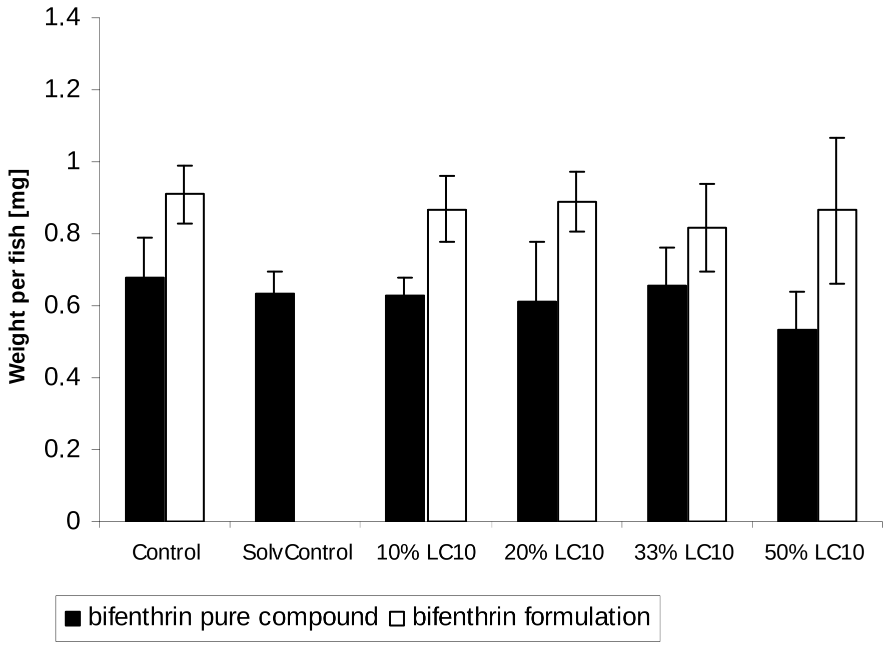

Figure 3: Average dry weight per fish after 24-h exposure to bifenthrin and Talstar® and 6 day recovery. Fish exposed to 0.35 μg/L pure bifenthrin (50% LC_{10}) showed slightly lower average weight compared to the solvent control. Differences were statistically significant ($p<0.05$) compared to the control, but not solvent control.

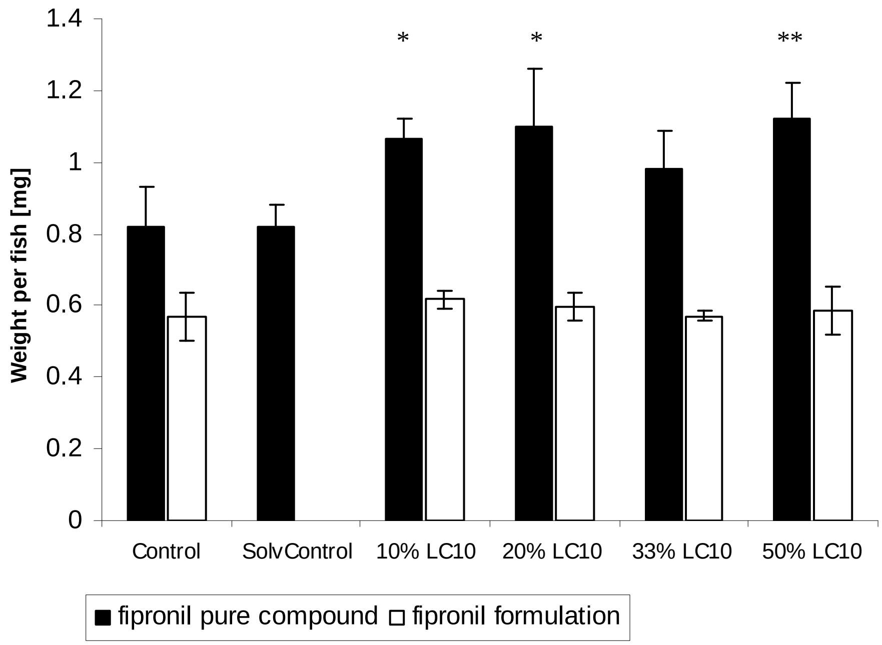

Figure 4: Average dry weight per fish after 24-h exposure to fipronil and Termidor® and 6-d recovery. Fish exposed to pure fipronil had significantly higher average weight than fish in control treatments (*: p<0.05. **: p<0.01). Fish exposed to Termidor® showed no statistically differences in weight after the 7-day growth period.

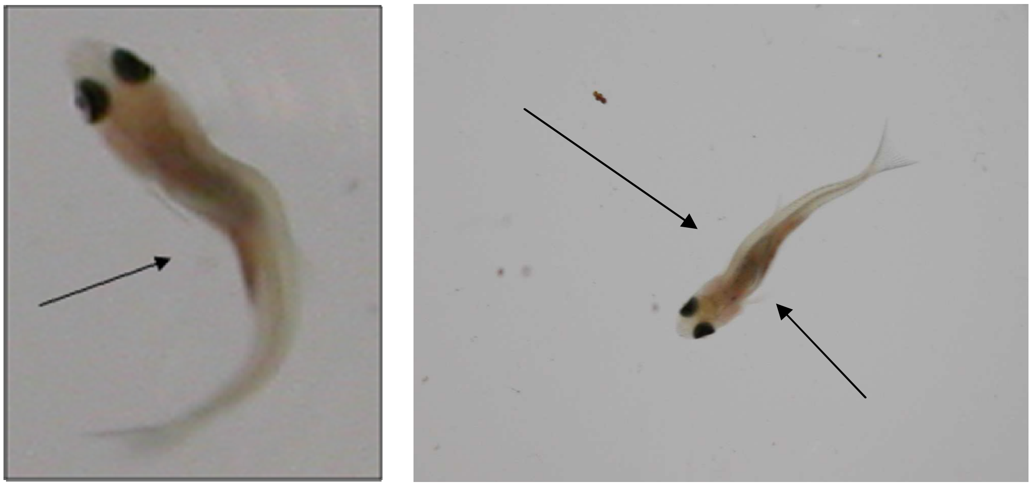

Figure 5: Abnormal spinal development in fish exposed to fipronil and Termidor®.

9.2

Molecular biomarkers in endangered species: neuromuscular impairments following sublethal copper exposures in the delta smelt (*Hypomesus transpacificus)*

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Abstract:

The delta smelt (*Hypomesus transpacificus*) is a pelagic fish species endemic to the Sacramento-San Joaquin Estuary in Northern California. It is listed as threatened under both the USA Federal and Californian State Endangered Species Acts and considered an indicator of ecosystem health in its habitat range. Copper is a contaminant of concern in Californian waterways, common in urban storm-water runoff, present from mining activities and is regularly used as a pesticide in many agricultural areas. To understand the effects of contaminants on *H. transpacificus* we have constructed a microarray with 8,448 Expressed Sequence Tags (ESTs). We applied this tool to measure gene responses in 60-day old juveniles exposed to 50µg.L^{-1} copper chloride for 7 days. Responding genes were predominantly involved in digestion and metabolism, and neuromuscular activity with further effects on immune system, redox, and metal ion binding. Selected genes were assessed using q-PCR on 57-day old juveniles, exposed for 96 h to copper concentrations ranging from 2.0 to 32.0 µg.L^{-1}, concentrations which resulted in no mortality. Quantitative PCR expression analyses corroborated neuromuscular impairments.

Our results support the use of molecular biomarkers such as amylase-3, myozenin, calpain, sarcoendoplasmic reticulum calcium ATPase (SER-Ca) and creatine kinase in delta smelt in the determination of digestive and neuromuscular responses to sublethal contaminant exposure.

We hypothesize that the measured responses are indicative of direct effects on swimming ability, feeding, and other behavioral parameters, that impact on reproductive success and population growth rates. We present here the application of microarrays, discuss their use in screening species health, and in identifying specific biomarkers for researching factors contributing to the decline in numbers of the delta smelt.

Keywords: 'Hypomesus transpacificus', 'delta smelt', microarray, biomarker, copper

Introduction.

The Delta smelt (*H. transpacificus*) is a pelagic fish species endemic to the Northern Sacramento-San Joaquin Estuary, California, and considered an “indicator species” for ecosystem health in this system. Abundance has dramatically declined since the 1980s and it was listed as threatened in 1993, under both the Federal Endangered Species Act (ESA) and California Endangered Species Act (CESA). Delta smelt have been reared since 1992 at the Fish Conservation and Culture Laboratory (FCCL), UC Davis, providing a refuge population as well as a supply for research. A more recent step decline of the delta smelt population (Sommer et al. 2007) has prompted considerable efforts to understand the causative factors of this decline. A number of complex factors, known and unknown have potentially been affecting populations of delta smelt in its native habitat. Pollution, in the form of agricultural, pharmaceutical and industrial chemicals, along with the effects of water exports for agricultural irrigation and urban uses, toxic algal blooms and habitat destruction, are among the potential causes for the decline in pelagic organisms.

Identifying the impacts of such stressors and their mechanistic effects on individuals and populations is a main challenge in ecotoxicology. Stress responses to toxic chemicals are often preceded by alterations in gene expression, thus gene expression studies offer insights into the overall health of an organism. Microarray gene profiling is a powerful tool for defining genome-wide effects of environmental change on biological function. This technology is being applied successfully to the field of ecotoxicology in a number of other species and links are being forged between what is measured at the gene expression level and life history parameters, such as metabolism, growth and reproduction (Connon et al. 2008; Heckmann et al. 2008). The predictive value of microarrays as screening tools is becoming more powerful as our understanding of these responses grows. Gene expression studies carried out over short-term exposures allow for the prediction of chronic effects that stressors may have on the health of the individual, their survival capacity, fecundity and somatic growth. Specific gene responses in individual delta smelt, indicative of their health status, could highlight potential causes for the population decline.

Our aims are to determine specific and general responses to a suite of stressors and develop molecular biomarkers applicable in the delta smelt and relevant to the varying contaminants found in the Californian watersheds. In order to understand the effects of contaminants upon *H.* transpacificus we have constructed a microarray with over 8,000 Expressed Sequence Tags (ESTs), described in Connon et al. (in review) and Werner et al. (2008). No sequence information was available on any database at the time this project was started.

We used copper to generate stress because biochemical responses to this heavy metal, and adverse effects on the whole organism, are relatively well understood and therefore would aid interpretation of results in this “proof of principle” test. Furthermore, copper is a contaminant of concern in Californian waterways, it is a common contaminant in urban storm-water runoff, is present from mining activities and is regularly used as a pesticide in agricultural areas. We expected neurological responses, respiration, growth and metabolism to be affected by exposure to this contaminant. Reported concentrations of copper in the Sacramento River are above 6μg μg $Cu^{+}.L^{-1}$ (USGS 1998) though there are seasonal fluctuations due to its application as a pesticide, where concentrations have been reported to exceed 500 μg $Cu^+.L^{-1}$in rice field effluents, following copper application (California-DFG 1998).

We present here responses to relatively high levels of copper (50μgCu^+L^{-1}) examined to establish confidence in significant responses, along with expression analyses carried out a select group of genes at environmentally relevant concentrations.

Methods

Fish Exposures and water chemistry.

Delta smelt were obtained from the Fish Conservation and Culture Laboratory, UC Davis and maintained for 24 hours in experimental conditions as described below.

i. Acute toxicity (exposures used for microarray analyses): 60-day old juveniles were exposed to a control and four concentrations of copper chloride (CuCl2); equivalent to nominal concentrations of 5, 10, 25 and 50 μg $Cu^+.L^-1$ for 7 days. Only controls and surviving organisms from the highest exposure concentration (50 μg $Cu^+.L^{-1}$) were assessed with the microarray in order to identify genes specifically responding to copper exposure, eliminating any possible hormetic responses.

ii. Sublethal toxicity (exposures used for quantitative PCR analyses): 57-day old juveniles were exposed to a control and four concentrations of copper chloride ($CuCl_2$); equivalent to nominal concentrations of 2, 4, 8 16 and 32 μg $Cu^+.L^{-1}$ for 4 days.

For both tests, replicate experimental treatment (n=4) were initiated with 10, juveniles in 7L of water at 20°C. Fish were fed twice daily with artemia (<48 h old). The light:dark cycle was 16h:8h. Approximately 80 percent of the water in each replicate was renewed on the second day for the 4-day exposures and on days 2, 4, and 6, for 7-day exposures.

Water temperature, pH, and DO were measured daily. Ammonia nitrogen (NH3-N) was measured prior to each water renewal. At test end, fish were snap-frozen and storage at –80oC for subsequent analyses.

RNA isolation, cDNA synthesis and fluorescence labeling.

RNA was extracted from whole, individual organisms using a standard phenol:chloroform protocol with Trizol Reagent (Invitrogen). Fifteen micrograms of total RNA were used for cDNA synthesis, spiked with control RNA (CAB, RCA, RBCL and LTP4 (SpotReport, Stratagene) and labeled with Alexa fluor dyes, using SuperScript^{tm} Plus Indirect cDNA labeling System (Invitrogen). Each experimental sample and control was combined with a reference pool cDNA prior to hybridization using an automated Tecan HS4800 hybridization station. Slides were scanned using a GenePix 4000B scanner (Axon Instruments).

Microarray images and data from esfenvalerate exposed delta smelt can be accessed at http://www.vetmed.ucdavis.edu/apc/WernerLab/subpage/pelagic_organism_decline.html; POD archive data.

Microarray Analyses

Normalization and analytical methods are described in Loguinov et al. (2004). In brief, print tip normalization was carried out within slides and sequential single slide data analysis was carried out as an alternative to between-slide normalization. An □-outlier-generating model was used to identify differentially expressed genes by applying the following decision rule for multiple-slide data analysis: a given gene was selected as a candidate if it was detected as significantly up- or downregulated in 4 of 4 replicates (raw p-value = 0.0625 using exact binomial test and considering outcomes as Bernoulli trials). The approach did not use scale estimator for statistical inference and, therefore, it did not require between-slide normalization.

Sequencing and Annotation

Sequencing was carried out at the CA&ES Genomic Facility, UC Davis. Basic Local Alignment Search Tool; translated nucleotide (BLASTx) searches were performed on specific fragments that responded significantly to the exposure treatments. Only genes that were differentially expressed following esfenvalerate exposure were sequenced. Sequences were annotated according to homologies to protein database searches using translated nucleotide sequences and direct nucleotide queries (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were only annotated if they were found to have a BLASTx match with the expect value smaller than $1x10^{-5}$ and a score above 50.

Functional Classifications

Differentially expressed genes were classified according to the Kyoto Encyclopedia of Genes and genomes (KEGG - http://www.genome.jp/kegg/kegg2.html) and Gene Ontology (GO - http://www.uniprot.org/uniprot), and information gathered from literature, into functional groups. Classification was carried out based on gene expression changes in respect of control subjects, regardless of whether these were up or downregulated, or exposure concentrations. Specific genes of interest were selected for further investigation using quantitative PCR (see below).

Biomarker development

Genes were selected according to level of expression significance, knowledge base from literature, and functional classification. Primer and probes for qPCR analyses were designed using Roche Universal Probe Library Assay Design Center (https://www.roche-applied-science.com). Designed primers were obtained from Eurofins MWG Operon (http://www.eurofinsdna.com), and TaqMan probes were supplied by Roche. Sequences for all genes assessed by qPCR analyses have been submitted to genbank (http://www.ncbi.nlm.nih.gov). Primers and probes for investigated biomarkers are detailed in table 1.

Gene		**q-PCR Primer Sequences**	**Roche Probe No.**
Vitronectin	F	AGTTGTCCCAAGTGTAGGTCTGG	38
	R	AAGTGCCGTTTGAGTCTGGG	
Amylase-3	F	GATCACCATGTTCTTGATCTGACG	99
	R	CCATCAATCCTGACCAAACCTG	
TNF	F	CTTTTTCCGCTGTTCCATGTTC	2
	R	GTTACCAGCATACGCAGTGTCC	
SER-Ca	F	CATGATCATTGGGGGAGCA	148
	R	TGCTGTGATGACAACGAGGAC	
TGF-□	F	CAACGGCATAGTGCATGTGG	76
	R	GAATGTGTGCACGTTGTTGGT	
Chitinase	F	TGTGATCAAGTTCCTCCGTCAGT	147
	R	CCGGGGTATTCCCAGTCAAT	
Calpain	F	CCCTCCGACATGGGAAGAGT	30
	R	ACCAACTATGCCTTGCCCAA	
Aspartoacylase	F	GGAGGCACACATGGGAATG	109
	R	CTTCCTCTGAATCTCTGTTCCATTATC	
Myozenin	F	CCAATGTCGTGCTGGTACACC	106
	R	CTGCCAGACATTGATGTAGCCA	
Creatine Kinase	F	CGATCGGCGTTGGAGATG	163
	R	GCCAAGTTCAACGAGATTCTGG	
□-Actin	F	CCTGCCTCGTCGTACTCCTG	12
	R	CATCCTGGCTTCCCTGTCC	

Table 1. Molecular biomarkers: Primer and probe sequences used for quantitative-PCR analyses of gene expression in striped bass.

Quantitative PCR

A total of 1.5 µg RNA was cDNA synthesized using random primers, and diluted to a total of 50 µl with nuclease free to generate sufficient template for qPCR analysis. TaqMan Universal PCR Mastermix (Applied Biosystems) was used in q-PCR amplifications in a reaction containing

10mMTris–HCl (pH 8.3), 50mM KCl, 5mM MgCl2, 2.5mM deoxynucleotide triphosphates, 0.625U AmpliTaq Gold DNA polymerase per reaction, 0.25U AmpErase UNG per reaction and 5µL of cDNA sample in a final volume of 12µL. The samples were placed in 384 well plates and cDNA was amplified in an automated fluorometer (ABI PRISM 7900 Sequence Detection System, Applied Biosystems). Amplification conditions were 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C and 60s at 60°C. Fluorescence of samples was measured every 7 s and signals were considered positive if fluorescence intensity exceeded 10 times the standard deviation of the baseline fluorescence (threshold cycle, CT). SDS 2.2.1 software (Applied Biosystems) was used to quantify transcription.

Statistical analyses

We use the geNorm algorithm [10] to estimate the variability of the reference genes, and to discover an optimal normalization gene. GeNorm estimates reference gene variability of candidate reference genes. (Vandesompele et al. 2002).

Quantitative PCR data was analyzed using the relative quantification 2(-Delta Delta CT) method (Livak and Schmittgen 2001). Expression was calculated relative to a-actin determined by GeNorm as the least variable gene in this study. One-way ANOVA was used to assess differences in gene expression through out the exposure concentrations, and data were further assessed using Student's T-test at individual concentrations in respect to controls.

Results and discussion

Fish Exposures and water chemistry.

Water chemistry remained stable throughout the exposures except for low concentrations of ammonia at the highest exposure in the acute toxicity tests (see table 2), which was attributed to high mortality and therefore lower number of remaining fish.

Calculated $EC_{50\text{-}96h}$ was 33.5 µg $Cu^{+}.L^{-1}$ and $EC_{50\text{-}7day}$ was 24.7 µg $Cu^{+}.L^{-1}$. The LC50s of juvenile delta smelt for copper are far below the 96-h LC50 value reported by the California Department of Fish and Game of 1.4 mg/L for larval delta smelt (Werner et al. 2008). Our experimental results and other available data indicate that delta smelt is one of the most sensitive fish species to copper. No significant differences were observed in length and weight after the 7-d exposure, though slight weight increase was observed at the higher concentrations attributed to fewer surviving organisms resulting in a relative increase of food and space compared to controls (results not shown).

Treatment	Lab Temp (^{o}C)	Lab pH	Lab EC (µmhos/cm)	Lab DO (mg/L)	Ammonia (mg/L)
Lab. Control (Dilute Well Water)	21	8.4	431	8.8	0.28
5 ppb Cu^+	21	8.49	456	8.7	0.24
10 ppb Cu^+	21	8.48	461	9	0.23
25 ppb Cu^+	21	8.46	455	8.8	0.37
50 ppb Cu^+	21	8.39	457	8.9	0.14

Table 2. Water chemistry: summary of water chemistry measurements taken on termination of the delta smelt Cu^+ reference toxicant test.

Microarray responses

Differentially expressed genes resulting for exposure to 50 µg $Cu^+.L^{-1}$ are presented in table 3. A functional classification based on KEGG and GO of up- and down-regulated genes responding to copper exposure are presented in table 4 and figure 1.

Primary responses were seemingly involved in cardiac muscle contraction (e.g. □-actin), muscle activity (e.g creatine kinase, myozenin, titin a) and neurological effects resulting in calcium and phosphate signaling (e.g. sarcoendoplasmic reticulum calcium ATPase, m-calpain, cyclophilin-a). Digestion was also affected by copper exposure and was the largest affected functional classification of genes. Digestive genes encoding a number of proteins involved in glycolisis, cholesterol efflux, lipid transport, chymotripsin activity, proteolysis (e.g. amylase-3, gastric chitinase). Other responses indicate compromised immunity (e.g. TNF, TGF-□) and cellular homeostasis and tumor malignancy (e.g. vitronectin), changes in expression of these proteins have been implicated in a variety of diseases.

Peptidylproplyl isomerase A (commonly known as Cyclophilin A). is a complex that inhibits calcium dependent phosphatases, which is though to halt the production of the TNF-□ pro-inflammatory molecules. Interestingly, Cyclophilin A was significantly up-regulated by copper exposure whilst a TNF receptor was significantly down-regulated, supporting detrimental effects of copper on immune responses.

Gene classification from KEGG Orthology analyses identify the majority of gene expression effects are involved in the Peroxisome Proliferator-Activated Receptor (PPAR) pathway (figure 2 and 3). Peroxisome Proliferator-Activated Receptors are a group of nuclear receptors that function as transcription factors regulating gene expression, playing an essential role in the regulation of cellular differentiation, development, metabolism of carbohydrate, lipids and proteins, and tumorgenesis. This pathway integrates the majority of genes classified into digestion and metabolism; the largest classification effect observed on copper exposure, as well as genes with various other cellular functions.

Gene most similar to	Species Match	Accession No	E-Value	Score	Kegg Orthology	Response	Fold
1-acylglycerol-3-phosphate O-acyltransferase 3	*Danio rerio*	NP_998590	4.00E-68	261	K00629	Up	2.36
actin alpha 2, skeletal muscle	*Pagrus major*	BAF80060	1.00E-94	384	K10354	Up	4.88
actin, alpha 2, smooth muscle, aorta	*Danio rerio*	AAH75896	e-107	391	K12314	Up	3.75
actin, alpha, cardiac muscle 1 like	*Danio rerio*	NP_001001409	e-127	458	K12314	Up	6.10
actin, beta	*Acanthopagrus schlegelii*	AAR84618	e-122	441	K05692	Up	2.51
aldolase a, fructose-bisphosphate	*Danio rerio*	NP_919358	e-124	447	K01623	Up	3.47
alpha tubulin, (protein LOC573122)	*Danio rerio*	NP_001098596	e-120	434	K07374	Up	1.86
amylase-3 protein	*Pseudopleuronectes americanus*	AAF65827	e-144	513	K01176	Up	3.06
APEX nuclease 2	*Xenopus tropicalis*	NP_001006804	6.00E-25	118	K10772	Down	4.54
apolipoprotein	*Tetraodon nigroviridis*	CAG03661	1.00E-38	78	K08757	Up	1.80
apolipoprotein A-I	*Danio rerio*	NP_571203	1.00E-81	306	K08758	Up	2.28
apolipoprotein A-I-1 precursor (Apo-AI-1)	*Oncorhynchus mykiss*	O57523	8.00E-76	286	K08759	Up	3.99
apolipoprotein A-I-2 precursor	*Oncorhynchus mykiss*	O57524	4.00E-71	271	K08760	Up	4.81
apolipoprotein A-IV	*Danio rerio*	AAH93239	1.00E-73	279	K08761	Up	2.72
apolipoprotein CII	*Oncorhynchus mykiss*	AAG11410	3.00E-19	99	K08763	Up	2.17
apolipoprotein Eb	*Danio rerio*	NP_571173	2.00E-38	162	K08764	Up	4.16
c1q-like protein	*Dissostichus mawsoni*	ABN45966	3.00E-38	162	K08765	Up	2.17
calpain 1	*Danio rerio*	AAH91999	2.00E-68	262	K08766	Up	2.27
chitin binding Peritrophin-A domain	*Danio rerio*	AAH45331	4.00E-69	264	K08767	Up	2.34
chymotrypsinogen 2-like protein	*Sparus aurata*	AAT45254	1.00E-20	101	K08768	Up	3.93
dopachrome tautomerase	*Salmo salar*	ABD73808	1.00E-85	318	K08769	Down	1.78
F-type lectin	*Morone saxatilis*	ABB29997	1.00E-46	188	K08770	Up	3.73
gastric chitinase	*Morone saxatilis*	ABU93585	4.00E-164	581	K08771	Up	4.25
intestinal fatty acid binding protein	*Danio rerio*	AAF00925	3.00E-56	221	K08772	Up	2.82
isocitrate dehydrogenase 3 (NAD+) gamma	*Danio rerio*	NP_001017713	2.00E-14	83	K08773	Down	1.89
lipoxygenase 12R	*Ornithorhynchus anatinus*	XP_001518171	8.00E-06	55	K08774	Up	4.17
m-calpain	*Oncorhynchus mykiss*	BAD77825	e-108	396	K08775	Down	1.99
muscle creatine kinase	*Danio rerio*	CAM16434	e-112	406	K08776	Up	2.21
myozenin 1	*Danio rerio*	NP_991241	2.00E-25	119	K08777	Up	3.91
NADH dehydrogenase subunit 5	*Osmerus mordax*	ABI35911	e-107	390	K08779	Up	3.88
NADH dehydrogenase subunit 6	*Salangichthys microdon*	NP_795843	e-107	392	K08780	Up	3.03
pancreatic protein with 2 somatomedin B domains	*Paralichthys olivaceus*	BAA88246	2.00E-95	352	K08781	Up	7.54
pepsin A2	*Trematomus bernacchii*	CAD80096	2.00E-88	253	K08782	Up	4.05
pepsinogen	*Paralichthys olivaceus*	BAC87742	3.00E-77	291	K08783	Up	3.04
pepsinogen A form IIa	*Pseudopleuronectes americanus*	AAD56283	3.00E-89	331	K08785	Up	4.65
pepsinogen C (progastricsin)	*Salvelinus fontinalis*	AAG35646	e-107	390	K08786	Up	3.41
peptidylprolyl isomerase A (cyclophilin)	*Danio rerio*	AAQ91263	1.00E-61	239	K08788	Up	2.77
phosphoglucose isomerase-2	*Plecoglossus altivelis*	BAF91566	e-120	435	K08789	Up	4.86
proteasome (macropain) 26S subunit, ATPase 4	*Danio rerio*	AAI53480	e-109	396	K08790	Down	2.92
proteasome subunit alpha type 7	*Danio rerio*	NP_998331	e-112	409	K08791	Down	2.89
sarcoendoplasmic reticulum calcium ATPase	*Silurus lanzhouensis*	ABG90496	8.00E-87	323	K08795	Up	2.71
simple type II keratin K8b (S2)	*Oncorhynchus mykiss*	CAA63300	3.00E-74	281	K08799	Up	3.28
SPARC: secreted protein, acidic, rich in cysteine	*Danio rerio*	AAT01213	2.00E-31	139	K08800	Up	4.14
suppressor of ypt1	*Danrio rerio*	NP_878281	e-122	442	K08801	Down	2.14
suppressor of ypt1	*Danrio rerio*	NP_878281	e-123	445	K08802	Down	3.01
titin a	*Danio rerio*	ABG48500	e-125	451	K08805	Up	2.80
TNF (tumor necrosis factor) decoy receptor	*Oncorhynchus mykiss*	AAK91758	2.00E-67	258	K08807	Down	4.23
transforming growth factor, beta-induced	*Danio rerio*	NP_878282	3.00E-21	105	K08808	Up	1.59
tripartite motif-containing 45	*Xenopus tropicalis*	NP_001011026	3.00E-27	125	K08809	Up	2.20
zinc finger protein 503	*Danio rerio*	NP_942137	3.00E-63	245	K08810	Down	2.58
zona pellucida protein X	*Sparus aurata*	AAY21008	1.00E-68	263	K08811	Down	1.99

Table 4. Annotation, fold-change in expression, and functional KEGG Orthology codes of delta smelt genes significantly differing ($p<0.05$) on exposure to copper (50 µg $Cu^{+}.L^{-1}$).

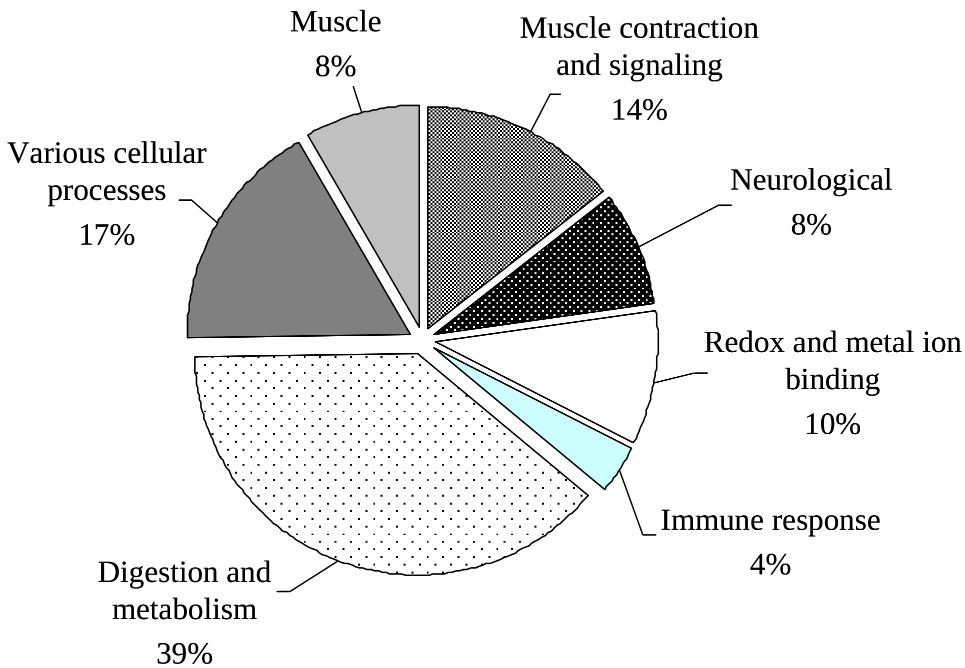

Figure 1. Microarray responses**:** systematic analysis of KEGG Orthology and Gene Ontology based functional classification of delta smelt genes significantly differing on exposure to copper (50 μg $Cu^{+}.L^{-1}$).

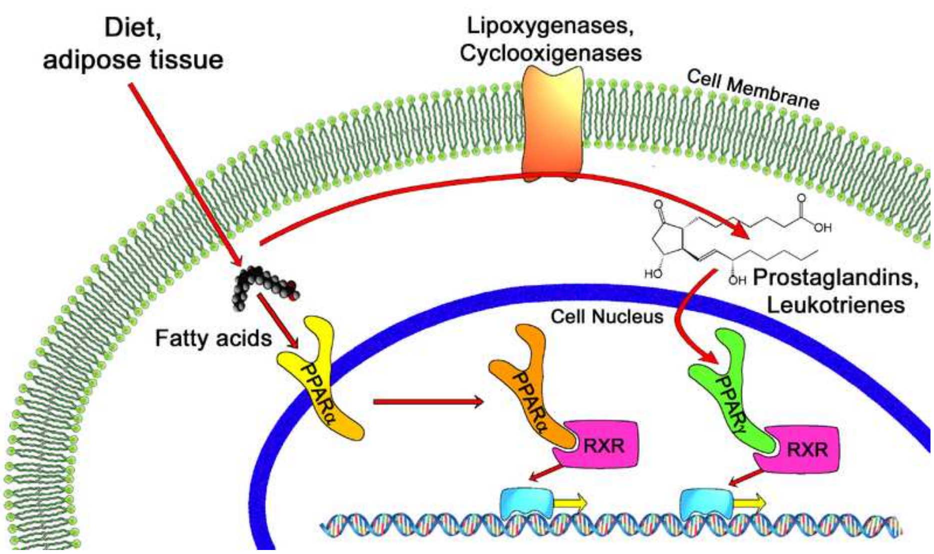

Figure 2. Peroxisome Proliferator-Activated Receptor showing involvement in metabolism. Genes involved in the PPAR pathway are further highlighted in figure 3.

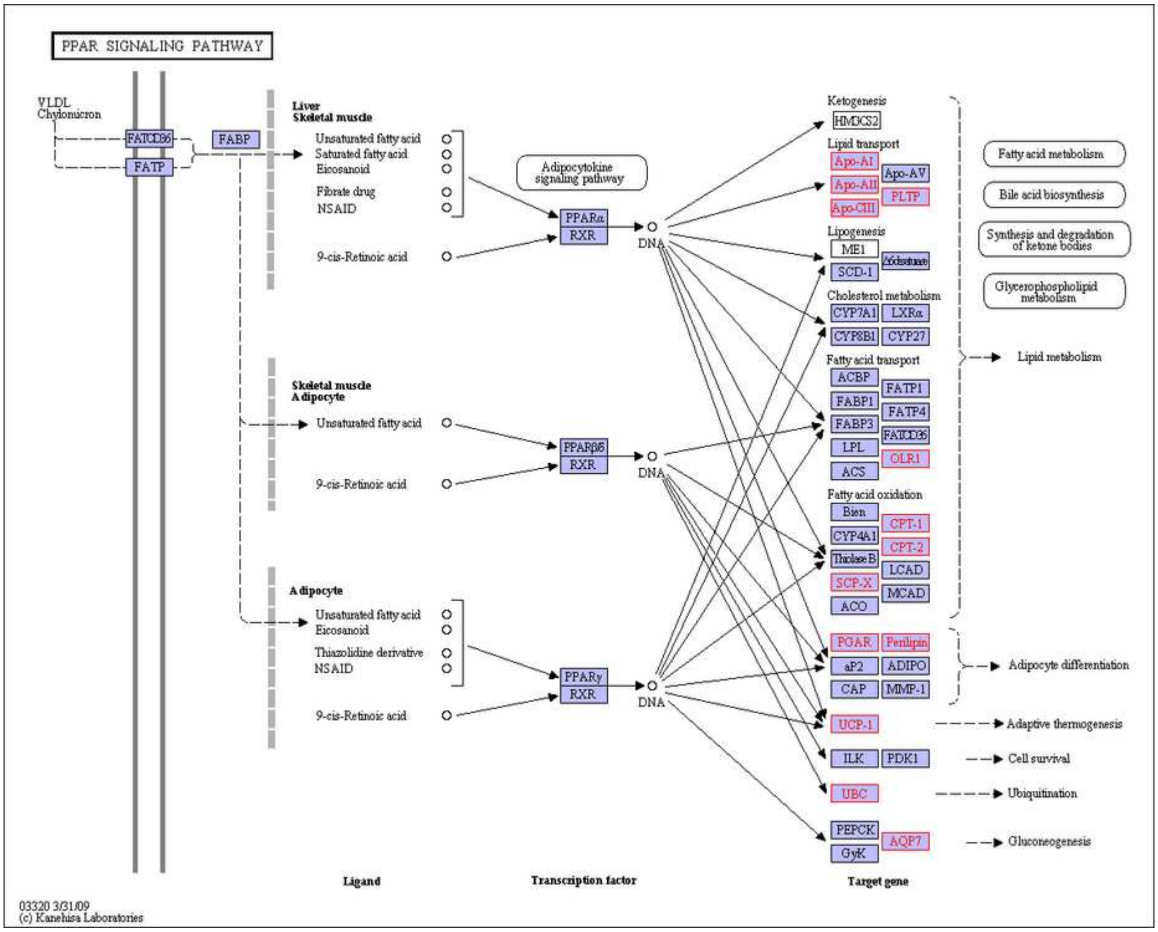

Figure 3. Peroxisome Proliferator-Activated Receptor (PPAR) highlighting genes from microarray analysis, involved in signaling pathway, through KEGG pathways.

In summary, the overall responses to copper exposure in the delta smelt appear to be from genes involved in regulation of cellular differentiation, development, metabolism of carbohydrate, lipids and proteins, and tumorgenesis. Furthermore, neuromuscular responses were identified as hypothesized. There is also probable compromise to the immune system and suggestions that excess copped may lead to tissue damage.

Confirmation tests were carried out on selected genes identified through the microarray application, and investigated as probable biomarkers using real-time quantitative PCR assessing responses to copper exposure at environmentally relevant concentrations (presented below).

Biomarker responses

Genes selected from the microarray functional classification were assessed as probable biomarkers of copper exposure. Genes were selected to cover neuromuscular, digestive and immune system responses to copper exposure.

Quantitative PCR responses to sublethal copper exposure are presented in figure 4. Results confirm microarray identification of neuromuscular effects of sublethal copper concentrations on the delta smelt. Environmentally relevant concentrations elicited significant responses in sarcoendoplasmic reticulum calcium ATPase (SerCa), muscle creatine kinase and myelin aspartoacylase. Furthermore, the response profiles for these three genes display a significant difference in expression ($p<0.01$) at 8 $\mu g\ Cu^{+}.L^{-1}$ with respect to controls.

Compensatory responses are generally observed at low contaminant exposure concentrations, as an organism is capable of metabolizing and detoxifying the chemicals in question.

Hormesis, defined as a biphasic dose response phenomenon (Calabrese 2008), is often observed at low exposure concentrations, with opposing responses to those observed at higher concentrations (Connon et al. 2008; Connon et al. in review; Heckmann et al. 2008). This shockwave response may result from non-specific responses resulting from signaling receptors being triggered. At higher levels of exposure, the responses become more specific, as the organism directly responds to the stressor. Thus, low concentrations of contaminants, may not necessarily have direct detrimental effects upon the organism, but the change in this biphasic response, to a more specific dose-response may be indicative of concentrations at which contaminants begin to be detrimental to overall health. We observe a biphasic response at the lower concentrations in the majority of genes assesses by qPCR, with fluctuating responses at low doses leading to a dose-response relationship at concentrations known to be detrimental.

Four of the investigated biomarkers displayed a dose-response relationship with copper, Calpain, Myozenin, TNF and Amylase. The physiological roles of calpains are still poorly understood. They have been shown to participate in cell mobility and cell cycle progression, potentiation in neurons and cell fusions in myoblasts. Myozenin is involved in muscle contraction. It is a Z-line, α-actinin- and γ-filamin-binding protein expressed predominantly in skeletal muscle, and has been suggested as a biomarker for muscular dystrophy and other neuromuscular disorders. Tumor Necrosis Factor - □ (TNF) is a cytokine in systemic inflammation. The primary role of TNF is in the regulation of immune cells, inducing apoptosis to induce inflammation and inhibiting tumorgenesis and viral replication. Amylase is an enzyme that breaks down starch into sugars thus directly involved in digestion.

In summary, the selected biomarkers confirm expression of genes identified through microarray screening and corroborate effects of copper exposure upon digestion, metabolism, neuromuscular activity and immune responses, proving to be useful candidates to investigate effects of contaminants upon the delta smelt.

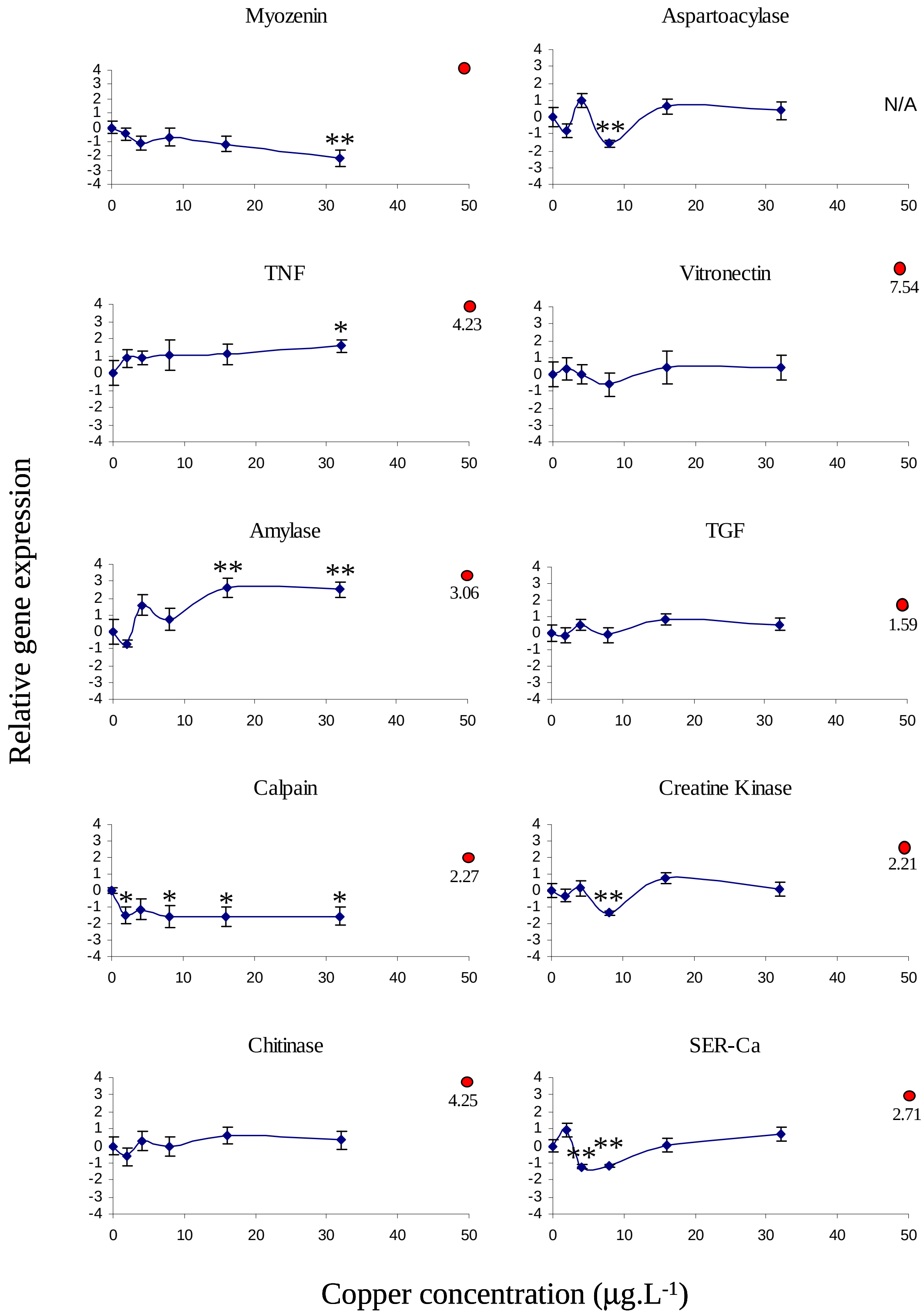

Figure 4. Biomarkers of sublethal toxicity: Quantitative PCR expression profiling of selected delta smelt genes responding to environmentally relevant concentrations of copper. Circular dots indicate comparative responses from exposure to 50 µg $Cu^{+}.L^{-1}$, as identified through microarray analysis.

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9.3

Molecular Evaluation of Environmental Contaminant Extracts in Striped bass collected from Semi Permeable Membrane Devices (SPMD) in the San Francisco Estuary.

Connon R.E., D'Abronzo L.S. and Werner I.
(in collaboration with Dr. David Ostrach's research group)

This work was carried out on striped bass samples obtained from Dr. David Ostrach.

Background

Semipermeable membrane devices (SPMDs) are used to assess environmental pollutants from water and air, through the accumulation of hydrophobic organic compounds, such as PCBs, PAHs, and organochlorine pesticides. The principal advantage of SPMD is its sampling of the truly-dissolved and thus bio available phase of these pollutants. SPMDs estimate bioconcentration factors of organic compounds over a period of time, representing a time-weighted average. SPMD derived extracts can be used for conventionally applied aquatic toxicological bioassays.

In an effort to assess bioavailable lipophilic contaminants in the estuary, SPMDs were deployed and extracts used in toxicant bioassays of juvenile striped bass (*Morone saxitilis*). This study was carried out by Dr. David Ostrach. Tissue samples were assessed for gene expression by Dr. Inge Werner's laboratory, in a collaborative approach. Additional tissues will be analyzed in the near future.

Methods

Exposure details:
SPMD extracts dissolved in peanut oil were injected intraperitoneally (100 µL/fish) into 4 and 6 month old hatchery juvenile striped bass in two different exposure experiments.
Fish were exposed for 7-days and test terminated by humanely euthanizing the fish in MS222. Livers from each fish were dissected, snap frozen in liquid nitrogen and stored at -80°C for molecular analyses.

RNA extraction and cDNA synthesis
Total RNA from was extracted from liver tissue using a Qiagen RNeasy Mini kit, with on-column DNase digestion following manufacturer's protocols. Complementary DNA (cDNA) was synthesized using 1µg total RNA, with 50 units of Superscript III reverse transcriptase, 600ng random primers, 10 units of RNaseOut, and 1mM dNTPs (all Invitrogen). Reactions were incubated for 50 min at 50ºC, followed by a 5 min denaturation step at 95ºC, and were later diluted 3-fold for subsequent real time - PCR assessments.

Real-time quantitative PCR (rt-qPCR)

Genes investigated in this study were based on sequences, primers and probes previously developed and validated by (Geist et al. 2007), with the addition of -actin, used as reference gene, for which primer pairs and fluorescent probes were designed using Roche Applied Science Universal ProbeLibrary Assay Design. All rt-qPCR systems were validated for specificity and amplification efficiencies as described in (Leutenegger et al. 1999). Briefly, a 2-fold dilution series of cDNA samples were tested in triplicate with the respective real-time TaqMan PCR system. The amplification efficiency was calculated using the formula $E=2^{1/S}-1$, where S is the slope curve. All amplification efficiencies were above 90%, validating the specificity of the rt-qPCR systems.

Molecular biomarkers (summarized in table 1) were used to evaluate sublethal stress response of proteotoxicity (HSP70), phase I detoxification mechanism (CYP1a), metal-binding (Metallothionein), endocrine disruption (Vitellogenin) and pathogen-defense (Mx protein).

Gene	**Primer Sequences**	**Roche Probe Number and Sequence**
HSP 70	F: CATCCTTTCTGGGGACAAGTCAG R: ACACCTCCAGCGGTCTCAATAC	62 ACCTGCTG
CYP1A1	F: GCGGCACAACCCCAGAGTA R: CAGCTTTCATGACGGTGTTGAG	65 CTGGAGGA
Metallothionein	F: GCGGAGGATCCTGCACTTG R:CAGCCAGAGGCACACTTGGT	68 CTGCTCCT
Vitellogenin	F: CTGATCTGAATTTGGCCTGAGG R: ACCTGTATCCCAAGGACAGTGC	156 GCTGATGG
β-Actin	F: CAATGAGAGGTTCCGTTGC R: CAGGACTCCATACCGAGGAA	11 CTTCCAGC

Table 1. Molecular Biomarkers: List of real-time Quantitative PCR primers and probes used on Striped bass (*Morone saxatilis*)

Real-time TaqMan PCR reactions were prepared with 400nM of each of two primers and 80nM of the appropriate TaqMan probe, and TaqMan Universal PCR Mastermix (Applied Biosystems, Foster City, CA, USA) containing 10mMTris–HCl (pH 8.3), 50mM KCl, 5mM MgCl2, 2.5mM deoxynucleotide triphosphates, 0.625U AmpliTaq Gold DNA polymerase per reaction, 0.25U AmpErase UNG per reaction. A total of 5µl of cDNA was combined with 7µl of the above mix and amplified in 384-well plates with an automated fluorometer (ABI HT 7900 A FAST Sequence Detection System, Applied Biosystems). Amplification conditions were 2 min initial primer annealing at 50ºC and 10 min denaturation at 95ºC, followed by 40 cycles of 15 sec denaturing at 95ºC and 60 sec annealing at 60ºC. SDS 2.2.1 software (Applied Biosystems) was used to quantify product amplification.

Relative quantitation and statistical analyses.
A comparative cycle threshold (*C*T) method as described in (User Bulletin #2, Applied Biosystems) was applied to quantify gene transcription of investigated stress response genes and

values are therefore expressed as relative transcription to □-actin reference gene and *n*-fold transcription relative to oil controls. Both Analysis of Variance (ANOVA) and student-T tests were carried out between SPMD site samples and oil controls, as well as between SPMD dialysis and oil controls. Differences between the two tests dates were also assessed through ANOVA and student t-tests.

Results and Discussion

There were significant temporal variations in gene expression over the four SMPD deployment periods (Figure 1 and 2 – presented separately due to expression scale differences)

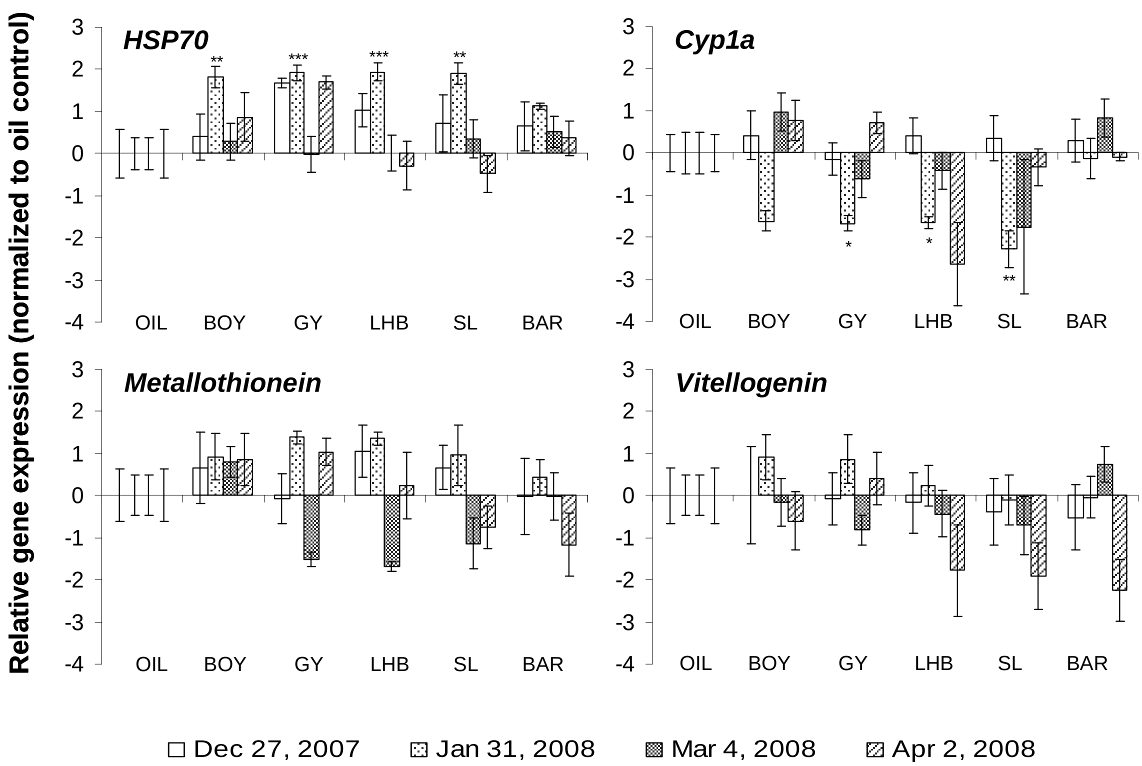

Figure 1. Gene Expression: Biomarker transcription of four selected genes in Striped bass responding to intraperitoneal doses of SPMD accumulated contaminants from five sites in the San Francisco Estuary. Site keys: BAR = Barbie Slough/North Cache Slough; LHB = Little Honker Bay; BY = Boyngton Slough; GY = Goodyear Slough & SL = Sherman Lake. (* p<0.05, ** p<0.01, ***p<0.001).

Heat Shock Proteins (HSP70) were predominantly up-regulated confirming contaminant induced stress, and that protein increase protein synthesis was still induced at the end of the tests. Expression levels were significantly up-regulated at all sites except for Barbie Slough/North Cache Slough (BAR).

CYP1a were predominantly down-regulated at sites Little Honker Bay (LHB) and Sherman Lake (SL) suggesting probable short term induction leading to sufficient protein synthesis for detoxification purposes. Goodyear Slough (GY), LHB and SL displayed significant down-regulation in respect of oil controls.

Metallothionein displayed both up and down regulations, with temporal variations. Down-regulation, though not significant at test termination, may be indicative of sufficient protein synthesis for metal sequestration at lower doses, whilst mRNA levels were still highly expressed at 48 hour with elevated contaminants.

Interestingly vitellogenin was down-regulated at LHB, SL and BAR at similar time-points in April 2008, though the expression levels were not significantly different to oil controls.

The cytokine encoding for MX protein (presented in fig 2), was significantly up-regulated at Boynton Slough (BOY) and SL, suggesting effects upon the immune system.

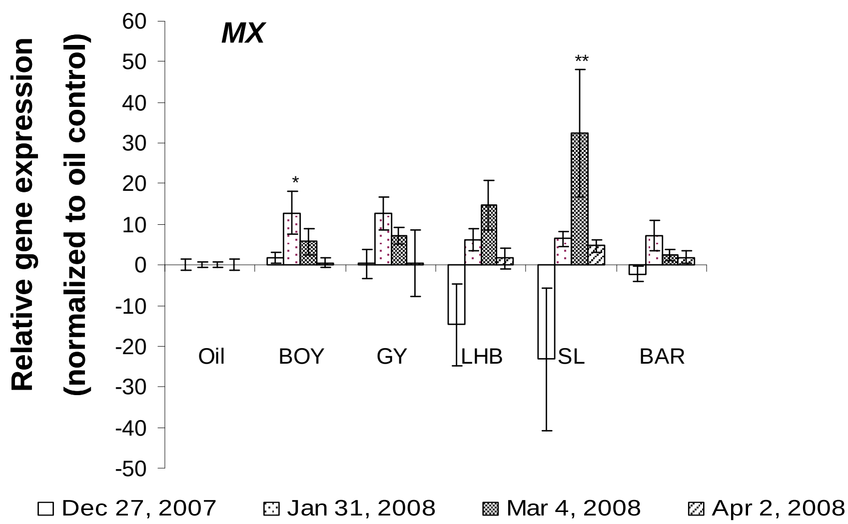

Figure 2. Gene Expression: Biomarker transcription of MX in Striped bass responding to intraperitoneal doses of SPMD accumulated contaminants from five sites in the San Francisco Estuary. Site keys: BAR = Barbie Slough/North Cache Slough; LHB = Little Honker Bay; BY = Boyngton Slough; GY = Goodyear Slough & SL = Sherman Lake. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

In summary, HSP70 up-regulation confirms general stress at sites BOYS, GY, LHB and SL, with little to no variation in BAR. Interestingly, the same sites display a down-regulation in Cyp1a, a probable indication that processes have synthesized sufficient protein for this phase I detoxification enzyme. Both BOY and SL samples appear to have further effects upon the striped bass immune system.

It would be of great interest to compare the obtained results with rainfall and flow data for the examined sites, for the duration of the SPMD deployment. This would offer some indication of dilution factors and water volumes to which the membranes were exposed.

Protein analysis data from corresponding samples will enable us to confirm the molecular results and hypothesized conclusions.

References.

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10. Quality Assurance/Quality Control

Quality Assurance/Quality Control (QA/QC) measures are included in this project to assess the reliability of the data collected. These QA/QC procedures include positive control tests (i.e., reference toxicant tests), field duplicates, bottle blanks and trip blanks. The components of these QA/QC measures are outlined below.

Reference toxicant tests: Positive control tests (reference toxicant tests) are conducted to ascertain whether organism responses fall within the acceptable range as dictated by US EPA.
Hyalella azteca: Reference toxicant tests with *H. azteca* using sodium chloride as the toxicant were performed once a month. The LC_{50} for each reference toxicant test survival endpoint was plotted to determine whether it fell within the 95% confidence interval of the running mean. If the LC_{50} falls out of the 95% confidence interval, or plus or minus two standard deviations around a running mean, test organism sensitivity is considered atypical and results of toxicity tests conducted during the month of reference toxicant outliers may be considered suspect. From January 1 to June 30, 2009, *H. azteca* performed normally within each reference toxicant test.
Delta Smelt: Two reference toxicant tests with Delta smelt using copper chloride (EC = 900 µS/cm, T = 16 ± 2ºC) as the toxicant were performed in June, 2009. The average control survival for the reference toxicant test conducted on June 24, 2009, did not meet this project's test acceptability criterion of 60%. The LC_{50} was plotted to determine whether it fell within the 95% confidence interval of the running mean. Excluding the reference toxicant test conducted on June 24, 2009, Delta smelt performed normally within each reference toxicant test.

Table 10-1. Delta smelt RT

Test Date	**Mean Survival**	**96-h LC50**	**NOEC**	**LOEC**
6/10/09	93.3 %	150.3 ppb	106 ppb	213 ppb
6/24/09	53.3 %	133 ppb	213 ppb	>213 ppb

Field duplicates: Field duplicate samples were collected to assess precision. For this report, these QA/QC samples were collected on the following dates:

Table 10-2. Field duplicate collection dates

Sample Date	**Field Duplicate Primary Site**
January 7	602
January 22	711
February 4	Light 55
February 17	Rough & Ready
March 4	340
April 23	902
May 26	Suisun
May 27	609
June 11	815
June 24	405
June 24	508

Field duplicate samples are in agreement when the primary sample and its duplicate are both either statistically similar or statistically different from the control. The frequency of field duplicates sharing equivalent results is outlined in Table 3.

Bottle blanks: Bottle blank samples were included to evaluate potential incidental contamination due to the sample container. Bottle blanks are analyte-free water samples that are transferred to a clean sample container that is prepared in the laboratory. For this project, bottle blanks were comprised of de-ionized water amended with dry salts to US EPA moderately hard specifications (DIEPAMHR). A bottle blank sample is in agreement when it is statistically similar to the control. The frequency of bottle blanks sharing equivalent results is outlined in Table 3.

Trip blanks: Trip blank samples were included in this project to evaluate potential incidental contamination that can occur during field sampling and sample processing. A trip blank is an analyte-free water sample that is transferred into a clean sample container that is prepared in the laboratory, brought out into the field, and treated like any other collected sample throughout the course of the trip. For this project, trip blanks were comprised of DIEPAMHR. A trip blank sample is in agreement when it is statistically similar to the control. The frequency of trip blanks sharing equivalent results is outlined in Table 3.

Table 10-3. Frequency of QA/QC samples sharing equivalent results

Quality Assurance Samples	***H. azteca*** **Survival**		***H. azteca*** **Weight**	
	Sample Size	% Agreement	Sample Size	% Agreement
Field Duplicates	11	100	11	91
Bottle Blanks	13	100	13	92
Trip Blanks	5	100	5	100

In a field duplicate of site Light 55, collected on February 4, 2009, animals exhibited reduced weight when compared to animals in the primary sample. The reason for this discrepancy is unknown. However, as both the primary sample and its duplicate were statistically similar to the control, the results are considered equivalent.

In a field duplicate of site 340, collected on March 4, 2009, animals exhibited reduced weight when compared to the control, whereas animals in the primary sample did not. The mean weight of the animals in the primary sample was 0.040 mg/individual, and the mean weight of animals in the duplicate was 0.034 mg/individual. As the difference in weight between animals in the primary sample and its duplicate is small, we believe that this is an instance where the weights fell on the border between statistically significant and not statistically significant, where the primary sample's weight was not significant, and the duplicate's weight was. Although the results are not equivalent, we believe these data are reliable.

In a bottle blank collected on April 23, 2009, animals exhibited reduced weight when compared to the control. The mean weight of the animals in the control was 0.084 mg/individual, and the mean weight of animals in the bottle blank was 0.057 mg/individual. As there was low variability among replicates within this test, the ability to detect smaller statistical differences between samples increased. We believe that this difference is due to extra sensitivity in the test, rather than contamination from the sample container.

Precision: Precision is the degree to which the primary sample agrees with its duplicate. Precision can be measured by calculating the Relative Percent Difference (RPD) between sample measurements. The RPD between a sample and its duplicate can be calculated by using the following equation:

$$RPD = \left(\frac{[2 * |Dup1 - Dup2|]}{[Dup1 + Dup2]} \right) * 100$$

For this project, RPDs were calculated using the aforementioned equation on water chemistry measurements such as DO, pH, EC, hardness, alkalinity and ammonia. Both the individual and average RPDs between duplicates are listed in detail in Tables 4 and 5. Please note that the individual RPD between Site Rough & Ready and its duplicate (collected February 17, 200) and Site 902 and its duplicate (collected April 23, 2009) for ammonia is unusually high at 151% and 100%, respectively (noted with a superscript [A] within Tables 4A and 4B). Caution should be applied when interpreting water quality precision data. This high RPD is due to unusually small amounts of ammonia being measured, rather than lack of precision.

Deviations: Two deviations occurred during this reporting period. The first deviation occurred on April 1, 2009, in which samples 602 and 609 were received at the lab with temperatures of 6.9º C and 7.2º C, respectively, above the EPA criterion of 6º C. This deviation occurred due to a shortage of ice in the transport cooler. Upon receipt, samples were immediately transferred to an environmental chamber maintained between 0-6 ºC and stored in the dark until test initiation, which reduced the chance of sample degradation. Additionally, because the receiving temperatures were very close to the EPA criterion of 0-6 ºC, and the amount of time the samples were out of range was minimal, we believe that sample integrity was maintained. Therefore, we consider the data reliable.

The second deviation occurred on May 16, 2009, in which the 72-hr holding time was exceeded for test initiation. This deviation occurred because the toxicity test that was initiated within the proper holding time (May 14, 2009) had contamination in the PBO-manipulated samples. Tests with the un-manipulated ambient samples were continued until the scheduled test termination; however the test had to be repeated. It was determined that the PBO stock solution had become contaminated, and a new stock solution was made. The test initiated on May 16, 2009, was a re-test of all samples, using the new PBO stock solution. This test did meet all TAC, and the data are considered reliable.

Table 10-4A. Individual Relative Percent Differences (RPDs) of water chemistry measurements between field duplicates. [A] High RPD

Field Duplicate & Sample Date	EC	DO			pH		Hardness	Alkalinity	Ammonia
Site 602 January 7, 2009	3.94 2.96	2.41 0.00 1.31	1.21 6.71 6.45	4.37 4.94 0.00 1.24	0.26 0.13 0.51	0.00 0.13 0.13	4.48	0.00	4.08
Site 711 January 22, 2009	1.80 13.26	5.85 3.64 0.00	2.41 2.60 3.43	5.06 2.67 1.48 1.32	2.64 0.25 0.49	1.01 0.63 0.62	0.00	8.28	14.29
Site Light 55 February 4, 2009	0.30 4.60	2.53 4.82 4.08	4.76 3.77 2.44	2.63 2.44 0.00 1.29	1.61 0.74 0.24	0.62 0.12 0.00	8.00	7.41	14.08
Rough & Ready February 17, 2009	0.00 1.95	3.77 0.00 5.33	0.00 3.92 7.69	1.26 0.00 5.13 2.70	1.33 0.86 0.12	0.37 0.50 0.00	3.70	66.67	151.02[A]
Site 340 March 4, 2009	1.47 4.27	4.82 1.26 5.41	1.26 1.26 7.06	1.27 3.43 6.54 2.53	0.38 0.13 0.13	0.00 0.00 0.00	10.91	2.15	0.00

Table 10-4B. Individual Relative Percent Differences (RPDs) of water chemistry measurements between field duplicates. [A] High RPD

Field Duplicate & Sample Date	EC	DO			pH		Hardness	Alkalinity	Ammonia
Site 902 April 23, 2009	1.19 0.29	0.00 1.21 0.00	1.20 1.24 4.65	1.26 3.73 2.56 2.70	0.00 0.38 0.12	0.12 0.64 0.63	4.88	0.00	100.00[A]
Site Suisun May 26, 2009	0.72 0.94	1.26 1.24 0.00	1.21 3.92 2.33	2.60 2.38 1.32 2.63	1.15 0.12 0.50	0.62 0.37 0.12	7.41	8.11	52.63
Site 609 May 27, 2009	0.81 1.60	1.24 2.41 1.29	0.00 1.32 3.55	2.60 5.78 2.67 1.31	0.50 0.38 0.38	0.25 0.64 0.38	0.00	9.09	6.45
Site 815 June 11, 2009	0.62 4.02	2.44 1.24 2.63	1.21 1.29 3.51	0.00 1.20 2.74 0.00	2.25 1.01 0.26	0.00 0.91 0.78	6.45	3.51	0.00
Site 405 June 24, 2009	1.78 2.84	5.13 4.88 5.88	3.77 6.71 10.13	9.66 7.59 2.86 1.40	0.39 2.94 0.82	1.72 0.66 1.21	0.00	2.35	29.79
Site 508 June 24, 2009	6.23 5.46	1.26 3.59 2.82	2.41 0.00 5.26	2.44 3.51 22.56 2.70	1.53 1.39 0.41	2.51 2.80 1.60	0.00	0.00	22.22

Table 10-5A. Average Relative Percent Difference (RPD) of water chemistry measurements between field duplicates.

Field Duplicate & Sample Date	EC			DO			pH		
	Sample Size	Average	SD	Sample Size	Average	SD	Sample Size	Average	SD
Site 602 January 7, 2009	2	3.45	0.70	10	2.87	2.55	6	0.19	0.17
Site 711 January 22, 2009	2	7.53	8.11	10	2.85	1.75	6	0.94	0.87
Site Light 55 February 4, 2009	2	2.45	3.04	10	2.88	1.53	6	0.55	0.59
Rough & Ready February 17, 2009	2	0.97	1.38	10	2.98	2.66	6	0.53	0.50
Site 340 March 4, 2009	2	2.87	1.98	10	3.48	2.31	6	0.11	0.15

Table 10-5B. Average Relative Percent Difference (RPD) of water chemistry measurements between field duplicates.

Field Duplicate & Sample Date	EC			DO			pH		
	Sample Size	Average	SD	Sample Size	Average	SD	Sample Size	Average	SD
Site 902 April 23, 2009	2	0.74	0.63	10	1.86	1.53	6	0.32	0.28
Site Suisun May 26, 2009	2	0.83	0.55	10	1.89	1.09	6	0.48	0.38
Site 609 May 27, 2009	2	1.20	0.56	10	2.22	1.61	6	0.42	0.13
Site 815 June 11, 2009	2	2.32	2.40	10	1.63	1.17	6	0.87	0.78
Site 405 June 24, 2009	2	2.31	0.75	10	8.50	2.81	6	1.29	0.93
Site 508 June 24, 2009	2	5.85	0.54	10	4.65	6.44	6	1.71	0.86

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Data Appendices
A – I

Pelagic Organism Decline 2008 – 2010
Progress Report III
Project Period: January – June 2009

Appendix A

Final Report:
Acute Toxicity of Ammonia, Copper, and Pesticides to Eurytemora affinis, of the San Francisco Estuary
(Swee et al., 2009)

Final Report

Acute Toxicity of Ammonia, Copper, and Pesticides to *Eurytemora affinis*, of the San Francisco Estuary

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Executive Summary

Toxicity testing (96-h) of ambient surface waters in April-May 2008 from several locations in the North and South Delta-San Francisco Estuary (SFE) was shown to significantly affect the survival of *Eurytemora affinis.* Although chemical contaminants such as ammonia, bifenthrin, copper diuron, lambda cyhalothrin, and polyaromatic hydrocarbons have been detected in ambient waters, the impacts of these contaminants to pelagic organisms in the SFE food web are critically unknown particularly to the dominant zooplankton, i.e., *E. affinis*. The acute toxicity of ammonia, bifenthrin, chlorpyrifos, copper, cyfluthrin and permethrin to *E. affinis* was addressed in the current study as shown by the results of 96hr-LC50 values of the different contaminants: 1) ammonia - 10.97 mg/L total ammonia or 0.78 mg/L unionized ammonia at pH 8.1, 7.56 mg/L total ammonia or 0.12 mg/L unionized ammonia at pH7.6, and 10.93 mg/L total ammonia or 0.068 mg/L unionized ammonia at pH7.2; 2) bifenthrin - 11.37 ng/L, 3) chlorpyrifos - 803.20 ng/L 4) copper - 3.48 μg/L, 5) cyfluthrin - 12.72 ng/L and 6) permethrin -158.08 ng/L. Current findings indicated that *E. affinis* were sensitive to ammonia, copper, and pyrethroid pesticides (bifenthrin, cyfluthrin, and permethrin) and organophosphate insecticide (chlorpyrifos). Based on the results of this study, it is likely that the toxicities observed in *E. affinis* in 2008 may have been due, in part, to the presence of some of these chemicals in examined ambient waters. The potential impact of one or additive effects of these chemicals pose serious implications to the health and survival of zooplankton as important components of the SFE food web.

Introduction

Eurytemora affinis is an important food source to higher trophic level pelagic fish such as delta smelt, threadfin shad, and longfin smelt in the San Francisco Estuary (SFE). Previous study in this laboratory revealed that ambient surface waters from several locations in the North and South Delta in April-May 2008 showed significant effects to *E. affinis* survival (Teh *et al*., 2008). The initial detection of several chemical contaminants including ammonia, bifenthrin, chlorpyrifos, copper, cyfluthrin and permethrin in ambient waters prompted the need to examine their acute toxicity to *E. affinis*. Assessing the 96-hour LC50 values to establish the toxicity of these contaminants to *E. affinis* under controlled laboratory conditions was the main objective of the current study.

Experimental Details

1. Copepods

Brood stock of *E. affinis* was grown in aerated 120 L tanks placed in an environmentally controlled room at 20 ± 1 ^{o}C. Water quality in the tank including dissolved oxygen (>8 mg/L), pH (8.0 ± 0.1), water hardness (100 mg/L), salinity (2.0 ppt), and ammonia (<1 μg/L) were monitored weekly. An equal biovolume of the Instant Algae (*Nannochloropsis* and *Pavlova*) mix was given as food at 400 μg C.L^{-1}.day-1.

2. *Chemicals*

Stock solutions of ammonium chloride (10.0 g/L), bifenthrin (8.0 mg/L), chlorpyrifos (4.0 mg/L) copper chloride (4.0 mg/L), cyfluthrin (4.0 mg/L), and permethrin (8.0 mg/L) were prepared by personnel of Aquatic Toxicology Laboratory at UC Davis. The concentrations of the chemical used were: 1) bifenthrin (methanol control, 4.0, 8.0, 16.0, 32.0, and 64.0 ng/L), 2) chlorpyrifos (methanol control, 300, 600, 900, 1200, 1500 ng/L), 3) cyfluthrin (methanol control, 1.0, 3.0, 5.0, 7.0, 9.0 ng/L), and 4) permethrin (methanol control, 150, 175, 200, 225, 250 ng/L). Methanol was used as solvent for these chemicals, and therefore served as control using the highest concentration in each of the chemical treatments. The concentrations used for ammonia were: 1) 0.0, 10.0, 15.0, 20.0, 25.0, and 30.0 mg/L at pH 8.1, 2) 0.0, 10.0, 15.0, 20.0, 25.0, and 30.0 mg/L at pH7.6, and 3) 0.0, 4.0, 6.0, 8.0, 10.0, and 12.0 mg/L at pH7.2 that were prepared by diluting the ammonium chloride stock solution with culture water and the pH adjusted with 1N HCl. The concentrations used for copper chloride were 0.0, 1.0, 2.0, 4.0, 6.0, and 8.0 μg/L. Graded concentrations of these chemicals were prepared by diluting the stock solution with culture water (same source of water as used for culturing the *E. affinis*) 30-45 minutes prior to the initiation of the 96-hour exposures.

3. *Acute Toxicity Test*

Groups of juvenile *E. affinis* (N = 20 per replicate; three replicates per concentration) were exposed separately to ammonia, bifenthrin, chlorpyrifos, copper, cyfluthrin and permethrin using the standard static renewal method for acute toxicity testing (1993). The test conditions used for the acute toxicity tests for ammonia, bifenthrin, chlorpyrifos, copper, cyfluthrin and permethrin are shown in Table 1. Briefly, Copepods were fed with nutritious algae and 80% of the tested water was replaced at 24, 48, and 72 h with newly prepared corresponding treatment solutions previously acclimated to 20 ^{o}C. Mortalities were recorded daily for 4 days. At the end of 96 hr, the number of survivors in each beaker was counted to derive the mean percentage survival of *E. affinis* exposed to each chemical concentration. The estimated 96-hour LC50 values (Lethal Concentration causing 50% mortality of the *E. affinis*) were calculated using the U.S. Environmental Protection Agency Probit Analysis Program v1.5 (http://www.epa.gov/nerleerd/stat2.htm).

4. *Water parameters and chemical analysis*

Water quality was monitored and recorded daily for each of the acute toxicity trials. Unionized ammonia was calculated from total ammonia nitrogen using free ammonia calculator (http://cobweb.ecn.purdue.edu/~piwc/w3-research/free-ammonia/nh3.html). The concentrations of the chemicals used for the toxicity trials will be verified at the Aquatic Toxicology Laboratory at UC Davis by testing I L subsamples of each of the chemical concentrations prior to the exposure trials.

Results and Discussions

The mean survival (%) of *E. affinis* at the end of 96 hour of toxicity testing is given in Table 2. The 96hr-LC10 and 96hr-LC50 values with 95% confidence intervals as calculated using the USEPA Probit Analysis Program v1.5 are shown in Table 3.
The data demonstrates that juvenile *E. affinis* are sensitive to the ammonia, copper, pyrethroid pesticides (.bifenthrin, cyfluthrin and permethrin), and organophosphate insecticide (chlorpyrifos). This pilot study aimed to establish LC50 values for *E. affinis* to support the hypothesis that ambient water samples from certain locations in the SFE are toxic to *E. affinis.* Based on the results of this study, it is likely that the toxicities observed in *E. affinis* in 2008 may have been due, in part, to the presence of these chemicals in examined ambient waters. The potential impact of one or additive effects of these chemicals pose serious implications to the health and survival of zooplankton as important components of the SFE food web.

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Table 1 Test conditions used for *Eurytemora affinis*

Temperature (°C)	**20 ± 0.1**
Salinity (ppt)	**2**
pH	**8.0 ± 0.1**
Conductivity (µmhos)	**3000**
Hardness (mg/L)	**360**
Alkalinity (mg/L)	**60**
Acceptability in control survival	**≥80%**
Size of test beaker (mL)	**600**
Volume of test solution (mL)	**500**
Life stage of copepods	**Juvenile**
# of copepods	**20**
# of replicates per concentration	**3**
# of concentrations	**6**
Feeding regime	**Daily**
Static-renewal test Duration	**24-96 h**

Table 2 Mean % survivorship of *E. affinis* at the end of 96 hour exposure

Chemicals	Concentration	% Survivorship
Ammonia mg/L at pH 8.1	Control	96.66
	10	56.66
	15	20.00
	20	5.00
	25	0
	30	0
Ammonia mg/L at pH 7.6	Control	88.33
	10	16.66
	15	0
	20	0
	25	0
	30	0
Ammonia mg/L at pH 7.2	Control	88.33
	4	60.00
	6	56.66
	8	55.00
	10	46.66
	12	35.00
Bifenthrin ng/L (pptr)	Methanol control	85.00
	4	75.00
	8	43.33
	16	38.33
	32	16.67
	64	3.33
Chlorpyrifos ng/L (pptr)	Methanol control	83.33
	300	76.66
	600	65.00
	900	26.66
	1200	18.33
	1500	15.00
Copper µg/L (ppb)	Control	88.33
	1	88.33
	2	61.66
	4	23.33
	6	30.00
	8	13.33

Cyfluthrin ng/L (pptr)	Methanol control	88.33
	1	85.00
	3	68.33
	5	56.66
	7	68.33
	9	46.66
Permethrin ng/L (pptr)	methanol Control	88.33
	150	46.66
	175	35
	200	31.66
	225	25
	250	11.66

Table 3 Estimates LC 10 and 50 values of *E. affinis* calculated using Probit Analysis (95% confidence intervals are indicated in parentheses)

Chemicals	96hr-LC10	96hr-LC50
Total Ammonia (mg/L; pH8.1)	7.01 (5.50, 8.71)	10.97 (9.76, 11.96)
Unionized Ammonia (mg/L; pH8.1)	0.46 (0.35, 0.55)	0.78 (0.68, 0.86)
Total Ammonia (mg/L; pH7.6)	5.02 (1.42, 6.85)	7.56 (4.07, 8.95)
Unionized Ammonia (mg/L; pH7.6)	0.08 (0.02, 0.11)	0.12 (0.06, 0.14)
Total Ammonia (mg/L; pH7.2)	1.82 (0, 2.79)	10.93 (7.34,49.0)
Unionized Ammonia (mg/L; pH7.2)	0.011 (0.0, 0.017)	0.068 (0.046, 0.306)
Bifenthrin (ng/L; pptr)	2.76 (1.27, 4.43)	11.37 (8.04, 14.80)
Chlorpyrifos (ng/L; pptr)	384.49 (211.81, 515.58)	803.20 (640.17, 926.41)
Copper (µg/L; ppb)	1.42 (0.61, 1.45)	3.48 (2.85, 4.15)
Cyfluthrin (ng/L; pptr)	1.40 (0.05, 2.89)	12.72 (8.05, 55.55)
Permethrin (ng/L; pptr)	83.37 (38.71, 110.83)	158.08 (125.55, 175.99

Appendix B

Hyalella azteca
Ambient Sample Toxicity
10-day Survival and Weight

Table B1-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 1/08/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 1/06/09 - 1/07/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	98	2.5	100	0.0	NS
High EC Control @ 12.46 mS/cm	98	2.5	100	0.0	NS
High EC Control @ 19.42 mS/cm	98	2.5	98	2.5	NS
Suisun Slough at Rush Ranch[3]	100	0.0	100	0.0	NS
Rough and Ready DWR station, Stockton	100	0.0	100	0.0	NS
Sacramento River at Hood DWR Station	98	2.5	100	0.0	NS
Napa River at River Park Blvd.[4]	100	0.0	100	0.0	NS
Suisun Bay off Chipps Island (508)[3]	100	0.0	100	0.0	NS
Grizzly Bay at Dolphin (602)[4]	98	2.5	93	4.8	NS
Field Dup.: Grizzly Bay at Dolphin (602)[4]	100	0.0	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.042	0.006	0.076	0.009	S (181%)*
High EC Control @ 12.46 mS/cm	0.037	0.005	0.061	0.007	S (165%)*
High EC Control @ 19.42 mS/cm	0.065	0.003	0.038	0.013	NS
Suisun Slough at Rush Ranch[3]	0.105	0.011	0.078	0.010	NS
Rough and Ready DWR station, Stockton	0.117	0.006	0.064	0.015	S (55%)*
Sacramento River at Hood DWR Station	0.100	0.004	0.088	0.005	NS
Napa River at River Park Blvd.[4]	0.032	0.007	0.041	0.005	NS
Suisun Bay off Chipps Island (508)[3]	0.066	0.006	0.106	0.008	S (161%)**
Grizzly Bay at Dolphin (602)[4]	0.028†	0.006	0.069	0.008	S (246%)**
Field Dup.: Grizzly Bay at Dolphin (602)[4]	0.047	0.014	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.
   *: $P < 0.05$
   **: $P < 0.01$
2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.
3. These high conductivity samples were compared to the High EC Control @ 12.46 mS/cm.
4. These high conductivity samples were compared to the High EC Control @ 19.42 mS/cm.

†. This treatment showed lower weight compared to the High EC Control, but not compared to the normal EC Control.

Table B1-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 1/06/09 - 1/07/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (°C)	pH	DO (mg/L)			
Suisun Slough at Rush Ranch	11140	8.5	7.43	11.9	23.8	0.23	0.001
Rough and Ready DWR station, Stockton	983	8.2	7.25	11.2	5.6	0.12	0.000
Sacramento River at Hood DWR Station	216	8.2	7.25	11.3	13.0	0.56	0.002
Napa River at River Park Blvd.	18370	9.6	7.24	11.4	38.6	0.23	0.001
Suisun Bay off Chipps Island (508)	12330	8.3	7.37	10.2	13.1	0.31	0.001
Grizzly Bay at Dolphin (602)	19800	8.6	7.58	11.5	13.9	0.29	0.001
Field Dup.: Grizzly Bay at Dolphin (602)	19800	8.6	7.58	11.5	13.0	0.30	0.001

Table B1-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 1/08/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 1/06/09 - 1/07/09.

Treatment	Laboratory Chemistry							Hardness	Alkalinity	Unionized
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH	(mg/L as $CaCO_3$)	(mg/L as $CaCO_3$)	Ammonia (mg/L)[1]
DIEPAMHR	355	21.0	23.4	7.2	8.7	7.80	7.97	104	62	-
High EC Control @ 12.46 mS/cm	11840	21.4	24.0	7.2	8.5	7.75	7.82	1380	74	-
High EC Control @ 19.42 mS/cm	17925	21.1	24.1	6.6	8.4	7.74	7.83	2200	83	-
Suisun Slough at Rush Ranch	10520	20.7	23.9	6.4	8.4	7.75	8.14	1320	164	0.004
Rough and Ready DWR station, Stockton	988	20.9	23.4	7.1	8.9	7.97	8.15	200	118	0.004
Sacramento River at Hood DWR Station	262	21.0	23.8	7.5	8.7	7.84	8.02	80	88	0.017
Napa River at River Park Blvd.	17400	20.9	24.1	7.6	8.4	7.62	7.91	2160	116	0.003
Suisun Bay off Chipps Island (508)	11545	20.8	23.4	7.6	8.5	7.74	7.93	1380	96	0.005
Grizzly Bay at Dolphin (602)	18230	20.5	23.7	6.7	8.4	7.72	7.86	2280	102	0.004
Field Dup.: Grizzly Bay at Dolphin (602)	18120	20.7	23.6	7.0	8.3	7.74	7.87	2180	102	0.005
DIEPAMHR + 25 ppb PBO	395	20.8	22.7	7.3	8.5	7.80	8.02	-	-	-
High EC Control @ 12.46 mS/cm + 25 ppb PBO	11485	20.5	22.7	7.3	8.5	7.76	7.82	-	-	-
High EC Control @ 19.42 mS/cm + 25 ppb PBO	18055	20.7	23.1	6.6	8.3	7.74	7.82	-	-	-
Suisun Slough at Rush Ranch + 25 ppb PBO	10140	20.4	22.6	7.5	8.4	7.70	8.14	-	-	-
Rough and Ready DWR station, Stockton + 25 ppb PBO	978	20.6	22.8	7.7	8.7	7.98	8.15	-	-	-
Sacramento River at Hood DWR Station + 25 ppb PBO	262	20.5	22.9	7.5	8.7	7.85	8.02	-	-	-
Napa River at River Park Blvd. + 25 ppb PBO	17070	20.5	22.7	7.6	8.5	7.66	7.89	-	-	-
Suisun Bay off Chipps Island (508) + 25 ppb PBO	11135	20.2	22.4	7.0	8.6	7.76	7.92	-	-	-
Grizzly Bay at Dolphin (602) + 25 ppb PBO	18165	21.1	22.6	6.8	8.5	7.75	7.87	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B2-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 1/09/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 1/08/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	100	0.0	100	0.0	NS
Sacramento R. Deep Water Channel, Light 55	97	2.8	100	0.0	NS
Sacramento River at tip of Grand Island (711)	100	0.0	98	2.5	NS
Upper Cache Slough at mouth of Ulatis Creek	100	0.0	100	0.0	NS
Confluence of Lindsey Sl. And Cache Sl.	98	2.5	100	0.0	NS
San Joaquin River at Potato Slough (815)	100	0.0	100	0.0	NS
Old River, western arm at railroad bridge (902)	100	0.0	98	2.5	NS
Old River at mouth of Holland Cut (915)	100	0.0	100	0.0	NS
Trip Blank	100	0.0	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.069	0.002	0.056	0.008	NS
Sacramento R. Deep Water Channel, Light 55	0.105	0.008	0.106	0.008	NS
Sacramento River at tip of Grand Island (711)	0.085	0.012	0.097	0.014	NS
Upper Cache Slough at mouth of Ulatis Creek	0.124	0.008	0.107	0.004	NS
Confluence of Lindsey Sl. And Cache Sl.	0.111	0.010	0.096	0.010	NS
San Joaquin River at Potato Slough (815)	0.130	0.011	0.126	0.018	NS
Old River, western arm at railroad bridge (902)	0.129	0.007	0.129	0.006	NS
Old River at mouth of Holland Cut (915)	0.125	0.011	0.119	0.002	NS
Trip Blank	0.063	0.004	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

Table B2-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 1/08/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Sacramento R. Deep Water Channel, Light 55	297	7.9	7.52	12.5	13.0	0.31	0.002
Sacramento River at tip of Grand Island (711)	313	7.9	7.42	12.1	11.2	0.39	0.002
Upper Cache Slough at mouth of Ulatis Creek	444	7.3	7.46	12.7	15.6	0.10	0.000
Confluence of Lindsey Sl. And Cache Sl.	261	7.9	7.34	12.1	11.0	0.39	0.001
San Joaquin River at Potato Slough (815)	474	7.8	7.24	12.0	4.7	0.25	0.001
Old River, western arm at railroad bridge (902)	784	7.6	7.48	11.8	4.5	0.12	0.001
Old River at mouth of Holland Cut (915)	745	7.6	7.47	12.6	3.8	0.12	0.000
Trip Blank	363	14.9	7.94	9.6	0.1	0.00	0.000

Table B2-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 1/09/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 1/08/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	329	21.2	22.6	7.0	8.3	7.76	8.04	104	62	-
Sacramento R. Deep Water Channel, Light 55	280	21.7	22.7	7.6	8.7	7.90	8.17	104	104	0.017
Sacramento River at tip of Grand Island (711)	258	21.7	23.1	7.3	8.7	7.83	8.18	84	86	0.012
Upper Cache Slough at mouth of Ulatis Creek	438	22.4	23.2	7.2	8.6	8.10	8.29	144	132	0.007
Confluence of Lindsey Sl. And Cache Sl.	245	21.1	22.7	7.4	8.5	7.85	8.11	94	97	0.021
San Joaquin River at Potato Slough (815)	433	22.5	22.8	6.9	8.6	7.79	8.15	100	84	0.013
Old River, western arm at railroad bridge (902)	715	21.9	22.5	7.1	8.6	7.91	8.06	136	88	0.005
Old River at mouth of Holland Cut (915)	691	22.2	22.6	7.5	8.4	7.90	8.11	132	85	0.006
Trip Blank	336	22.7	22.8	7.1	8.4	7.75	8.07	108	58	0.000
DIEPAMHR + 25 ppb PBO	340	22.7	22.7	7.6	8.2	7.76	8.08	-	-	-
Sacramento R. Deep Water Channel, Light 55 + 25 ppb PBO	289	22.4	23.0	7.5	8.3	7.90	8.15	-	-	-
Sacramento River at tip of Grand Island (711) + 25 ppb PBO	278	22.7	22.9	7.5	8.5	7.80	8.11	-	-	-
Upper Cache Slough at mouth of Ulatis Creek + 25 ppb PBO	448	22.7	22.9	6.8	8.8	8.05	8.27	-	-	-
Confluence of Lindsey Sl. And Cache Sl. + 25 ppb PBO	256	23.1	23.2	7.3	8.6	7.80	8.13	-	-	-
San Joaquin River at Potato Slough (815) + 25 ppb PBO	460	22.9	22.9	7.3	8.5	7.82	8.22	-	-	-
Old River, western arm at railroad bridge (902) + 25 ppb PBO	756	23.2	23.2	7.4	8.6	7.89	8.06	-	-	-
Old River at mouth of Holland Cut (915) + 25 ppb PBO	733	22.6	23.1	7.4	8.6	7.89	8.08	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B3-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 1/22/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 1/20/09 - 1/21/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	100	0.0	100	0.0	NS
High EC Control @ 12.68 mS/cm	100	0.0	98	2.5	NS
High EC Control @ 20.85 mS/cm	92	4.8	78*	4.8	NS
Suisun Slough at Rush Ranch[3]	100	0.0	100	0.0	NS
Rough and Ready DWR station, Stockton	98	2.5	100	0.0	NS
Napa River at River Park Blvd.[4]	100	0.0	100	0.0	NS
Suisun Bay off Chipps Island (508)[3]	100	0.0	100	0.0	NS
Grizzly Bay at Dolphin (602)[4]	100	0.0	97	2.8	NS
Montezuma Slough at Nurse Slough (609)	100	0.0	100	0.0	NS
Bottle Blank	100	0.0	-	-	NS

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.075	0.008	0.067	0.009	NS
High EC Control @ 12.68 mS/cm	0.054*	0.003	0.040	0.005	S* (74%)
High EC Control @ 20.85 mS/cm	0.043**	0.003	0.057	0.004	S* (133%)
Suisun Slough at Rush Ranch[3]	0.118	0.003	0.121	0.008	NS
Rough and Ready DWR station, Stockton	0.087	0.011	0.115	0.007	NS
Napa River at River Park Blvd.[4]	0.068	0.009	0.080	0.003	NS
Suisun Bay off Chipps Island (508)[3]	0.045	0.006	0.073	0.004	S** (167%)
Grizzly Bay at Dolphin (602)[4]	0.040	0.005	0.060	0.001	S** (150%)
Montezuma Slough at Nurse Slough (609)	0.110	0.008	0.123	0.005	NS
Bottle Blank	0.062	0.005	-	-	NS

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.

   *: $P < 0.05$

   **: $P < 0.01$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.
3. These high conductivity samples were compared to the High EC Control @ 12.68 mS/cm.
4. These high conductivity samples were compared to the High EC Control @ 20.85 mS/cm.

Table B3-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 1/20/09 - 1/21/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Suisun Slough at Rush Ranch	11780	10.3	7.24	9.8	20.5	0.17	0.000
Rough and Ready DWR station, Stockton	1022	9.8	7.31	11.3	2.4	0.09	0.000
Napa River at River Park Blvd.	20870	11.9	7.46	10.4	36.7	0.11	0.000
Suisun Bay off Chipps Island (508)	12440	9.3	7.65	11.4	9.1	0.24	0.001
Grizzly Bay at Dolphin (602)	19140	9.6	7.69	11.7	8.3	0.22	0.001
Montezuma Slough at Nurse Slough (609)	7870	9.5	7.4	11.9	24.4	0.25	0.001
Bottle Blank	-	-	-	-	0.4	0.01	-

Table B3-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 1/22/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 1/20/09 - 1/21/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (^oC)	Max Temp (^oC)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	347	19.5	23.6	7.3	8.3	7.85	8.11	100	60	-
High EC Control @ 12.68 mS/cm	11945	19.3	23.5	7.1	8.8	7.74	7.98	1400	74	-
High EC Control @ 20.85 mS/cm	20050	20.3	23.5	7.2	8.3	7.73	7.98	2360	86	-
Suisun Slough at Rush Ranch	11210	20.4	23.2	7.2	8.6	7.60	8.23	1360	152	0.002
Rough and Ready DWR station, Stockton	1072	20.9	23.6	7.1	8.3	8.03	8.18	204	116	0.004
Napa River at River Park Blvd.	20080	21.0	23.6	6.8	8.5	7.63	8.00	2360	122	0.002
Suisun Bay off Chipps Island (508)	11900	20.0	23.5	6.9	8.5	7.72	8.02	1440	96	0.004
Grizzly Bay at Dolphin (602)	18730	21.5	23.7	7.0	8.3	7.81	7.94	2280	102	0.005
Montezuma Slough at Nurse Slough (609)	7660	21.0	23.7	6.9	8.5	7.77	8.02	880	96	0.005
Bottle Blank	367	20.8	23.9	7.1	8.9	7.83	8.09	108	58	0.001
DIEPAMHR + 25 ppb PBO	365	21.2	22.9	7.2	8.3	7.86	8.03	-	-	-
High EC Control @ 12.68 mS/cm + 25 ppb PBO	12215	21.9	22.9	7.1	8.2	7.74	7.95	-	-	-
High EC Control @ 20.85 mS/cm + 25 ppb PBO	20285	21.3	23.3	7.0	8.2	7.76	7.93	-	-	-
Suisun Slough at Rush Ranch + 25 ppb PBO	11330	21.0	23.3	6.8	8.3	7.66	8.15	-	-	-
Rough and Ready DWR station, Stockton + 25 ppb PBO	1066	21.9	23.4	6.9	8.7	8.10	8.24	-	-	-
Napa River at River Park Blvd. + 25 ppb PBO	20315	21.6	23.3	6.8	8.0	7.59	7.97	-	-	-
Suisun Bay off Chipps Island (508) + 25 ppb PBO	11820	21.3	23.4	7.0	8.4	7.84	7.99	-	-	-
Grizzly Bay at Dolphin (602) + 25 ppb PBO	18775	22.0	23.3	7.1	8.0	7.71	7.95	-	-	-
Montezuma Slough at Nurse Slough (609) + 25 ppb PBO	7750	21.4	23.6	6.9	8.3	7.88	8.05	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B4-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 1/23/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 1/22/09 - 1/23/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	92	4.8	95	3.1	NS
Low EC Control @ 136.5 uS/cm	98	2.3	97	2.8	NS
Confluence of Lindsey Sl. And Cache Sl.	98	2.5	100	0.0	NS
Old River at mouth of Holland Cut (915)	98	2.5	100	0.0	NS
San Joaquin River at Potato Slough (815)	98	2.5	100	0.0	NS
Upper Cache Slough at mouth of Ulatis Creek	98	2.5	100	0.0	NS
Sacramento R. Deep Water Channel, Light 55	95	2.9	100	0.0	NS
Old River, western arm at railroad bridge (902)	100	0.0	100	0.0	NS
Sacramento River at tip of Grand Island (711)	100	0.0	100	0.0	NS
Sacramento River at Hood DWR Station[3]	98	2.5	100	0.0	NS
Field Dup.: Sacramento River at tip of Grand Island (711)	98	2.5	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.075	0.006	0.053	0.009	NS
Low EC Control @ 136.5 uS/cm	0.112	0.005	0.067	0.007	S** (60%)
Confluence of Lindsey Sl. And Cache Sl.	0.115	0.018	0.105	0.013	NS
Old River at mouth of Holland Cut (915)	0.084	0.016	0.127	0.005	S* (151%)
San Joaquin River at Potato Slough (815)	0.122	0.006	0.126	0.006	NS
Upper Cache Slough at mouth of Ulatis Creek	0.125	0.012	0.135	0.012	NS
Sacramento R. Deep Water Channel, Light 55	0.115	0.005	0.083	0.013	NS
Old River, western arm at railroad bridge (902)	0.127	0.006	0.075	0.008	S** (59%)
Sacramento River at tip of Grand Island (711)	0.107	0.006	0.078	0.008	S* (73%)
Sacramento River at Hood DWR Station[3]	0.134	0.008	0.093	0.013	S* (69%)
Field Dup.: Sacramento River at tip of Grand Island (711)	0.098	0.009	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.
   *: $P < 0.05$
   **: $P < 0.01$
2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.
3. This low conductivity sample was compared to the Low EC Control.

Table B4-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 1/22/09 - 1/23/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (°C)	pH	DO (mg/L)			
Confluence of Lindsey Sl. And Cache Sl.	271	10.2	7.61	11.3	6.4	0.35	0.003
Old River at mouth of Holland Cut (915)	741	9.5	7.51	11.5	4.0	0.04	0.000
San Joaquin River at Potato Slough (815)	392	9.5	7.42	11.4	5.1	0.18	0.001
Upper Cache Slough at mouth of Ulatis Creek	566	9.8	7.95	11.5	9.1	0.00	0.000
Sacramento R. Deep Water Channel, Light 55	303	10.0	7.90	11.2	14.1	0.25	0.003
Old River, western arm at railroad bridge (902)	830	9.4	7.52	11.4	4.2	0.05	0.000
Sacramento River at tip of Grand Island (711)	266	10.1	7.51	11.0	4.3	0.45	0.003
Sacramento River at Hood DWR Station	207	11.0	7.49	10.4	8.9	0.49	0.003
Field Dup.: Sacramento River at tip of Grand Island (711)	266	10.1	7.51	11.0	4.4	0.39	0.002

Table B4-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 1/23/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 1/22/09 - 1/23/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (^oC)	Max Temp (^oC)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	356	22.6	23.4	7.1	8.6	7.84	8.04	100	60	-
Low EC Control @ 136.5 uS/cm	154	22.5	23.8	7.3	8.8	7.45	7.87	44	24	-
Confluence of Lindsey Sl. And Cache Sl.	278	22.7	23.4	6.8	8.9	8.00	8.14	92	99	0.020
Old River at mouth of Holland Cut (915)	742	22.7	23.7	7.0	8.9	7.95	8.08	132	98	0.002
San Joaquin River at Potato Slough (815)	557	22.8	23.2	6.8	8.9	7.88	8.04	116	88	0.006
Upper Cache Slough at mouth of Ulatis Creek	569	22.8	23.0	7.1	8.9	8.07	8.34	168	150	0.000
Sacramento R. Deep Water Channel, Light 55	318	22.7	23.4	6.9	8.8	8.00	8.11	92	104	0.011
Old River, western arm at railroad bridge (902)	836	22.8	23.7	6.9	8.8	7.96	8.08	140	88	0.002
Sacramento River at tip of Grand Island (711)	258	22.8	23.7	6.7	8.9	7.84	8.00	84	88	0.014
Sacramento River at Hood DWR Station	217	22.8	23.5	6.9	8.7	7.60	8.01	72	78	0.009
Field Dup.: Sacramento River at tip of Grand Island (711)	278	22.8	23.6	6.8	8.6	7.90	8.05	84	81	0.020
DIEPAMHR + 25 ppb PBO	345	22.8	23.0	7.0	8.4	7.81	8.01	-	-	-
Low EC Control @ 136.5 uS/cm + 25 ppb PBO	156	22.8	23.5	7.1	8.7	7.49	7.86	-	-	-
Confluence of Lindsey Sl. And Cache Sl. + 25 ppb PBO	486	22.7	22.9	6.8	8.8	7.95	8.17	-	-	-
Old River at mouth of Holland Cut (915) + 25 ppb PBO	736	22.8	22.9	7.1	8.9	7.98	8.09	-	-	-
San Joaquin River at Potato Slough (815) + 25 ppb PBO	567	22.9	22.9	6.9	8.7	7.89	8.02	-	-	-
Upper Cache Slough at mouth of Ulatis Creek + 25 ppb PBO	559	22.2	22.8	6.9	8.6	8.09	8.34	-	-	-
Sacramento R. Deep Water Channel, Light 55 + 25 ppb PBO	318	22.5	22.8	6.9	8.5	8.02	8.17	-	-	-
Old River, western arm at railroad bridge (902) + 25 ppb PBO	828	22.3	22.8	7.7	8.5	7.95	8.07	-	-	-
Sacramento River at tip of Grand Island (711) + 25 ppb PBO	247	22.6	22.9	6.9	8.6	7.93	8.07	-	-	-
Sacramento River at Hood DWR Station + 25 ppb PBO	230	22.4	22.9	6.8	8.7	7.59	8.02	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B5-1a. Summary of 10-day *H. azteca* water column toxicity test initiated on 2/05/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 2/03/09 - 2/04/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	95	3.1	98	2.3	NS
High EC Control @ 10.21 mS/cm	100	0.0	100	0.0	NS
High EC Control @ 20.48 mS/cm	69**	6.8	83*	3.6	NS
Napa River at River Park Blvd.[4]	98	2.5	95	2.8	NS
Sacramento River at Hood DWR Station	95	2.9	97	2.8	NS
Suisun Slough at Rush Ranch[3]	100	0.0	100	0.0	NS
Rough and Ready DWR station, Stockton	100	0.0	98	2.5	NS
San Joaquin River at Potato Slough (815)	98	2.5	100	0.0	NS
Sacramento River at tip of Grand Island (711)	100	0.0	100	0.0	NS
Upper Cache Slough at mouth of Ulatis Creek	100	0.0	100	0.0	NS
Old River, western arm at railroad bridge (902)	95	2.9	98	2.5	NS
Confluence of Lindsey Sl. And Cache Sl.	100	0.0	98	2.5	NS
Old River at mouth of Holland Cut (915)	100	0.0	100	0.0	NS
Sacramento R. Deep Water Channel, Light 55	100	0.0	100	0.0	NS
Field Dup.: Sacramento R. Deep Water Channel, Light 55	100	0.0	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.
   *: $P < 0.05$
   **: $P < 0.01$
   ***: $P < 0.001$
2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.
3. This high conductivity sample was compared to the High EC Control @ 10.21 mS/cm
4. This high conductivity sample was compared to the High EC Control @ 20.48 mS/cm

†. The mean weight of animals exposed to the Field Duplicate of the Light 55 site was significantly lower than that of animals exposed to the original sample.

Table B5-1b. Summary of 10-day *H. azteca* water column toxicity test initiated on 2/05/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 2/03/09 - 2/04/09.

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.066	0.009	0.052	0.003	NS
High EC Control @ 10.21 mS/cm	0.057	0.004	0.028*	0.008	S* (49%)
High EC Control @ 20.48 mS/cm	0.064	0.008	0.025***	0.002	S** (39%)
Napa River at River Park Blvd.[4]	0.066	0.007	0.022	0.012	S* (33%)
Sacramento River at Hood DWR Station	0.108	0.012	0.069	0.012	NS
Suisun Slough at Rush Ranch[3]	0.068	0.006	0.051	0.004	NS
Rough and Ready DWR station, Stockton	0.088	0.001	0.092	0.005	NS
San Joaquin River at Potato Slough (815)	0.117	0.012	0.087	0.006	NS
Sacramento River at tip of Grand Island (711)	0.104	0.005	0.087	0.014	NS
Upper Cache Slough at mouth of Ulatis Creek	0.121	0.011	0.063	0.005	S** (52%)
Old River, western arm at railroad bridge (902)	0.119	0.014	0.044	0.012	S** (37%)
Confluence of Lindsey Sl. And Cache Sl.	0.105	0.004	0.060	0.002	S*** (57%)
Old River at mouth of Holland Cut (915)	0.097	0.014	0.062	0.006	NS
Sacramento R. Deep Water Channel, Light 55	0.079	0.005	0.050	0.005	S** (63%)
Field Dup.: Sacramento R. Deep Water Channel, Light 55 †	0.051	0.005	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.
   *: $P < 0.05$
   **: $P < 0.01$
   ***: $P < 0.001$
2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.
3. This high conductivity sample was compared to the High EC Control @ 10.21 mS/cm
4. This high conductivity sample was compared to the High EC Control @ 20.48 mS/cm

†. The mean weight of animals exposed to the Field Duplicate of the Light 55 site was significantly lower than that of animals exposed to the original sample.

Table B5-2. Water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 2/03/09 - 2/04/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Napa River at River Park Blvd.	20180	12.6	7.48	11.2	8.1	0.11	0.001
Sacramento River at Hood DWR Station	303	10.6	7.43	10.5	12.8	0.44	0.002
Suisun Slough at Rush Ranch	10090	11.5	7.45	11.1	24.0	0.10	0.000
Rough and Ready DWR station, Stockton	1045	11.0	7.89	11.3	3.7	0.05	0.001
San Joaquin River at Potato Slough (815)	572	10.5	7.56	11.4	4.0	0.16	0.001
Sacramento River at tip of Grand Island (711)	417	10.9	7.68	10.8	5.3	0.26	0.002
Upper Cache Slough at mouth of Ulatis Creek	490	10.7	8.40	13.8	9.1	0.00	0.000
Old River, western arm at railroad bridge (902)	723	10.9	7.60	11.1	3.8	0.06	0.000
Confluence of Lindsey Sl. And Cache Sl.	260	10.7	7.53	10.9	7.1	0.35	0.002
Old River at mouth of Holland Cut (915)	664	10.7	7.63	11.1	3.1	0.02	0.000
Sacramento R. Deep Water Channel, Light 55	278	10.6	7.57	11.5	8.2	0.33	0.002
Field Dup.: Sacramento R. Deep Water Channel, Light 55	278	10.6	7.57	11.5	7.6	0.38	0.003

Table B5-3. Water chemistry during a *H. azteca* initial screening toxicity test initiated on 2/05/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 2/03/09 - 2/04/09.

Treatment	Laboratory Chemistry							Hardness	Alkalinity	Unionized
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH	(mg/L as $CaCO_3$)	(mg/L as $CaCO_3$)	Ammonia (mg/L)[1]
DIEPAMHR	335	20.0	23.1	7.5	8.5	7.76	8.07	108	60	-
High EC Control @ 10.21 mS/cm	9570	19.7	23.5	7.6	8.6	7.80	8.08	1160	74	-
High EC Control @ 20.48 mS/cm	19625	20.5	23.3	7.4	8.1	7.79	8.04	2400	88	-
Napa River at River Park Blvd.	18730	20.9	23.3	7.0	8.1	7.72	8.19	2360	126	0.002
Sacramento River at Hood DWR Station	249	20.6	23.5	7.4	8.3	7.97	8.15	80	92	0.019
Suisun Slough at Rush Ranch	9445	20.4	23.4	7.3	8.3	7.77	8.37	1200	164	0.002
Rough and Ready DWR station, Stockton	967	21.1	23.2	7.4	8.3	8.09	8.32	212	116	0.003
San Joaquin River at Potato Slough (815)	543	20.1	23.3	7.6	8.4	7.80	8.26	124	94	0.004
Sacramento River at tip of Grand Island (711)	376	20.4	23.3	7.4	8.3	8.03	8.27	100	96	0.013
Upper Cache Slough at mouth of Ulatis Creek	474	20.8	23.3	7.5	8.5	8.23	8.55	148	146	0.000
Old River, western arm at railroad bridge (902)	707	21.2	23.4	7.7	8.6	7.90	8.21	140	92	0.002
Confluence of Lindsey Sl. And Cache Sl.	261	21.1	23.1	7.5	8.6	8.00	8.22	96	100	0.021
Old River at mouth of Holland Cut (915)	680	21.3	23.4	7.6	8.4	7.96	8.26	140	92	0.001
Sacramento R. Deep Water Channel, Light 55	275	21.1	23.4	7.5	8.6	8.07	8.90	104	112	0.021
Field Dup.: Sacramento R. Deep Water Channel, Light 55	268	21.2	23.4	7.2	8.3	8.02	8.29	96	104	0.018
DIEPAMHR + 25 ppb PBO	342	21.1	23.0	7.6	8.6	7.79	8.12	-	-	-
High EC Control @ 10.21 mS/cm + 25 ppb PBO	9685	21.4	23.2	7.6	8.3	7.80	8.05	-	-	-
High EC Control @ 20.48 mS/cm + 25 ppb PBO	19620	21.1	23.3	7.4	7.9	7.81	8.04	-	-	-
Napa River at River Park Blvd. + 25 ppb PBO	18885	21.3	23.4	7.2	8.1	7.81	8.19	-	-	-
Sacramento River at Hood DWR Station + 25 ppb PBO	236.6	21.1	23.3	7.4	8.5	7.94	8.19	-	-	-
Suisun Slough at Rush Ranch + 25 ppb PBO	9530	21.7	23.1	7.5	8.4	7.84	8.32	-	-	-
Rough and Ready DWR station, Stockton + 25 ppb PBO	1011	20.9	23.1	7.6	8.6	8.08	8.36	-	-	-
San Joaquin River at Potato Slough (815) + 25 ppb PBO	549	21.5	23.0	7.6	8.8	7.98	8.22	-	-	-
Sacramento River at tip of Grand Island (711) + 25 ppb PBO	376.9	21.3	23.5	7.4	8.8	7.96	8.22	-	-	-
Upper Cache Slough at mouth of Ulatis Creek + 25 ppb PBO	487.7	21.6	23.1	7.5	8.4	8.23	8.54	-	-	-
Old River, western arm at railroad bridge (902) + 25 ppb PBO	716	21.4	22.8	7.6	8.6	7.95	8.22	-	-	-
Confluence of Lindsey Sl. And Cache Sl. + 25 ppb PBO	266.3	21.3	23.7	7.4	8.7	8.00	8.21	-	-	-
Old River at mouth of Holland Cut (915) + 25 ppb PBO	709	21.4	23.7	7.3	8.3	8.03	8.27	-	-	-
Sacramento R. Deep Water Channel, Light 55 + 25 ppb PBO	284.5	21.4	23.8	7.3	8.6	8.00	8.30	-	-	-

Table B6-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 2/06/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 2/05/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	98	2.5	95	2.9	NS
High EC Control @ 13.57 mS/cm	98	2.5	97	2.8	NS
High EC Control @ 19.22 mS/cm	80	9.1	79	9.4	NS
Suisun Bay off Chipps Island (508)[3]	100	0.0	97	3.1	NS
Montezuma Slough at Nurse Slough (609)	100	0.0	98	2.5	NS
Grizzly Bay at Dolphin (602)[4]	94	3.4	98	2.5	NS
Trip Blank	98	2.5	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.046	0.004	0.027	0.006	S* (59%)
High EC Control @ 13.57 mS/cm	0.029*	0.006	0.034	0.004	NS
High EC Control @ 19.22 mS/cm	0.025**	0.005	0.035	0.011	NS
Suisun Bay off Chipps Island (508)[3]	0.028	0.004	0.046	0.003	S* (164%)
Montezuma Slough at Nurse Slough (609)	0.042	0.007	0.038	0.009	NS
Grizzly Bay at Dolphin (602)[4]	0.039	0.009	0.034	0.007	NS
Trip Blank	0.049	0.005	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.
   *: $P < 0.05$
   **: $P < 0.01$
2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.
3. This high conductivity sample was compared to the High EC Control @ 13.57 mS/cm.
4. This high conductivity sample was compared to the High EC Control @ 19.22 mS/cm.

Table B6-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 2/05/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^{o}C)	pH	DO (mg/L)			
Suisun Bay off Chipps Island (508)	12810	10.6	7.7	10.8	18.4	0.25	0.002
Montezuma Slough at Nurse Slough (609)	5140	10.7	7.55	10.8	29.0	0.27	0.001
Grizzly Bay at Dolphin (602)	17210	10.8	7.79	11.0	13.9	0.21	0.002
Trip Blank	345	16.1	8.01	10.1	0.3	0.00	0.000

Table B6-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 2/06/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 2/05/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	327	22.1	24.1	7.7	8.2	7.83	8.09	108	60	-
High EC Control @ 13.57 mS/cm	13030	19.9	23.9	7.4	8.6	7.77	7.84	1760	80	-
High EC Control @ 19.22 mS/cm	18995	22.5	23.9	7.2	8.1	7.77	7.84	2280	86	-
Suisun Bay off Chipps Island (508)	13075	22.2	23.1	7.4	8.2	7.73	7.95	1800	106	0.005
Montezuma Slough at Nurse Slough (609)	5030	22.4	23.7	7.4	8.1	7.83	8.02	680	100	0.007
Grizzly Bay at Dolphin (602)	17090	21.8	23.0	7.0	8.1	7.71	7.94	2200	106	0.003
Trip Blank	355	22.5	23.9	7.6	8.6	7.80	8.15	108	48	0.000
DIEPAMHR + 25 ppb PBO	327	22.1	22.5	7.1	8.5	7.78	8.06	-	-	-
High EC Control @ 13.57 mS/cm + 25 ppb PBO	13090	22.1	23.3	7.4	8.2	7.77	7.82	-	-	-
High EC Control @ 19.22 mS/cm + 25 ppb PBO	18380	22.0	23.1	7.1	8.1	7.78	7.86	-	-	-
Suisun Bay off Chipps Island (508) + 25 ppb PBO	12365	20.9	23.6	7.2	8.5	7.77	7.96	-	-	-
Montezuma Slough at Nurse Slough (609) + 25 ppb PBO	5006	21.5	23.2	7.6	8.2	7.91	8.00	-	-	-
Grizzly Bay at Dolphin (602) + 25 ppb PBO	16875	20.4	23.0	7.2	8.2	7.74	7.94	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B7-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 2/19/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 2/17/09 - 2/18/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	95	2.9	97	2.8	NS
High EC Control @ 12.50 mS/cm	97	2.8	92	2.7	NS
High EC Control @ 21.92 mS/cm	69*	8.1	73	13.0	NS
High EC Control @ 24.63 mS/cm	54**	10.9	32*	10.5	NS
Napa River at River Park Blvd.	94	6.3	94	6.3	NS
Sacramento River at Hood DWR Station	95	2.9	93	4.8	NS
Suisun Slough at Rush Ranch	100	0.0	100	0.0	NS
Rough and Ready DWR station, Stockton	100	0.0	95	5.0	NS
Suisun Bay off Chipps Island (508)	100	0.0	100	0.0	NS
Montezuma Slough at Nurse Slough (609)	98	2.3	100	0.0	NS
Carquinez Strait, West of Benicia army dock (405)[4]	93	2.5	98	2.5	NS
Grizzly Bay at Dolphin (602)[3]	100	0.0	100	0.0	NS
Napa River at Vallejo Seawall (340)[5]	91	6.0	88	7.5	NS
Field Dup.: Rough and Ready DWR station, Stockton	100	0.0	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.039	0.004	0.032	0.006	NS
High EC Control @ 12.50 mS/cm	0.019**	0.003	0.026	0.005	NS
High EC Control @ 21.92 mS/cm	0.008***	0.002	0.009*	0.000	NS
High EC Control @ 24.63 mS/cm	0.025*	0.002	0.041	0.021	NS
Napa River at River Park Blvd.	0.052	0.002	0.044	0.004	NS
Sacramento River at Hood DWR Station	0.051	0.004	0.060	0.004	NS
Suisun Slough at Rush Ranch	0.035	0.006	0.060	0.001	S** (171%)
Rough and Ready DWR station, Stockton	0.040	0.003	0.050	0.004	NS
Suisun Bay off Chipps Island (508)	0.046	0.009	0.045	0.008	NS
Montezuma Slough at Nurse Slough (609)	0.036	0.004	0.043	0.003	NS
Carquinez Strait, West of Benicia army dock (405)[4]	0.020	0.003	0.033	0.003	NS
Grizzly Bay at Dolphin (602)[3]	0.031	0.003	0.023	0.003	NS
Napa River at Vallejo Seawall (340)[5]	0.023	0.004	0.052	0.007	S* (226%)
Field Dup.: Rough and Ready DWR station, Stockton	0.067	0.012	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.

   *: $P < 0.05$

   **: $P < 0.01$

   ***: $P < 0.001$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

Table B7-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 2/17/09 - 2/18/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Napa River at River Park Blvd.	454	10.0	7.31	11.4	713.3	0.35	0.001
Sacramento River at Hood DWR Station	196	9.5	7.55	12.0	20.2	0.06	0.000
Suisun Slough at Rush Ranch	7310	9.9	7.47	10.4	41.4	0.08	0.000
Rough and Ready DWR station, Stockton	1107	11.0	7.93	11.2	3.7	0.43	0.006
Suisun Bay off Chipps Island (508)	6780	9.9	7.87	11.7	10.7	0.23	0.002
Montezuma Slough at Nurse Slough (609)	8000	9.8	7.65	11.4	25.0	0.21	0.001
Carquinez Strait, West of Benicia army dock (405)	23650	10.2	7.83	11.0	24.4	0.22	0.002
Grizzly Bay at Dolphin (602)	12200	10.0	7.85	11.6	13.1	0.23	0.002
Napa River at Vallejo Seawall (340)	22400	10.0	7.88	10.8	44.2	0.23	0.002
Field Dup.: Rough and Ready DWR station, Stockton	1107	11.0	7.93	11.2	3.5	0.06	0.001

Table B7-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 2/19/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 2/17/09 - 2/18/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (^oC)	Max Temp (^oC)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	335	22.6	23.8	7.5	8.0	7.80	8.10	100	62	-
High EC Control @ 12.50 mS/cm	12110	22.7	24.0	7.3	8.0	7.76	7.90	1440	70	-
High EC Control @ 21.92 mS/cm	21710	22.5	24.1	7.1	7.7	7.38	7.82	2520	84	-
High EC Control @ 24.63 mS/cm	23825	22.7	23.9	6.8	7.6	7.69	7.91	2840	86	-
Napa River at River Park Blvd.	474	22.7	24.1	7.6	8.0	7.52	7.81	72	284	0.011
Sacramento River at Hood DWR Station	229	22.7	24.3	7.2	8.0	7.82	8.10	72	78	0.004
Suisun Slough at Rush Ranch	7045	22.7	23.8	7.3	8.0	7.16	8.17	840	172	0.003
Rough and Ready DWR station, Stockton	1102	22.7	23.5	7.3	8.4	7.16	8.18	212	240	0.027
Suisun Bay off Chipps Island (508)	6310	22.6	23.4	7.3	8.4	7.87	7.95	840	100	0.007
Montezuma Slough at Nurse Slough (609)	7595	22.6	23.8	7.3	8.2	7.84	7.99	880	92	0.008
Carquinez Strait, West of Benicia army dock (405)	20210	22.7	23.5	7.0	7.7	7.70	7.92	2400	104	0.006
Grizzly Bay at Dolphin (602)	11600	22.7	24.0	7.3	8.2	7.85	7.94	1480	102	0.007
Napa River at Vallejo Seawall (340)	22665	22.6	23.3	6.9	7.7	7.75	7.83	2760	104	0.005
Field Dup.: Rough and Ready DWR station, Stockton	1091	22.7	23.8	7.5	8.4	8.05	8.29	220	120	0.005
DIEPAMHR + 25 ppb PBO	1791	22.6	22.9	7.6	8.3	7.85	8.06	-	-	-
High EC Control @ 12.50 mS/cm + 25 ppb PBO	11925	22.6	23.3	7.4	8.0	7.74	7.92	-	-	-
High EC Control @ 21.92 mS/cm + 25 ppb PBO	21195	22.6	23.5	7.0	7.8	7.69	7.89	-	-	-
High EC Control @ 24.63 mS/cm + 25 ppb PBO	23640	22.6	23.3	6.9	7.7	7.68	7.91	-	-	-
Napa River at River Park Blvd. + 25 ppb PBO	450	22.6	23.2	7.5	8.2	7.56	7.87	-	-	-
Sacramento River at Hood DWR Station + 25 ppb PBO	209	22.7	23.3	7.1	8.2	7.84	8.19	-	-	-
Suisun Slough at Rush Ranch + 25 ppb PBO	6980	22.6	23.4	7.3	8.1	7.96	8.17	-	-	-
Rough and Ready DWR station, Stockton + 25 ppb PBO	1079	22.5	23.5	7.3	8.3	8.08	8.20	-	-	-
Suisun Bay off Chipps Island (508) + 25 ppb PBO	6445	22.7	23.0	7.3	8.0	7.86	8.06	-	-	-
Montezuma Slough at Nurse Slough (609) + 25 ppb PBO	7605	22.6	23.2	7.3	8.1	7.84	7.90	-	-	-
Carquinez Strait, West of Benicia army dock (405) + 25 ppb PBO	19965	22.7	23.5	6.9	7.8	7.73	7.88	-	-	-
Grizzly Bay at Dolphin (602) + 25 ppb PBO	11825	22.6	23.2	7.3	8.2	7.84	7.95	-	-	-
Napa River at Vallejo Seawall (340) + 25 ppb PBO	23020	22.6	23.2	6.9	7.8	7.75	7.84	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B8-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 2/20/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 2/19/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	92	4.8	98	2.5	NS
Low EC Control @ 152.2 uS/cm	86	5.5	98	2.5	NS
Sacramento R. Deep Water Channel, Light 55	83	13.7	100	0.0	NS
Sacramento River at tip of Grand Island (711)[3]	95	3.1	70	23.4	NS
Old River, western arm at railroad bridge (902)	100	0.0	100	0.0	NS
San Joaquin River at Potato Slough (815)	95	2.9	100	0.0	NS
Upper Cache Slough at mouth of Ulatis Creek	98	2.5	95	3.1	NS
Old River at mouth of Holland Cut (915)	100	0.0	89	11.1	NS
Confluence of Lindsey Sl. And Cache Sl.	100	0.0	95	2.9	NS
Bottle Blank	97	2.8	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.034	0.006	0.030	0.006	NS
Low EC Control @ 152.2 uS/cm	0.042	0.003	0.034	0.003	NS
Sacramento R. Deep Water Channel, Light 55	0.084	0.005	0.094	0.015	NS
Sacramento River at tip of Grand Island (711)[3]	0.055	0.012	0.068	0.006	NS
Old River, western arm at railroad bridge (902)	0.077	0.002	0.077	0.013	NS
San Joaquin River at Potato Slough (815)	0.056	0.005	0.098	0.007	S** (175%)
Upper Cache Slough at mouth of Ulatis Creek	0.074	0.008	0.042	0.006	S* (57%)
Old River at mouth of Holland Cut (915)	0.090	0.010	0.098	0.007	NS
Confluence of Linsey Sl. And Cache Sl.	0.085	0.010	0.143	0.060	NS
Bottle Blank	0.035	0.002	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.

   *: $P < 0.05$

   **: $P < 0.01$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

3. This low conductivity sample was compared to the Low EC Control.

Table B8-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 2/19/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Sacramento R. Deep Water Channel, Light 55	385	9.5	8.03	11.5	17.4	0.19	0.003
Sacramento River at tip of Grand Island (711)	145	8.9	7.38	11.0	82.8	0.33	0.001
Old River, western arm at railroad bridge (902)	590	10.5	7.88	11.3	5.1	0.09	0.001
San Joaquin River at Potato Slough (815)	354	10.5	7.74	11.0	5.6	0.25	0.002
Upper Cache Slough at mouth of Ulatis Creek	377	9.7	7.81	10.0	138.3	0.23	0.002
Old River at mouth of Holland Cut (915)	628	10.1	7.8	10.6	4.4	0.08	0.001
Confluence of Lindsey Sl. And Cache Sl.	300	9.3	7.82	11.0	30.8	0.23	0.002
Bottle Blank	-	-	-	-	0.2	0.00	-

Table B8-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 2/20/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 2/19/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	326	21.1	23.9	7.6	8.0	7.78	8.04	100	62	-
Low EC Control @ 152.2 uS/cm	149	20.8	23.6	7.6	8.4	7.46	7.81	44	26	-
Sacramento R. Deep Water Channel, Light 55	365	22.2	23.4	7.3	8.2	8.05	8.28	120	104	0.015
Sacramento River at tip of Grand Island (711)	143	22.2	23.8	7.5	8.2	7.62	7.80	60	52	0.010
Old River, western arm at railroad bridge (902)	557	22.3	23.9	7.3	8.4	7.91	8.00	124	90	0.004
San Joaquin River at Potato Slough (815)	340	22.2	23.6	7.4	8.4	7.83	8.00	100	86	0.008
Upper Cache Slough at mouth of Ulatis Creek	369	22.2	23.7	7.3	8.3	7.92	8.12	124	100	0.012
Old River at mouth of Holland Cut (915)	604	22.0	23.5	7.5	8.2	7.92	8.05	128	90	0.003
Confluence of Lindsey Sl. And Cache Sl.	292	22.4	23.6	7.5	8.3	7.96	8.09	100	108	0.010
Bottle Blank	338	22.5	23.5	7.5	8.2	7.77	8.02	104	58	0.000
DIEPAMHR + 25 ppb PBO	330	22.4	22.4	7.4	8.2	7.78	8.01	-	-	-
Low EC Control @ 152.2 uS/cm + 25 ppb PBO	149	22.3	22.4	7.4	8.1	7.48	7.81	-	-	-
Sacramento R. Deep Water Channel, Light 55 + 25 ppb PBO	362	22.2	22.4	7.4	8.3	8.08	8.17	-	-	-
Sacramento River at tip of Grand Island (711) + 25 ppb PBO	141	22.3	22.3	7.4	8.3	7.71	7.82	-	-	-
Old River, western arm at railroad bridge (902) + 25 ppb PBO	556	22.1	22.1	7.3	8.2	7.95	8.03	-	-	-
San Joaquin River at Potato Slough (815) + 25 ppb PBO	336	21.9	22.0	7.4	8.3	7.84	8.02	-	-	-
Upper Cache Slough at mouth of Ulatis Creek + 25 ppb PBO	359	21.9	22.1	7.4	8.0	7.91	8.09	-	-	-
Old River at mouth of Holland Cut (915) + 25 ppb PBO	590	21.5	22.3	7.5	8.1	7.92	8.05	-	-	-
Confluence of Lindsey Sl. And Cache Sl. + 25 ppb PBO	289	21.7	22.1	7.4	8.4	7.95	8.08	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B9-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 3/05/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/03/09 - 3/04/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	95	2.9	98	2.5	NS
Napa River at River Park Blvd.	98	2.5	98	2.5	NS
Sacramento River at Hood DWR Station	100	0.0	98	2.3	NS
Suisun Slough at Rush Ranch	100	0.0	98	2.5	NS
Rough and Ready DWR station, Stockton	98	2.5	93	4.8	NS
Suisun Bay off Chipps Island (508)	100	0.0	100	0.0	NS
Montezuma Slough at Nurse Slough (609)	100	0.0	100	0.0	NS
Carquinez Strait, West of Benicia army dock (405)	98	2.5	98	2.5	NS
Grizzly Bay at Dolphin (602)	100	0.0	100	0.0	NS
Napa River at Vallejo Seawall (340)	98	2.5	100	0.0	NS
Field Dup.: Napa River at Vallejo Seawall (340)	100	0.0	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.057	0.007	0.046	0.005	NS
Napa River at River Park Blvd.	0.128	0.005	0.102	0.013	NS
Sacramento River at Hood DWR Station	0.099	0.010	0.072	0.008	NS
Suisun Slough at Rush Ranch	0.100	0.004	0.087	0.013	NS
Rough and Ready DWR station, Stockton	0.126	0.008	0.106	0.008	NS
Suisun Bay off Chipps Island (508)	0.131	0.006	0.083	0.013	S* (63%)
Montezuma Slough at Nurse Slough (609)	0.123	0.005	0.093	0.014	NS
Carquinez Strait, West of Benicia army dock (405)	0.045	0.004	0.054	0.004	NS
Grizzly Bay at Dolphin (602)	0.100	0.007	0.101	0.006	NS
Napa River at Vallejo Seawall (340)	0.040	0.010	0.065	0.008	NS
Field Dup.: Napa River at Vallejo Seawall (340)	0.034*	0.009	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.
*: $P < 0.05$
2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

Table B9-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/03/09 - 3/04/09

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^{o}C)	pH	DO (mg/L)			
Napa River at River Park Blvd.	237	12.0	7.33	10.7	88.9	0.09	0.000
Sacramento River at Hood DWR Station	187	12.8	7.28	9.5	43.9	0.25	0.001
Suisun Slough at Rush Ranch	2673	12.6	7.26	8.3	63.9	0.19	0.001
Rough and Ready DWR station, Stockton	878	13.2	7.42	8.7	5.8	0.15	0.001
Suisun Bay off Chipps Island (508)	401	12.1	7.35	10.4	39.8	0.16	0.001
Montezuma Slough at Nurse Slough (609)	2229	12.8	7.14	11.6	68.9	0.24	0.001
Carquinez Strait, West of Benicia army dock (405)	6510	11.9	7.47	13.0	115.3	0.24	0.001
Grizzly Bay at Dolphin (602)	1060	11.9	7.64	13.4	90.8	0.18	0.001
Napa River at Vallejo Seawall (340)	9460	11.9	7.52	13.0	77.9	0.23	0.001
Field Dup.: Napa River at Vallejo Seawall (340)	9460	11.9	7.52	13.0	76.2	0.23	0.001

Table B9-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 3/05/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/03/09 - 3/04/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	333	21.6	22.9	7.7	8.7	7.78	8.09	100	56	-
Napa River at River Park Blvd.	230	21.9	23.1	7.8	8.8	7.68	7.87	70	54	0.002
Sacramento River at Hood DWR Station	180	21.9	23.6	7.5	8.5	7.79	7.98	72	74	0.009
Suisun Slough at Rush Ranch	2556	21.9	23.9	7.6	8.7	7.70	8.14	380	130	0.004
Rough and Ready DWR station, Stockton	844	21.3	23.8	7.5	8.6	7.88	8.15	186	110	0.005
Suisun Bay off Chipps Island (508)	394	22.3	24.0	7.6	8.7	7.86	8.00	96	74	0.006
Montezuma Slough at Nurse Slough (609)	2159	21.6	23.7	7.5	8.5	7.05	7.94	292	80	0.006
Carquinez Strait, West of Benicia army dock (405)	6160	22.4	23.8	7.4	8.4	7.82	7.92	800	88	0.006
Grizzly Bay at Dolphin (602)	972	21.6	23.7	7.4	8.8	7.90	8.05	152	74	0.009
Napa River at Vallejo Seawall (340)	9110	22.1	23.9	7.6	8.9	7.78	7.90	1040	92	0.005
Field Dup.: Napa River at Vallejo Seawall (340)	8850	21.7	24.0	7.2	8.6	7.81	7.90	1160	94	0.006
DIEPAMHR + 25 ppb PBO	345	22.3	23.5	7.6	8.2	7.80	8.06	-	-	-
Napa River at River Park Blvd. + 25 ppb PBO	228	22.0	23.3	7.5	8.6	7.69	7.89	-	-	-
Sacramento River at Hood DWR Station + 25 ppb PBO	185	22.7	23.8	7.4	8.5	7.75	7.96	-	-	-
Suisun Slough at Rush Ranch + 25 ppb PBO	2608	22.0	23.6	7.5	8.5	7.88	8.15	-	-	-
Rough and Ready DWR station, Stockton + 25 ppb PBO	847	21.9	24.0	7.4	8.6	7.95	8.14	-	-	-
Suisun Bay off Chipps Island (508) + 25 ppb PBO	397	22.5	23.7	7.6	8.9	7.83	7.97	-	-	-
Montezuma Slough at Nurse Slough (609) + 25 ppb PBO	2121	21.6	24.1	7.3	8.6	7.78	7.92	-	-	-
Carquinez Strait, West of Benicia army dock (405) + 25 ppb PBO	6175	22.4	24.1	7.4	8.4	7.82	7.94	-	-	-
Grizzly Bay at Dolphin (602) + 25 ppb PBO	966	21.9	24.0	7.6	8.6	7.87	8.00	-	-	-
Napa River at Vallejo Seawall (340) + 25 ppb PBO	9015	21.5	23.8	7.7	8.5	7.81	7.91	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B10-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 3/06/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/05/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	100	0.0	95	5.0	NS
Sacramento R. Deep Water Channel, Light 55	98	2.5	100	0.0	NS
Sacramento River at tip of Grand Island (711)	86	14.3	95	3.1	NS
Upper Cache Slough at mouth of Ulatis Creek	97	3.1	95	2.9	NS
Confluence of Linsey Sl. And Cache Sl.	98	2.5	95	2.9	NS
San Joaquin River at Potato Slough (815)	97	2.8	100	0.0	NS
Old River, western arm at railroad bridge (902)	100	0.0	100	0.0	NS
Old River at mouth of Holland Cut (915)	98	2.5	98	2.5	NS
Trip Blank	98	2.5	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.045	0.002	0.060	0.026	NS
Sacramento R. Deep Water Channel, Light 55	0.089	0.005	0.094	0.009	NS
Sacramento River at tip of Grand Island (711)	0.064	0.009	0.040	0.007	NS
Upper Cache Slough at mouth of Ulatis Creek	0.073	0.004	0.040	0.005	S** (55%)
Confluence of Linsey Sl. And Cache Sl.	0.083	0.009	0.068	0.004	NS
San Joaquin River at Potato Slough (815)	0.089	0.008	0.094	0.008	NS
Old River, western arm at railroad bridge (902)	0.101	0.007	0.086	0.009	NS
Old River at mouth of Holland Cut (915)	0.085	0.010	0.055	0.007	NS
Trip Blank	0.056	0.005	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.
   *: $P < 0.05$
   **: $P < 0.01$
2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

Table B10-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/05/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (°C)	pH	DO (mg/L)			
Sacramento R. Deep Water Channel, Light 55	258	11.7	7.44	10.1	45.8	0.15	0.001
Sacramento River at tip of Grand Island (711)	137	11.4	7.1	10.1	146.3	0.20	0.000
Upper Cache Slough at mouth of Ulatis Creek	343	11.4	7.48	9.3	151.3	0.21	0.001
Confluence of Linsey Sl. And Cache Sl.	211	11.7	7.35	10.1	37.8	0.14	0.001
San Joaquin River at Potato Slough (815)	209	12.5	7.39	9.7	16.7	0.15	0.001
Old River, western arm at railroad bridge (902)	337	12.3	7.48	10.3	12.0	0.08	0.000
Old River at mouth of Holland Cut (915)	425	12.6	7.42	10.3	9.2	0.07	0.000
Trip Blank	335	16.9	8.03	9.0	0.3	0.00	0.000

Table B10-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 3/06/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/05/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	344	22.4	24.0	7.1	8.2	7.77	8.04	100	56	-
Sacramento R. Deep Water Channel, Light 55	261	22.4	23.9	7.3	8.3	7.83	7.94	106	90	0.005
Sacramento River at tip of Grand Island (711)	136	22.4	23.9	7.6	8.5	7.63	7.82	68	54	0.006
Upper Cache Slough at mouth of Ulatis Creek	347	22.4	23.9	7.3	8.2	7.96	8.08	128	112	0.009
Confluence of Linsey Sl. And Cache Sl.	215	22.2	23.9	7.3	8.3	7.83	7.94	78	78	0.005
San Joaquin River at Potato Slough (815)	216	22.1	24.0	7.2	8.5	7.73	7.83	84	60	0.004
Old River, western arm at railroad bridge (902)	344	22.2	24.3	7.6	8.2	7.87	7.94	84	88	0.003
Old River at mouth of Holland Cut (915)	423	22.2	24.0	7.5	8.5	7.86	7.95	108	84	0.003
Trip Blank	348	22.3	24.1	7.0	8.5	7.74	8.03	110	64	0.000
DIEPAMHR + 25 ppb PBO	343	22.2	22.9	7.4	8.1	7.73	8.03	-	-	-
Sacramento R. Deep Water Channel, Light 55 + 25 ppb PBO	259	22.2	22.9	7.2	8.6	7.83	7.99	-	-	-
Sacramento River at tip of Grand Island (711) + 25 ppb PBO	135	22.2	22.8	7.4	8.4	7.66	7.86	-	-	-
Upper Cache Slough at mouth of Ulatis Creek + 25 ppb PBO	339	22.2	22.7	7.4	8.5	8.00	8.14	-	-	-
Confluence of Linsey Sl. And Cache Sl. + 25 ppb PBO	212	22.2	22.6	7.6	8.8	7.83	7.98	-	-	-
San Joaquin River at Potato Slough (815) + 25 ppb PBO	210	22.2	22.4	7.5	8.6	7.78	7.85	-	-	-
Old River, western arm at railroad bridge (902) + 25 ppb PBO	340	22.2	22.4	7.0	8.4	7.86	7.92	-	-	-
Old River at mouth of Holland Cut (915) + 25 ppb PBO	412	22.0	22.4	7.4	8.5	7.88	8.02	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B11-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 3/19/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/17/09 - 3/18/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	95	2.8	98	2.5	NS
Suisun Bay off Chipps Island (508)	100	0.0	100	0.0	NS
Montezuma Slough at Nurse Slough (609)	100	0.0	91	6.4	NS
Grizzly Bay at Dolphin (602)	100	0.0	100	0.0	NS
Carquinez Strait, West of Benicia army dock (405)	98	2.5	100	0.0	NS
Napa River at Vallejo Seawall (340)	100	0.0	100	0.0	NS
Rough and Ready DWR station, Stockton	100	0.0	100	0.0	NS
Confluence of Lindsey Sl. and Cache Sl.	98	2.5	98	2.5	NS
Upper Cache Slough at mouth of Ulatis Creek	100	0.0	100	0.0	NS
Sacramento R. Deep Water Channel, Light 55	100	0.0	100	0.0	NS

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.048	0.009	0.063	0.011	NS
Suisun Bay off Chipps Island (508)	0.098	0.006	0.074	0.009	NS
Montezuma Slough at Nurse Slough (609)	0.068	0.009	0.058	0.006	NS
Grizzly Bay at Dolphin (602)	0.077	0.004	0.071	0.007	NS
Carquinez Strait, West of Benicia army dock (405)	0.075	0.005	0.061	0.003	S* (81%)
Napa River at Vallejo Seawall (340)	0.073	0.002	0.057	0.005	S* (78%)
Rough and Ready DWR station, Stockton	0.093	0.006	0.064	0.007	S* (69%)
Confluence of Lindsey Sl. and Cache Sl.	0.062	0.009	0.065	0.004	NS
Upper Cache Slough at mouth of Ulatis Creek	0.069	0.002	0.077	0.008	NS
Sacramento R. Deep Water Channel, Light 55	0.072	0.004	0.097	0.003	S** (135%)

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.

*: $P < 0.05$

**: $P < 0.01$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

Table B11-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/17/09 - 3/18/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^{o}C)	pH	DO (mg/L)			
Suisun Bay off Chipps Island (508)	358	13.6	6.91	10.3	38.4	0.16	0.000
Montezuma Slough at Nurse Slough (609)	2030	14.0	6.99	9.7	65.7	0.29	0.001
Grizzly Bay at Dolphin (602)	425	13.8	6.75	10.5	71.8	0.18	0.000
Carquinez Strait, West of Benicia army dock (405)	6750	13.2	7.14	10.5	97.9	0.24	0.001
Napa River at Vallejo Seawall (340)	11210	13.2	7.02	10.3	74.8	0.18	0.000
Rough and Ready DWR station, Stockton	740	15.7	7.68	8.5	6.0	0.10	0.001
Confluence of Lindsey Sl. and Cache Sl.	354	14.3	7.38	10.0	14.7	0.29	0.002
Upper Cache Slough at mouth of Ulatis Creek	614	14.3	7.97	9.9	32.1	0.10	0.002
Sacramento R. Deep Water Channel, Light 55	369	13.0	7.79	10.0	18.7	0.22	0.003

Table B11-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 3/19/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/17/09 - 3/18/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	332	21.8	23.5	7.6	8.4	7.77	8.10	100	58	-
Suisun Bay off Chipps Island (508)	370	21.7	23.7	7.6	8.4	7.81	8.04	92	72	0.006
Montezuma Slough at Nurse Slough (609)	2587	21.6	23.9	7.5	8.4	7.77	7.86	380	78	0.007
Grizzly Bay at Dolphin (602)	420	21.6	23.8	7.6	8.5	7.86	8.06	320	72	0.008
Carquinez Strait, West of Benicia army dock (405)	6590	21.3	24.0	7.5	8.6	7.77	7.88	800	80	0.005
Napa River at Vallejo Seawall (340)	10390	21.6	24.3	7.3	8.7	7.76	7.91	1260	88	0.005
Rough and Ready DWR station, Stockton	723	21.5	23.6	7.2	8.4	7.98	8.14	176	104	0.004
Confluence of Lindsey Sl. and Cache Sl.	283	21.5	24.2	7.5	8.8	7.89	8.18	100	100	0.016
Upper Cache Slough at mouth of Ulatis Creek	577	21.4	23.8	7.6	8.7	8.14	8.38	212	180	0.005
Sacramento R. Deep Water Channel, Light 55	355	21.5	23.6	7.5	8.4	8.04	8.21	124	124	0.013
DIEPAMHR + 25 ppb PBO	332	21.5	23.0	7.8	8.1	7.80	8.09	-	-	-
Suisun Bay off Chipps Island (508) + 25 ppb PBO	352	21.9	23.2	7.5	8.6	7.81	8.02	-	-	-
Montezuma Slough at Nurse Slough (609) + 25 ppb PBO	2530	21.7	23.1	7.5	8.3	7.70	7.84	-	-	-
Grizzly Bay at Dolphin (602) + 25 ppb PBO	418	21.9	23.2	7.6	8.5	7.84	8.08	-	-	-
Carquinez Strait, West of Benicia army dock (405) + 25 ppb PBO	6410	21.7	23.0	6.3	8.2	7.54	7.91	-	-	-
Napa River at Vallejo Seawall (340) + 25 ppb PBO	10275	21.7	23.4	7.3	8.2	7.73	7.83	-	-	-
Rough and Ready DWR station, Stockton + 25 ppb PBO	710	21.5	23.4	7.7	8.5	7.94	8.15	-	-	-
Confluence of Lindsey Sl. and Cache Sl. + 25 ppb PBO	286	21.4	23.4	7.6	8.7	7.90	8.23	-	-	-
Upper Cache Slough at mouth of Ulatis Creek + 25 ppb PBO	575	21.2	23.5	7.6	8.9	8.27	8.37	-	-	-
Sacramento R. Deep Water Channel, Light 55 + 25 ppb PBO	357	21.5	23.6	7.4	8.6	8.00	8.26	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B12-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 3/20/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/18/09 - 3/19/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	92	2.6	94	5.6	NS
Sacramento River at tip of Grand Island (711)	95	2.9	95	2.9	NS
San Joaquin River at Potato Slough (815)	100	0.0	100	0.0	NS
Old River, western arm at railroad bridge (902)	100	0.0	98	2.5	NS
Old River at mouth of Holland Cut (915)	100	0.0	98	2.5	NS
Napa River at River Park Blvd.	100	0.0	100	0.0	NS
Suisun Slough at Rush Ranch	100	0.0	100	0.0	NS
Sacramento River at Hood DWR Station	95	5.0	95	2.9	NS
Instant Ocean Control @ 150 mS/cm	98	2.5	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.026	0.005	0.046	0.005	S* (177%)
Sacramento River at tip of Grand Island (711)	0.061	0.005	0.061	0.008	NS
San Joaquin River at Potato Slough (815)	0.046	0.004	0.087	0.007	S** (189%)
Old River, western arm at railroad bridge (902)	0.064	0.013	0.065	0.010	NS
Old River at mouth of Holland Cut (915)	0.093	0.006	0.069	0.004	S* (74%)
Napa River at River Park Blvd.	0.084	0.011	0.100	0.012	NS
Suisun Slough at Rush Ranch	0.074	0.005	0.093	0.006	S* (126%)
Sacramento River at Hood DWR Station	0.092	0.003	0.067	0.006	S* (73%)
Instant Ocean Control @ 150 mS/cm	0.033	0.004	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.

*: $P < 0.05$

**: $P < 0.01$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

Table B12-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/18/09 - 3/19/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^{o}C)	pH	DO (mg/L)			
Sacramento River at tip of Grand Island (711)	210	14.1	7.16	10.1	5.9	0.34	0.001
San Joaquin River at Potato Slough (815)	218	14.4	6.94	10.0	10.3	0.11	0.000
Old River, western arm at railroad bridge (902)	258	14.8	7.39	10.1	10.9	0.05	0.000
Old River at mouth of Holland Cut (915)	319	14.8	7.21	9.8	7.6	0.03	0.000
Napa River at River Park Blvd.	466	16.6	7.96	10.5	42.3	0.05	0.001
Suisun Slough at Rush Ranch	4106	19.0	7.42	9.2	98.5	0.16	0.001
Sacramento River at Hood DWR Station	197	14.4	7.14	9.9	5.4	0.46	0.002

Table B12-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 3/20/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/18/09 - 3/19/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	334	22.1	23.9	7.6	8.4	7.29	8.14	100	58	-
Sacramento River at tip of Grand Island (711)	199	22.1	23.8	7.4	8.4	7.29	8.08	80	78	0.015
San Joaquin River at Potato Slough (815)	206	22.3	24.0	7.3	8.7	7.20	8.17	84	68	0.004
Old River, western arm at railroad bridge (902)	254	22.3	23.6	7.5	8.8	7.27	8.14	84	68	0.002
Old River at mouth of Holland Cut (915)	286	22.8	22.3	7.3	8.5	7.34	8.11	88	70	0.000
Napa River at River Park Blvd.	453	22.4	23.8	7.3	8.9	7.51	8.20	88	96	0.003
Suisun Slough at Rush Ranch	3885	22.3	23.9	6.9	8.4	7.85	8.31	620	222	0.009
Sacramento River at Hood DWR Station	219	22.4	24.0	6.9	8.7	7.28	8.22	72	80	0.029
Instant Ocean Control @ 150 mS/cm	156	22.3	23.5	7.5	8.3	5.94	8.69	40	5	-
DIEPAMHR + 25 ppb PBO	338	22.2	23.3	7.4	8.5	7.33	8.26	-	-	-
Sacramento River at tip of Grand Island (711) + 25 ppb PBO	204	22.3	23.5	7.6	8.5	7.39	8.25	-	-	-
San Joaquin River at Potato Slough (815) + 25 ppb PBO	207	22.4	23.6	7.4	8.7	7.18	8.15	-	-	-
Old River, western arm at railroad bridge (902) + 25 ppb PBO	244	22.5	23.7	7.3	8.7	7.29	8.10	-	-	-
Old River at mouth of Holland Cut (915) + 25 ppb PBO	289	22.3	23.4	7.2	8.6	7.34	8.14	-	-	-
Napa River at River Park Blvd. + 25 ppb PBO	450	22.3	23.7	7.3	8.6	7.51	8.23	-	-	-
Suisun Slough at Rush Ranch + 25 ppb PBO	4056	22.4	23.4	7.2	8.3	7.90	8.31	-	-	-
Sacramento River at Hood DWR Station + 25 ppb PBO	200	22.5	23.6	6.9	8.5	7.31	8.24	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B13-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 4/02/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/31/09 - 4/01/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	95	5.0	95	3.1	NS
High EC Control @ 19.70 mS/cm	89	4.2	89	7.0	NS
Sacramento River at Hood DWR Station	100	0.0	94	5.6	NS
Napa River at River Park Blvd.	98	2.5	98	2.5	NS
Suisun Slough at Rush Ranch	100	0.0	100	0.0	NS
Rough and Ready DWR station, Stockton	100	0.0	100	0.0	NS
Grizzly Bay at Dolphin (602)	100	0.0	100	0.0	NS
Montezuma Slough at Nurse Slough (609)	95	2.9	100	0.0	NS
Suisun Bay off Chipps Island (508)	98	2.1	100	0.0	NS
Carquinez Strait, West of Benicia army dock (405)	94	3.7	94	3.4	NS
Napa River at Vallejo Seawall (340)	100	0.0	95	3.1	NS

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.032	0.003	0.046	0.007	NS
High EC Control @ 19.70 mS/cm	0.032	0.005	0.039	0.009	NS
Sacramento River at Hood DWR Station	0.064	0.005	0.079	0.003	NS
Napa River at River Park Blvd.	0.084	0.005	0.073	0.005	NS
Suisun Slough at Rush Ranch	0.074	0.008	0.091	0.007	NS
Rough and Ready DWR station, Stockton	0.094	0.008	0.102	0.010	NS
Grizzly Bay at Dolphin (602)	0.094	0.006	0.085	0.010	NS
Montezuma Slough at Nurse Slough (609)	0.088	0.003	0.091	0.002	NS
Suisun Bay off Chipps Island (508)	0.087	0.008	0.130	0.017	NS
Carquinez Strait, West of Benicia army dock (405)	0.046	0.007	0.056	0.007	NS
Napa River at Vallejo Seawall (340)	0.059	0.009	0.053	0.003	NS

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

Table B13-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/31/09 - 4/01/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (°C)	pH	DO (mg/L)			
Sacramento River at Hood DWR Station	178	14.7	6.91	9.9	6.9	0.43	0.001
Napa River at River Park Blvd.	1206	17.4	7.77	9.6	105.0	0.22	0.004
Suisun Slough at Rush Ranch	3805	16.6	7.45	9.0	343.0	0.27	0.002
Rough and Ready DWR station, Stockton	913	16.3	7.94	10.1	7.2	0.02	0.000
Grizzly Bay at Dolphin (602)	5260	16.9	7.67	10.0	68.4	0.10	0.001
Montezuma Slough at Nurse Slough (609)	3439	15.9	7.12	9.0	80.3	0.25	0.001
Suisun Bay off Chipps Island (508)	5300	14.8	7.55	10.5	40.4	0.16	0.001
Carquinez Strait, West of Benicia army dock (405)	17740	15.7	7.53	9.8	155.7	0.21	0.001
Napa River at Vallejo Seawall (340)	18760	15.6	6.94	9.6	37.5	0.12	0.000

Table B13-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 4/02/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 3/31/09 - 4/01/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (^oC)	Max Temp (^oC)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	334	22.8	22.8	7.4	8.2	7.77	8.06	100	56	-
High EC Control @ 19.70 mS/cm	19135	22.4	23.1	7.0	8.2	7.72	7.78	2100	82	-
Sacramento River at Hood DWR Station	218	22.8	23.1	7.0	8.5	7.63	8.13	64	72	0.012
Napa River at River Park Blvd.	1325	22.1	23.1	7.3	8.5	7.89	8.12	240	118	0.007
Suisun Slough at Rush Ranch	3705	22.5	23.1	7.4	7.9	7.85	8.27	496	176	0.007
Rough and Ready DWR station, Stockton	931	23.1	23.1	7.3	8.3	7.95	8.14	164	104	0.001
Grizzly Bay at Dolphin (602)	4760	23.1	23.1	7.4	8.1	7.83	7.96	500	86	0.003
Montezuma Slough at Nurse Slough (609)	3382	22.7	23.1	7.4	8.4	7.71	8.00	388	84	0.005
Suisun Bay off Chipps Island (508)	5255	23.1	23.7	7.5	7.9	7.82	7.99	564	86	0.004
Carquinez Strait, West of Benicia army dock (405)	16675	23.1	23.4	7.1	8.1	7.65	7.88	1860	98	0.004
Napa River at Vallejo Seawall (340)	18515	23.1	23.9	7.2	7.7	7.65	7.92	1996	94	0.002
DIEPAMHR + 25 ppb PBO	391	22.6	23.0	7.3	7.9	7.77	8.03	-	-	-
High EC Control @ 19.70 mS/cm + 25 ppb PBO	19055	22.9	23.2	6.9	7.8	7.73	7.80	-	-	-
Sacramento River at Hood DWR Station + 25 ppb PBO	240	23.1	23.8	7.2	8.1	7.70	7.97	-	-	-
Napa River at River Park Blvd. + 25 ppb PBO	1362	23.2	23.4	7.4	8.2	7.93	8.15	-	-	-
Suisun Slough at Rush Ranch + 25 ppb PBO	3699	23.1	23.9	7.2	8.2	7.89	8.27	-	-	-
Rough and Ready DWR station, Stockton + 25 ppb PBO	916	23.1	23.5	7.3	8.5	7.96	8.17	-	-	-
Grizzly Bay at Dolphin (602) + 25 ppb PBO	4656	23.1	24.1	7.3	8.1	7.81	7.95	-	-	-
Montezuma Slough at Nurse Slough (609) + 25 ppb PBO	3458	23.5	23.5	7.0	8.5	7.76	7.98	-	-	-
Suisun Bay off Chipps Island (508) + 25 ppb PBO	5165	23.3	24.0	7.1	8.3	7.81	7.96	-	-	-
Carquinez Strait, West of Benicia army dock (405) + 25 ppb PBO	16845	23.2	23.6	6.9	8.3	7.66	7.87	-	-	-
Napa River at Vallejo Seawall (340) + 25 ppb PBO	18575	23.2	23.9	7.0	8.1	7.70	7.87	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B14-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 4/03/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/02/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	100	0.0	76	17.9	NS
Instant Ocean Control @ 150 uS/cm	100	0.0	100	0.0	NS
Sacramento R. Deep Water Channel, Light 55	98	2.5	100	0.0	NS
Upper Cache Slough at mouth of Ulatis Creek	100	0.0	98	2.5	NS
Confluence of Linsey Sl. And Cache Sl.	98	2.5	98	2.5	NS
Sacramento River at tip of Grand Island (711)	95	2.9	98	2.5	NS
San Joaquin River at Potato Slough (815)	98	2.5	98	2.5	NS
Old River, western arm at railroad bridge (902)	100	0.0	100	0.0	NS
Old River at mouth of Holland Cut (915)	98	2.5	98	2.5	NS

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.050	0.008	0.050	0.012	NS
Instant Ocean Control @ 150 uS/cm	0.030*	0.003	0.051	0.004	S** (170%)
Sacramento R. Deep Water Channel, Light 55	0.068	0.022	0.112	0.005	NS
Upper Cache Slough at mouth of Ulatis Creek	0.036	0.005	0.106	0.005	S*** (294%)
Confluence of Linsey Sl. And Cache Sl.	0.083	0.006	0.088	0.005	NS
Sacramento River at tip of Grand Island (711)	0.076	0.002	0.088	0.007	NS
San Joaquin River at Potato Slough (815)	0.090	0.003	0.105	0.006	NS
Old River, western arm at railroad bridge (902)	0.090	0.007	0.124	0.007	S* (138%)
Old River at mouth of Holland Cut (915)	0.100	0.013	0.105	0.006	NS

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.

*: $P < 0.05$

**: $P < 0.01$

***: $P < 0.001$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

Table B14-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/02/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Sacramento R. Deep Water Channel, Light 55	283	14.9	7.02	10.1	19.5	0.26	0.001
Upper Cache Slough at mouth of Ulatis Creek	605	15.3	7.96	10.2	29.3	0.04	0.001
Confluence of Linsey Sl. And Cache Sl.	272	15.5	6.90	9.9	14.2	0.28	0.001
Sacramento River at tip of Grand Island (711)	217	16.3	7.38	9.7	8.5	0.38	0.003
San Joaquin River at Potato Slough (815)	190	16.0	7.50	10.0	9.6	0.10	0.001
Old River, western arm at railroad bridge (902)	262	15.9	7.49	9.7	12.3	0.04	0.000
Old River at mouth of Holland Cut (915)	268	15.9	7.41	9.8	9.0	0.01	0.000

Table B14-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 4/03/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/02/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	335	22.1	23.5	7.5	8.5	7.69	8.12	100	56	-
Instant Ocean Control @ 150 uS/cm	155	22.6	23.4	7.5	9.1	6.74	7.08	32	4	-
Sacramento R. Deep Water Channel, Light 55	264	22.3	23.2	7.3	8.9	7.78	8.10	104	92	0.012
Upper Cache Slough at mouth of Ulatis Creek	571	22.3	23.5	7.3	8.6	8.24	8.39	196	172	0.003
Confluence of Linsey Sl. And Cache Sl.	248	22.6	23.4	7.0	8.8	7.79	8.13	92	88	0.017
Sacramento River at tip of Grand Island (711)	196	22.7	23.5	7.3	8.8	7.72	8.04	80	134	0.019
San Joaquin River at Potato Slough (815)	222	22.6	23.5	7.3	8.3	7.72	8.05	100	72	0.005
Old River, western arm at railroad bridge (902)	253	22.7	23.4	7.5	8.8	7.79	8.06	84	72	0.002
Old River at mouth of Holland Cut (915)	256	22.7	23.3	7.4	8.8	7.75	8.07	88	74	0.001
DIEPAMHR + 25 ppb PBO	334	22.7	23.5	7.6	8.3	7.72	8.05	-	-	-
Instant Ocean Control @ 150 uS/cm + 25 ppb PBO	154	22.6	22.8	7.5	8.6	6.75	7.09	-	-	-
Sacramento R. Deep Water Channel, Light 55 + 25 ppb PBO	258	22.6	23.1	7.0	8.4	7.74	8.06	-	-	-
Upper Cache Slough at mouth of Ulatis Creek + 25 ppb PBO	567	22.7	23.3	7.2	8.5	8.23	8.37	-	-	-
Confluence of Linsey Sl. And Cache Sl. + 25 ppb PBO	245	22.6	22.8	7.0	8.5	7.79	8.05	-	-	-
Sacramento River at tip of Grand Island (711) + 25 ppb PBO	197	22.7	23.1	7.3	8.5	7.19	8.07	-	-	-
San Joaquin River at Potato Slough (815) + 25 ppb PBO	232	22.6	23.2	7.3	8.4	7.74	8.05	-	-	-
Old River, western arm at railroad bridge (902) + 25 ppb PBO	253	22.6	22.9	7.4	9.0	7.81	8.12	-	-	-
Old River at mouth of Holland Cut (915) + 25 ppb PBO	258	22.7	23.1	7.2	8.8	7.78	7.97	-	-	-

Table B15-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 4/16/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/14/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	98	2.5	83	11.1	NS
High EC Control @ 19.88 mS/cm	87	6.3	81	10.8	NS
Rough and Ready DWR station, Stockton	100	0.0	97	2.8	NS
Montezuma Slough at Nurse Slough (609)	100	0.0	95	2.9	NS
Grizzly Bay at Dolphin (602)	95	2.7	100	0.0	NS
Napa River at Vallejo Seawall (340)[3]	95	3.1	88	4.8	NS
Carquinez Strait, West of Benicia army dock (405)	100	0.0	98	2.5	NS
Suisun Bay off Chipps Island (508)	98	2.3	98	2.5	NS

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.046	0.003	0.033	0.005	S*
High EC Control @ 19.88 mS/cm	0.023**	0.006	0.008**	0.001	S*
Rough and Ready DWR station, Stockton	0.095	0.005	0.105	0.006	NS
Montezuma Slough at Nurse Slough (609)	0.072	0.009	0.065	0.013	NS
Grizzly Bay at Dolphin (602)	0.044	0.007	0.049	0.003	NS
Napa River at Vallejo Seawall (340)[3]	0.052	0.004	0.032	0.007	S*
Carquinez Strait, West of Benicia army dock (405)	0.030*	0.007	0.044	0.008	NS
Suisun Bay off Chipps Island (508)	0.095	0.013	0.070	0.007	NS

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard single-concentration statistical protocols.

*: $P < 0.05$

**: $P < 0.01$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

3. This high conductivity sample was compared to the High EC Control.

Table B15-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/02/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Rough and Ready DWR station, Stockton	914	16.9	7.72	8.0	10.2	0.15	0.002
Montezuma Slough at Nurse Slough (609)	3895	15.4	7.05	9.3	93.5	0.34	0.001
Grizzly Bay at Dolphin (602)	5620	15.6	7.54	10.0	234.0	0.32	0.002
Napa River at Vallejo Seawall (340)	19420	14.0	6.99	10.2	46.3	0.59	0.001
Carquinez Strait, West of Benicia army dock (405)	9520	14.3	7.32	10.1	146.0	0.62	0.002
Suisun Bay off Chipps Island (508)	597	14.9	7.14	10.2	26.8	0.31	0.001

Table B15-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 4/03/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/02/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	331	22.0	23.4	7.6	8.4	7.73	8.02	100	56	-
High EC Control @ 19.88 mS/cm	19135	21.6	23.5	7.5	8.6	7.58	7.87	2680	82	-
Rough and Ready DWR station, Stockton	877	21.5	23.2	7.5	8.8	8.03	8.24	190	112	0.010
Montezuma Slough at Nurse Slough (609)	3577	20.2	23.4	7.4	8.4	7.82	8.01	452	88	0.013
Grizzly Bay at Dolphin (602)	6280	21.9	23.3	7.8	8.5	7.83	8.10	704	86	0.015
Napa River at Vallejo Seawall (340)	18650	22.6	23.2	6.4	8.1	7.56	7.91	2340	100	0.015
Carquinez Strait, West of Benicia army dock (405)	8875	22.7	23.1	7.4	8.5	7.80	8.02	1004	88	0.022
Suisun Bay off Chipps Island (508)	510	22.5	22.9	7.2	8.5	7.80	8.22	98	76	0.021
DIEPAMHR + 25 ppb PBO	338	22.5	22.5	7.7	8.6	7.83	8.12	-	-	-
High EC Control @ 19.88 mS/cm + 25 ppb PBO	19215	22.7	23.5	7.2	8.1	7.56	7.86	-	-	-
Rough and Ready DWR station, Stockton + 25 ppb PBO	917	22.7	24.4	7.4	8.8	8.00	8.32	-	-	-
Montezuma Slough at Nurse Slough (609) + 25 ppb PBO	3754	22.6	24.4	7.3	8.7	7.83	8.03	-	-	-
Grizzly Bay at Dolphin (602) + 25 ppb PBO	7805	22.7	23.7	7.6	8.4	7.88	8.03	-	-	-
Napa River at Vallejo Seawall (340) + 25 ppb PBO	18885	22.6	24.8	7.1	8.5	7.77	7.95	-	-	-
Carquinez Strait, West of Benicia army dock (405) + 25 ppb PBO	8820	22.7	24.2	7.6	8.4	7.79	7.97	-	-	-
Suisun Bay off Chipps Island (508) + 25 ppb PBO	524	22.6	24.1	7.1	8.6	7.82	8.21	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B16-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 4/17/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/15/09 - 4/16/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	100	0.0	100	0.0	NS
Low EC control @147.3 uS/cm	100	0.0	94	3.2	NS
Sacramento R. Deep Water Channel, Light 55[3]	100	0.0	100	0.0	NS
Upper Cache Slough at mouth of Ulatis Creek	95	5.0	93	2.5	NS
Confluence of Linsey Sl. And Cache Sl.	98	2.5	95	2.9	NS
Suisun Slough at Rush Ranch	100	0.0	98	2.5	NS
Napa River at River Park Blvd.	100	0.0	95	2.9	NS
Sacramento River at tip of Grand Island (711)	95	2.8	93	4.8	NS
Sacramento River at Hood DWR Station[3]	97	2.8	95	2.9	NS

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.065	0.006	0.056	0.001	NS
Low EC control @147.3 uS/cm	0.049*	0.005	0.033**	0.005	NS
Sacramento R. Deep Water Channel, Light 55[3]	0.092	0.010	0.092	0.015	NS
Upper Cache Slough at mouth of Ulatis Creek	0.101	0.011	0.096	0.009	NS
Confluence of Linsey Sl. And Cache Sl.	0.083	0.010	0.083	0.007	NS
Suisun Slough at Rush Ranch	0.050	0.014	0.090	0.005	S* (180%)
Napa River at River Park Blvd.	0.088	0.009	0.093	0.014	NS
Sacramento River at tip of Grand Island (711)	0.081	0.014	0.086	0.015	NS
Sacramento River at Hood DWR Station[3]	0.075	0.016	0.079	0.006	NS

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard statistical protocols.

*: $P < 0.05$

**: $P < 0.01$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

3. These low EC samples were compared to the Low EC Control.

Table B16-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/15/09 - 4/16/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Sacramento R. Deep Water Channel, Light 55	409	15.5	7.23	9.5	18.0	0.14	0.001
Upper Cache Slough at mouth of Ulatis Creek	674	15.3	8.61	11.0	46.1	0.08	0.007
Confluence of Linsey Sl. And Cache Sl.	674	15.3	7.23	11.0	30.0	0.16	0.001
Suisun Slough at Rush Ranch	4816	16.9	7.53	8.8	395.3	0.46	0.004
Napa River at River Park Blvd.	3892	17.9	7.98	9.9	12.7	0.09	0.002
Sacramento River at tip of Grand Island (711)	148	15.6	7.03	10.0	23.9	0.42	0.001
Sacramento River at Hood DWR Station	148	14.4	6.85	10.0	7.8	0.52	0.001

Table B16-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 4/17/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/15/09 - 4/16/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (^oC)	Max Temp (^oC)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	326	22.7	23.4	7.7	8.2	7.81	8.01	104	60	-
Low EC control @147.3 uS/cm	255	22.6	23.2	7.6	8.9	7.55	7.85	44	26	-
Sacramento R. Deep Water Channel, Light 55	280	22.8	23.9	7.6	8.8	8.06	8.17	124	108	0.007
Upper Cache Slough at mouth of Ulatis Creek	526	22.7	23.5	7.5	8.6	8.35	8.47	226	204	0.010
Confluence of Linsey Sl. And Cache Sl.	533	22.7	24.3	7.3	8.5	8.00	8.20	114	118	0.009
Suisun Slough at Rush Ranch	2492	22.7	23.9	7.4	8.7	7.90	8.40	650	248	0.014
Napa River at River Park Blvd.	4280	22.8	23.9	7.6	8.5	7.97	8.16	480	120	0.003
Sacramento River at tip of Grand Island (711)	1986	22.7	23.7	7.4	8.9	7.71	7.95	56	58	0.018
Sacramento River at Hood DWR Station	156	22.7	24.1	7.4	8.8	7.71	7.90	52	52	0.015
DIEPAMHR + 25 ppb PBO	240	22.7	23.6	7.6	8.5	7.80	8.01	-	-	-
Low EC control @147.3 uS/cm 25 ppb PBO	257	22.8	23.4	7.6	8.9	7.55	7.81	-	-	-
Sacramento R. Deep Water Channel, Light 55 + 25 ppb PBO	280	22.7	23.9	7.7	8.8	8.13	8.40	-	-	-
Upper Cache Slough at mouth of Ulatis Creek + 25 ppb PBO	533	22.7	23.6	7.5	8.7	8.34	8.49	-	-	-
Confluence of Linsey Sl. And Cache Sl. + 25 ppb PBO	533	22.7	24.1	7.4	8.7	8.10	8.23	-	-	-
Suisun Slough at Rush Ranch + 25 ppb PBO	2475	22.7	23.7	7.4	8.7	7.78	8.39	-	-	-
Napa River at River Park Blvd. + 25 ppb PBO	4308	22.8	24.1	7.6	8.4	7.96	8.13	-	-	-
Sacramento River at tip of Grand Island (711) + 25 ppb PBO	1984	23.1	23.8	7.4	8.7	7.78	7.93	-	-	-
Sacramento River at Hood DWR Station + 25 ppb PBO	154	23.2	23.9	7.4	8.9	7.68	7.92	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B17-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 4/24/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/23/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	95	2.9	98	2.5	NS
Low EC Control @ 191.2 µS/cm	89	0.3	98	2.5	NS
Old River at mouth of Holland Cut (915)	90	10.0	100	0.0	NS
San Joaquin River at Potato Slough (815)	95	2.9	97	2.8	NS
Old River, western arm at railroad bridge (902)	100	0.0	95	2.9	NS
Field Dup: Old River, western arm at railroad bridge (902)	98	2.5	-	-	NA
Bottle Blank: DIEPAMHR	84	5.2	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.084	0.005	0.025	0.005	S*** (30%)
Low EC Control @ 191.2 µS/cm	0.061	0.004	0.031	0.002	S*** (51%)
Old River at mouth of Holland Cut (915)	0.087	0.007	0.058	0.007	S* (67%)
San Joaquin River at Potato Slough (815)	0.069	0.007	0.054	0.005	NS
Old River, western arm at railroad bridge (902)	0.068	0.004	0.045	0.003	S** (66%)
Field Dup: Old River, western arm at railroad bridge (902)	0.061	0.007	-	-	NA
Bottle Blank: DIEPAMHR	0.057**	0.003	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control.
Unmanipulated samples were analyzed using one-way ANOVA and Tukey's Multiple Comparison Procedure ($P <$ 0.05).
Samples with PBO additions were analyzed using two-way ANOVA and Tukey's Multiple Comparison Procedure (P < 0.05).
2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

*: $P < 0.05$

**: $P < 0.01$

***: $P < 0.001$

Table B17-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/23/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
San Joaquin River at Potato Slough (815)	181	18.2	7.16	9.9	7.0	0.10	0.000
Old River, western arm at railroad bridge (902)	252	20.5	7.24	9.4	6.8	0.06	0.000
Old River at mouth of Holland Cut (915)	292	20.5	7.19	9.0	6.4	0.03	0.000
Field Dup: Old River, western arm at railroad bridge (902)	252	20.5	7.24	9.4	7.4	0.06	0.000

Table B17-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 4/24/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/23/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (^oC)	Max Temp (^oC)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	339	22.7	24.4	7.4	8.5	7.80	8.11	-	-	-
Low EC Control @ 191.2 µS/cm	209	22.7	24.1	7.5	8.7	7.64	7.98	64	34	-
Old River at mouth of Holland Cut (915)	230	22.8	24.4	7.3	8.9	7.87	8.09	84	72	0.002
San Joaquin River at Potato Slough (815)	187	22.7	24.2	7.3	8.5	7.78	8.03	72	64	0.005
Old River, western arm at railroad bridge (902)	243	22.6	24.3	7.5	8.4	7.88	8.10	84	70	0.004
Field Dup: Old River, western arm at railroad bridge (902)	244	22.5	24.4	7.3	8.8	7.83	8.10	80	70	0.001
Bottle Blank: DIEPAMHR	334	22.6	24.2	7.5	8.6	7.76	8.08	64	58	0.003
DIEPAMHR + 25 ppb PBO	335	22.7	24.2	7.4	8.4	7.79	8.10	-	-	-
Low EC Control @ 191.2 µS/cm + 25 ppb PBO	209	22.7	24.2	7.5	8.9	7.69	7.96	-	-	-
Old River at mouth of Holland Cut (915) + 25 ppb PBO	283	22.8	24.1	7.3	8.9	7.73	8.06	-	-	-
San Joaquin River at Potato Slough (815) + 25 ppb PBO	184	22.7	24.1	7.2	8.9	7.74	7.96	-	-	-
Old River, western arm at railroad bridge (902) + 25 ppb PBO	245	22.6	24.0	7.4	8.6	7.83	8.13	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B18-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 4/30/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/28/09 - 4/29/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	98	2.5	94	3.2	NS
Low EC Control @ 129.1 µS/cm	98	2.5	98	2.5	NS
High EC Control @ 15.30 mS/cm	95	2.9	100	0.0	NS
High EC Control @ 25.00 mS/cm	79*	4.8	82	7.7	NS
Suisun Slough at Rush Ranch	100	0.0	100	0.0	NS
Napa River at River Park Blvd.	100	0.0	97	2.8	NS
Sacramento River at Hood DWR Station[3]	100	0.0	92	5.3	NS
Rough and Ready DWR station, Stockton	100	0.0	100	0.0	NS
Carquinez Strait, West of Benicia army dock (405)[4]	100	0.0	98	2.5	NS
Suisun Bay off Chipps Island (508)	98	2.3	100	0.0	NS
Montezuma Slough at Nurse Slough (609)	97	2.8	100	0.0	NS
Grizzly Bay at Dolphin (602)	98	2.5	95	2.8	NS
Napa River at Vallejo Seawall (340)[5]	90	5.5	95	3.1	NS
Trip Blank: DIEPAMHR	100	0.0	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.055	0.009	0.069	0.006	NS
Low EC Control @ 129.1 µS/cm	0.055	0.002	0.057	0.006	NS
High EC Control @ 15.30 mS/cm	0.036	0.006	0.045*	0.005	NS
High EC Control @ 25.00 mS/cm	0.020	0.005	0.034*	0.012	NS
Suisun Slough at Rush Ranch	0.090	0.006	0.119	0.006	S* (132%)
Napa River at River Park Blvd.	0.087	0.015	0.107	0.009	NS
Sacramento River at Hood DWR Station[3]	0.077	0.005	0.099	0.005	S* (129%)
Rough and Ready DWR station, Stockton	0.100	0.009	0.120	0.009	NS
Carquinez Strait, West of Benicia army dock (405)[4]	0.061	0.003	0.069	0.004	NS
Suisun Bay off Chipps Island (508)	0.098	0.002	0.106	0.011	NS
Montezuma Slough at Nurse Slough (609)	0.113	0.014	0.099	0.007	NS
Grizzly Bay at Dolphin (602)	0.054	0.007	0.081	0.004	S* (150%)
Napa River at Vallejo Seawall (340)[5]	0.048	0.007	0.070	0.005	S* (146%)
Trip Blank: DIEPAMHR	0.068	0.008	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard statistical protocols.

*: $P < 0.05$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.
3. This low conductivity sample was compared to the Low EC Control.
4. This high conductivity sample was compared to the High EC Control @ 15.30 mS/cm.
5. This high conductivity sample was compared to the High EC Control @ 25.00 mS/cm.

Table B18-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/28/09 - 4/29/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Suisun Slough at Rush Ranch	4772	14.7	7.01	8.9	71.4	0.14	0.000
Napa River at River Park Blvd.	9100	17.5	7.27	10.0	33.1	0.00	0.000
Sacramento River at Hood DWR Station	120	16.4	7.08	8.7	12.7	0.02	0.000
Rough and Ready DWR station, Stockton	690	18.8	7.74	7.5	12.6	0.13	0.002
Carquinez Strait, West of Benicia army dock (405)	15240	15.0	7.55	10.0	424.3	0.37	0.002
Suisun Bay off Chipps Island (508)	4810	15.4	7.51	10.0	37.0	0.13	0.001
Montezuma Slough at Nurse Slough (609)	4000	16.5	7.01	9.3	119.7	0.17	0.000
Grizzly Bay at Dolphin (602)	8380	15.3	7.48	10.0	379.0	0.33	0.002
Napa River at Vallejo Seawall (340)	24360	14.4	7.49	9.7	57.3	0.11	0.001
Trip Blank: DIEPAMHR	-	-	-	-	0.4	0.00	-

Table B18-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 4/30/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/28/09 - 4/29/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	332	22.8	24.1	7.3	8.2	7.70	8.26	124	58	-
Low EC Control @ 129.1 µS/cm	132	22.8	23.6	7.2	8.6	7.43	8.20	36	20	-
High EC Control @ 15.30 mS/cm	14790	22.9	23.9	7.2	8.3	7.68	7.99	1680	70	-
High EC Control @ 25.00 mS/cm	24215	22.8	24.0	7.1	7.8	7.72	7.93	3080	80	-
Suisun Slough at Rush Ranch	4586	22.8	24.0	7.5	8.4	8.11	8.15	640	158	0.007
Napa River at River Park Blvd.	4892	22.7	24.0	7.1	8.6	7.79	7.96	1200	128	0.000
Sacramento River at Hood DWR Station	235	22.7	24.1	7.0	8.7	7.62	8.11	48	51	0.001
Rough and Ready DWR station, Stockton	863	22.7	23.6	7.2	8.5	7.80	8.07	144	93	0.007
Carquinez Strait, West of Benicia army dock (405)	14030	22.7	22.8	7.2	8.2	7.68	7.88	1720	88	0.009
Suisun Bay off Chipps Island (508)	4674	22.7	23.9	7.4	8.7	7.71	8.01	520	74	0.005
Montezuma Slough at Nurse Slough (609)	3830	22.8	23.4	7.4	8.3	7.85	7.93	500	86	0.006
Grizzly Bay at Dolphin (602)	7910	22.9	23.8	7.4	8.2	7.75	8.07	1000	82	0.014
Napa River at Vallejo Seawall (340)	22870	22.8	23.8	6.9	7.6	7.64	7.87	2880	102	0.003
Trip Blank: DIEPAMHR	446	22.9	23.9	7.3	8.6	7.73	8.16	100	57	0.000
DIEPAMHR + 25 ppb PBO	339	22.5	22.8	7.3	8.4	7.70	8.03	-	-	-
Low EC Control @ 129.1 µS/cm + 25 ppb PBO	161	22.5	22.8	7.2	8.5	7.44	8.12	-	-	-
High EC Control @ 15.30 mS/cm + 25 ppb PBO	14580	22.4	22.9	7.3	8.2	7.67	7.91	-	-	-
High EC Control @ 25.00 mS/cm + 25 ppb PBO	23910	22.5	22.9	7.0	8.0	7.75	7.96	-	-	-
Suisun Slough at Rush Ranch + 25 ppb PBO	4550	22.4	22.9	7.4	8.6	7.92	8.21	-	-	-
Napa River at River Park Blvd. + 25 ppb PBO	4820	22.3	22.9	7.3	8.8	7.86	7.97	-	-	-
Sacramento River at Hood DWR Station + 25 ppb PBO	166	22.2	22.9	7.1	8.6	7.52	8.16	-	-	-
Rough and Ready DWR station, Stockton + 25 ppb PBO	699	22.1	22.8	7.3	8.6	7.82	8.01	-	-	-
Carquinez Strait, West of Benicia army dock (405) + 25 ppb PBO	14075	22.0	22.8	7.1	8.1	7.72	7.91	-	-	-
Suisun Bay off Chipps Island (508) + 25 ppb PBO	4428	21.9	22.8	7.4	8.9	7.72	7.98	-	-	-
Montezuma Slough at Nurse Slough (609) + 25 ppb PBO	3710.5	21.8	22.8	7.2	8.3	7.81	7.93	-	-	-
Grizzly Bay at Dolphin (602) + 25 ppb PBO	7890	21.9	22.9	7.5	8.3	7.74	7.94	-	-	-
Napa River at Vallejo Seawall (340) + 25 ppb PBO	22215	21.4	22.8	6.7	8.4	7.62	7.88	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B19-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 5/01/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/30/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	98	2.5	98	2.5	NS
Low EC Control @ 120.5 uS/cm	97	2.8	100	0.0	NS
Sacramento R. Deep Water Channel, Light 55	100	0.0	98	2.5	NS
Upper Cache Slough at mouth of Ulatis Creek	100	0.0	100	0.0	NS
Confluence of Lindsey Sl. and Cache Sl.	100	0.0	98	2.5	NS
Sacramento River at tip of Grand Island (711)	97	2.8	98	2.5	NS
San Joaquin River at Potato Slough	98	2.5	100	0.0	NS
Old River, western arm at railroad bridge (902)	100	0.0	100	0.0	NS
Old River at mouth of Holland Cut (915)	100	0.0	98	2.5	NS

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.057	0.004	0.048	0.001	S*
Low EC Control @ 120.5 uS/cm	0.051	0.006	0.025**	0.004	S**
Sacramento R. Deep Water Channel, Light 55	0.095	0.005	0.090	0.004	NS
Upper Cache Slough at mouth of Ulatis Creek	0.083	0.009	0.087	0.005	NS
Confluence of Lindsey Sl. and Cache Sl.	0.106	0.006	0.085	0.009	S*
Sacramento River at tip of Grand Island (711)	0.092	0.008	0.093	0.005	NS
San Joaquin River at Potato Slough	0.105	0.006	0.114	0.010	NS
Old River, western arm at railroad bridge (902)	0.109	0.006	0.112	0.009	NS
Old River at mouth of Holland Cut (915)	0.118	0.010	0.116	0.003	NS

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control.
*: $P < 0.05$
**: $P < 0.01$
2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

Table B19-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/30/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Sacramento R. Deep Water Channel, Light 55	236	16.6	7.25	9.4	31.2	0.16	0.001
Upper Cache Slough at mouth of Ulatis Creek	329	16.8	6.88	9.9	45.9	0.03	0.000
Confluence of Lindsey Sl. and Cache Sl.	246	16.8	6.8	9.5	27.5	0.20	0.000
Sacramento River at tip of Grand Island (711)	120	17.1	6.88	9.8	10.1	0.04	0.000
San Joaquin River at Potato Slough (815)	196	19.0	6.82	10.0	4.4	0.07	0.000
Old River, western arm at railroad bridge (902)	243	18.3	6.81	9.3	6.1	0.04	0.000
Old River at mouth of Holland Cut (915)	294	19.2	6.86	9.3	5.9	0.02	0.000

Table B19-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 5/1/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 4/30/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	337	23.3	23.8	7.4	8.3	7.66	8.10	124	58	-
Low EC Control @ 120.5 uS/cm	125	23.2	23.8	7.2	8.6	7.29	8.05	44	20	-
Sacramento R. Deep Water Channel, Light 55	261	23.3	23.7	7.1	8.7	7.75	8.13	84	78	0.010
Upper Cache Slough at mouth of Ulatis Creek	320	23.4	23.9	7.3	8.3	7.95	8.24	100	102	0.002
Confluence of Lindsey Sl. and Cache Sl.	203	23.3	23.7	7.2	8.9	7.74	8.19	64	74	0.014
Sacramento River at tip of Grand Island (711)	120	23.3	23.8	6.9	8.4	7.52	8.11	48	46	0.002
San Joaquin River at Potato Slough (815)	195	23.1	23.7	7.2	8.8	7.73	8.14	64	66	0.004
Old River, western arm at railroad bridge (902)	240	23.3	23.9	7.3	8.5	7.75	8.20	56	74	0.003
Old River at mouth of Holland Cut (915)	289	23.3	23.7	7.2	8.4	7.79	8.17	88	78	0.001
DIEPAMHR + 25 ppb PBO	335	22.0	23.9	7.4	8.5	7.70	8.06	-	-	-
Low EC Control @ 120.5 uS/cm + 25 ppb PBO	117	22.0	23.6	7.2	8.5	7.29	7.95	-	-	-
Sacramento R. Deep Water Channel, Light 55 + 25 ppb PBO	245	22.2	23.8	7.1	8.4	7.72	8.13	-	-	-
Upper Cache Slough at mouth of Ulatis Creek (815) + 25 ppb PBO	303	21.8	23.7	7.2	8.6	7.97	8.29	-	-	-
Confluence of Lindsey Sl. and Cache Sl. + 25 ppb PBO	189	21.7	23.9	7.3	8.6	7.75	8.14	-	-	-
Sacramento River at tip of Grand Island (711) + 25 ppb PBO	112	21.8	23.7	7.0	8.6	7.57	8.10	-	-	-
San Joaquin River at Potato Slough + 25 ppb PBO	184	21.8	23.9	7.3	8.9	7.74	7.99	-	-	-
Old River, western arm at railroad bridge (902) + 25 ppb PBO	231	21.8	23.9	7.3	8.6	7.78	8.09	-	-	-
Old River at mouth of Holland Cut (915) + 25 ppb PBO	275	22.0	24.0	7.3	8.7	7.77	8.08	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B20-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 5/15/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/14/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	91	5.9	58	25.0	NS
Confluence of Lindsey Sl. and Cache Sl.	95	2.9	87	9.4	NS
Sacramento R. Deep Water Channel, Light 55	92	5.3	100	0.0	NS

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.073	0.005	0.076	0.008	NS
Confluence of Lindsey Sl. and Cache Sl.	0.090	0.007	0.089	0.010	NS
Sacramento R. Deep Water Channel, Light 55	0.098	0.005	0.109	0.008	NS

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard statistical protocols.
2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

Table B20-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/14/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Confluence of Lindsey Sl. and Cache Sl.	207	20.7	7.29	11.0	132.7	0.16	0.001
Sacramento R. Deep Water Channel, Light 55	261	21.8	7.82	8.8	96.9	0.17	0.005

Table B20-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 5/15/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/14/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	359	23.4	24.3	7.4	8.6	7.70	8.00	80	58	-
Confluence of Lindsey Sl. and Cache Sl.	254	23.5	24.2	7.3	8.6	7.70	8.17	76	74	0.011
Sacramento R. Deep Water Channel, Light 55	291	23.6	24.2	7.4	8.5	7.61	8.03	76	72	0.009
DIEPAMHR + 25 ppb PBO	362	23.4	24.0	7.3	8.5	7.72	8.02	-	-	-
Confluence of Lindsey Sl. and Cache Sl. + 25 ppb PBO	245	23.9	24.0	7.2	8.7	7.72	8.18	-	-	-
Sacramento R. Deep Water Channel, Light 55 + 25 ppb PBO	290	24.0	24.3	7.1	8.7	7.69	8.02	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B21-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 5/16/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/12/09 - 5/13/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	100	0.0	87	9.4	NS
Low EC Control @ 119.2 µS/cm	100	0.0	87	6.3	NS
High EC Control @ 17.30 mS/cm	73*	6.0	73	11.1	NS
Napa River at River Park Blvd.	100	0.0	98	2.5	NS
Suisun Slough at Rush Ranch	100	0.0	97	2.8	NS
Sacramento River at Hood DWR Station[3]	90	4.1	81	3.3	NS
Rough and Ready DWR station, Stockton	100	0.0	100	0.0	NS
Napa River at Vallejo Seawall (340)[4]	61	10.1	14**	9.0	S*

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.062	0.006	0.047	0.003	S*
Low EC Control @ 119.2 µS/cm	0.049	0.006	0.045	0.006	NS
High EC Control @ 17.30 mS/cm	0.039*	0.005	0.027**	0.004	NS
Napa River at River Park Blvd.	0.099	0.007	0.069	0.002	S**
Suisun Slough at Rush Ranch	0.101	0.006	0.089	0.012	NS
Sacramento River at Hood DWR Station[3]	0.070	0.008	0.043	0.007	S*
Rough and Ready DWR station, Stockton	0.085	0.004	0.084	0.007	NS
Napa River at Vallejo Seawall (340)[4]	0.063	0.006	0.097	0.013	NS

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard statistical protocols.

*: $P < 0.05$

**: $P < 0.01$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

3. This low conductivity sample was compared to the Low EC Control.

4. This high conductivity sample was compared to the High EC Control.

Table B21-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/12/09 - 5/13/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Napa River at River Park Blvd.	5780	20.8	6.51	10.6	47.8	0.14	0.000
Suisun Slough at Rush Ranch	4863	19.0	6.51	9.8	62.3	0.32	0.000
Sacramento River at Hood DWR Station	116	19.3	6.89	11.4	21.5	0.21	0.001
Rough and Ready DWR station, Stockton	491	21.3	7.43	6.9	9.6	0.09	0.001
Napa River at Vallejo Seawall (340)	16330	17.5	6.91	9.2	77.5	0.13	0.000

Table B21-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 5/16/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/12/09 - 5/13/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	347	23.9	24.0	7.5	8.5	7.72	8.06	108	60	-
Low EC Control @ 119.2 µS/cm	127	23.7	24.1	7.4	8.5	7.30	7.82	32	22	-
High EC Control @ 17.30 mS/cm	16505	23.9	24.1	6.9	8.1	7.56	7.82	2040	82	-
Napa River at River Park Blvd.	5410	23.8	24.0	6.9	8.2	7.78	7.98	652	116	0.005
Suisun Slough at Rush Ranch	4550	23.9	24.0	6.9	8.5	8.01	8.19	384	198	0.017
Sacramento River at Hood DWR Station	124	23.7	24.0	7.0	8.4	7.34	7.99	44	50	0.010
Rough and Ready DWR station, Stockton	483	23.9	24.1	7.2	8.4	7.66	7.91	112	70	0.003
Napa River at Vallejo Seawall (340)	15320	23.7	24.0	6.6	8.1	7.58	7.81	1920	94	0.003
DIEPAMHR + 25 ppb PBO	239	23.7	23.7	7.2	8.5	7.67	7.84	-	-	-
Low EC Control @ 119.2 µS/cm + 25 ppb PBO	240	23.6	24.0	7.4	8.5	7.37	8.08	-	-	-
High EC Control @ 17.30 mS/cm + 25 ppb PBO	16445	23.9	24.0	6.7	8.0	7.60	7.84	-	-	-
Napa River at River Park Blvd. + 25 ppb PBO	5330	23.8	24.0	7.2	8.4	7.78	7.98	-	-	-
Suisun Slough at Rush Ranch + 25 ppb PBO	4552	23.8	24.3	6.9	8.3	7.99	8.20	-	-	-
Sacramento River at Hood DWR Station + 25 ppb PBO	149	23.8	24.2	6.9	8.7	7.44	7.83	-	-	-
Rough and Ready DWR station, Stockton + 25 ppb PBO	485	23.9	24.2	7.0	8.5	7.65	7.94	-	-	-
Napa River at Vallejo Seawall (340) + 25 ppb PBO	15325	23.6	24.1	6.8	8.2	7.68	7.78	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B22-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 5/20/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/18/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	98	2.5	100	0.0	NS
High EC Control @ 20360 uS/cm	100	0.0	93	4.8	NS
Grizzly Bay at Dolphin (602)	100	0.0	100	0.0	NS
Suisun Bay off Chipps Island (508)	100	0.0	100	0.0	NS
Carquinez Strait, West of Benicia army dock (405)[3]	88	7.5	93	4.8	NS
Montezuma Slough at Nurse Slough (609)	100	0.0	100	0.0	NS

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.071	0.002	0.081	0.005	NS
High EC Control @ 20360 uS/cm	0.035*	0.012	0.051**	0.005	NS
Grizzly Bay at Dolphin (602)	0.096	0.002	0.103	0.007	NS
Suisun Bay off Chipps Island (508)	0.087	0.008	0.111	0.010	NS
Carquinez Strait, West of Benicia army dock (405)[3]	0.053	0.007	0.058	0.002	NS
Montezuma Slough at Nurse Slough (609)	0.106	0.009	0.102	0.004	NS

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard statistical protocols.

*: $P < 0.05$

**: $P < 0.01$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

3. This high conductivity sample was compared to the High EC Control.

Table B22-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/18/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^{o}C)	pH	DO (mg/L)			
Grizzly Bay at Dolphin (602)	6250	19.7	7.95	9.0	54.5	0.09	0.003
Suisun Bay off Chipps Island (508)	2366	19.5	6.98	9.0	28.3	0.00	0.000
Carquinez Strait, West of Benicia army dock (405)	19550	18.3	7.38	9.3	10.5	0.00	0.000
Montezuma Slough at Nurse Slough (609)	3368	21.4	7.66	8.2	47.0	0.00	0.000

Table B22-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 5/20/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/18/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	340	22.9	23.5	7.4	8.4	7.74	8.20	104	60	-
High EC Control @ 20360 uS/cm	19550	23.6	23.6	7.0	8.4	7.63	7.85	2400	90	-
Grizzly Bay at Dolphin (602)	6135	23.0	23.6	7.4	8.4	7.63	7.97	760	76	0.003
Suisun Bay off Chipps Island (508)	2237	23.4	23.6	7.5	8.8	7.65	8.06	256	64	0.000
Carquinez Strait, West of Benicia army dock (405)	18545	23.4	23.6	7.0	8.4	7.66	7.84	2200	94	0.000
Montezuma Slough at Nurse Slough (609)	3159	23.5	23.7	6.8	8.5	7.64	8.07	360	84	0.000
DIEPAMHR + 25 ppb PBO	331	23.1	23.4	7.3	8.4	7.72	8.05	-	-	-
High EC Control @ 20360 uS/cm + 25 ppb PBO	19385	23.2	23.5	6.9	8.0	7.58	7.78	-	-	-
Grizzly Bay at Dolphin (602) + 25 ppb PBO	5685	23.0	23.9	7.3	8.3	7.66	7.90	-	-	-
Suisun Bay off Chipps Island (508) + 25 ppb PBO	2217	23.0	24.0	7.1	8.9	7.68	7.97	-	-	-
Carquinez Strait, West of Benicia army dock (405) + 25 ppb PBO	18285	23.0	24.4	7.0	8.9	7.65	7.82	-	-	-
Montezuma Slough at Nurse Slough (609) + 25 ppb PBO	3173	23.2	23.6	7.4	8.5	7.69	8.01	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B23-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 5/21/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/20/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	97	2.8	97	2.8	NS
Low EC Control @ 149.4 µS/cm	98	2.5	95	2.9	NS
Upper Cache Slough at mouth of Ulatis Creek	100	0.0	100	0.0	NS
Sacramento River at tip of Grand Island (711)	100	0.0	93	4.8	NS
San Joaquin River at Potato Slough (815)	100	0.0	100	0.0	NS
Old River, western arm at railroad bridge (902)	100	0.0	100	0.0	NS
Old River at mouth of Holland Cut (915)	100	0.0	100	0.0	NS
Bottle Blank (amber cubitainer)	100	0.0	-	-	NA
Bottle Blank (clear cubitainer)	100	0.0	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.061	0.008	0.077	0.010	NS
Low EC Control @ 149.4 µS/cm	0.073	0.006	0.065	0.006	NS
Upper Cache Slough at mouth of Ulatis Creek	0.102	0.007	0.097	0.004	NS
Sacramento River at tip of Grand Island (711)	0.091	0.008	0.089	0.003	NS
San Joaquin River at Potato Slough (815)	0.082	0.009	0.095	0.010	NS
Old River, western arm at railroad bridge (902)	0.078	0.005	0.097	0.005	NS
Old River at mouth of Holland Cut (915)	0.087	0.011	0.104	0.009	NS
Bottle Blank (amber cubitainer)	0.067	0.003	-	-	NA
Bottle Blank (clear cubitainer)	0.065	0.005	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard statistical protocols.
2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

Table B23-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/20/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Upper Cache Slough at mouth of Ulatis Creek	282	19.9	7.24	8.3	56.6	0.09	0.001
Sacramento River at tip of Grand Island (711)	144	20.7	6.61	8.4	8.0	0.38	0.001
San Joaquin River at Potato Slough (815)	205	21.8	6.58	8.4	7.5	0.10	0.000
Old River, western arm at railroad bridge (902)	229	21.8	6.58	8.4	6.6	0.08	0.000
Old River at mouth of Holland Cut (915)	320	22.7	6.43	8.3	4.7	0.03	0.000
Bottle Blank (amber cubitainer)	-	-	-	-	-	0.05	-
Bottle Blank (clear cubitainer)	-	-	-	-	-	0.03	-

Table B23-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 5/21/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/20/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (^oC)	Max Temp (^oC)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	334	23.0	24.5	7.4	8.6	7.68	8.03	104	60	-
Low EC Control @ 149.4 µS/cm	145	23.2	24.0	7.2	8.6	7.33	7.69	44	28	-
Upper Cache Slough at mouth of Ulatis Creek	236	23.0	24.5	7.1	8.8	7.83	8.19	108	92	0.006
Sacramento River at tip of Grand Island (711)	103	22.4	24.4	6.9	8.7	7.46	7.91	48	56	0.014
San Joaquin River at Potato Slough (815)	183	22.8	24.1	7.0	8.8	7.58	7.99	60	58	0.004
Old River, western arm at railroad bridge (902)	167	23.7	24.1	7.0	8.7	7.64	8.03	72	64	0.004
Old River at mouth of Holland Cut (915)	242	23.0	24.2	6.9	8.7	7.65	8.11	84	76	0.002
Bottle Blank (amber cubitainer)	261	22.9	24.8	7.3	8.7	7.75	8.05	104	60	0.002
Bottle Blank (clear cubitainer)	313	22.6	24.6	7.4	8.7	7.68	8.03	104	60	0.001
DIEPAMHR + 25 ppb PBO	329	22.5	24.4	7.4	8.5	7.71	8.06	-	-	-
Low EC Control @ 149.4 µS/cm + 25 ppb PBO	144	22.3	23.8	7.3	8.6	7.32	7.76	-	-	-
Upper Cache Slough at mouth of Ulatis Creek + 25 ppb PBO	235	22.1	24.5	7.1	8.6	7.81	8.18	-	-	-
Sacramento River at tip of Grand Island (711) + 25 ppb PBO	134	22.1	24.4	6.8	8.6	7.46	7.93	-	-	-
San Joaquin River at Potato Slough (815) + 25 ppb PBO	221	22.2	24.4	7.1	8.6	7.60	7.96	-	-	-
Old River, western arm at railroad bridge (902) + 25 ppb PBO	205	22.6	25.1	7.0	8.8	7.62	8.04	-	-	-
Old River at mouth of Holland Cut (915) + 25 ppb PBO	280	22.1	25.5	7.0	8.8	7.74	8.07	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B24-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 5/28/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/26/09 - 5/27/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	95	3.1	100	0.0	NS
Low EC Control @ 157.5 µS/cm	100	0.0	100	0.0	NS
High EC Control @ 14.50 mS/cm	100	0.0	98	2.5	NS
High EC Control @ 23.36 mS/cm	94	3.3	94	6.3	NS
Suisun Slough at Rush Ranch	100	0.0	100	0.0	NS
Napa River at River Park Blvd.	100	0.0	98	2.5	NS
Sacramento River at Hood DWR Station[3]	94	3.3	98	2.5	NS
Grizzly Bay at Dolphin (602)	97	2.8	98	2.5	NS
Suisun Bay off Chipps Island (508)	100	0.0	98	2.5	NS
Napa River at Vallejo Seawall (340)[5]	90	5.8	94	3.2	NS
Montezuma Slough at Nurse Slough (609)	98	2.5	98	2.5	NS
Carquinez Strait, West of Benicia army dock (405)[4]	98	2.5	100	0.0	NS
Bottle Blank: DIEPAMHR	100	0.0	-	-	NA
Field Dup.: Suisun Slough at Rush Ranch	98	2.5	-	-	NA
Field Dup.: Montezuma Slough at Nurse Slough (609)	100	0.0	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.035	0.006	0.078	0.006	S** (223%)
Low EC Control @ 157.5 µS/cm	0.036	0.005	0.045	0.008	NS
High EC Control @ 14.50 mS/cm	0.037	0.003	0.055	0.004	S** (149%)
High EC Control @ 23.36 mS/cm	0.037	0.006	0.036	0.007	NS
Suisun Slough at Rush Ranch	0.097	0.010	0.123	0.008	S* (127%)
Napa River at River Park Blvd.	0.093	0.011	0.084	0.006	NS
Sacramento River at Hood DWR Station[3]	0.087	0.006	0.090	0.008	NS
Grizzly Bay at Dolphin (602)	0.087	0.010	0.097	0.005	NS
Suisun Bay off Chipps Island (508)	0.080	0.003	0.098	0.005	S* (123%)
Napa River at Vallejo Seawall (340)[5]	0.048	0.004	0.055	0.005	NS
Montezuma Slough at Nurse Slough (609)	0.090	0.001	0.075	0.005	S* (83%)
Carquinez Strait, West of Benicia army dock (405)[4]	0.073	0.000	0.117	0.041	NS
Bottle Blank: DIEPAMHR	0.070	0.008	-	-	NA
Field Dup.: Suisun Slough at Rush Ranch	0.112	0.009	-	-	NA
Field Dup.: Montezuma Slough at Nurse Slough (609)	0.103	0.008	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard statistical protocols.
*: $P < 0.05$
**: $P < 0.01$
2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.
3. This low conductivity sample was compared to the Low EC Control.
4. This high conductivity sample was compared to the High EC Control @ 14.50 mS/cm.
5. This high conductivity sample was compared to the High EC Control @ 23.36 mS/cm.

Table B24-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/26/09 - 5/27/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Suisun Slough at Rush Ranch	4755	18.5	6.82	7.1	54.7	0.12	0.000
Napa River at River Park Blvd.	10530	20.1	6.86	8.5	13.0	0.11	0.000
Sacramento River at Hood DWR Station	152	20.7	6.55	8.3	15.3	0.33	0.000
Grizzly Bay at Dolphin (602)	8100	22.1	6.80	9.1	28.4	0.06	0.000
Suisun Bay off Chipps Island (508)	3924	19.5	6.54	9.1	36.9	0.09	0.000
Napa River at Vallejo Seawall (340)	22870	18.6	6.58	8.7	32.8	0.09	0.000
Montezuma Slough at Nurse Slough (609)	3446	21.1	6.66	8.1	137.7	0.16	0.000
Carquinez Strait, West of Benicia army dock (405)	14080	18.5	7.45	9.2	288.7	0.21	0.001
Field Dup.: Suisun Slough at Rush Ranch	4755	18.5	6.82	7.1	47.7	0.07	0.000
Field Dup.: Montezuma Slough at Nurse Slough (609)	3446	21.1	6.66	8.1	138.0	0.15	0.000

Table B24-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 5/28/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/26/09 - 5/27/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	332	23.5	23.7	7.5	8.6	7.59	8.18	104	60	-
Low EC Control @ 157.5 µS/cm	157	23.6	23.6	7.6	8.8	7.42	7.97	52	26	-
High EC Control @ 14.50 mS/cm	14125	23.6	23.6	7.1	8.1	7.65	7.89	1720	74	-
High EC Control @ 23.36 mS/cm	22435	23.4	23.7	7.1	8.1	7.72	7.87	2760	90	-
Suisun Slough at Rush Ranch	4504	23.5	23.9	7.3	8.5	7.81	8.13	520	142	0.003
Napa River at River Park Blvd.	10125	23.4	23.7	7.0	8.6	7.84	7.98	1240	128	0.003
Sacramento River at Hood DWR Station	146	23.5	23.7	7.1	8.4	7.62	7.82	52	54	0.010
Grizzly Bay at Dolphin (602)	7690	23.4	23.7	7.3	8.4	7.67	7.92	960	68	0.002
Suisun Bay off Chipps Island (508)	4089	23.3	23.7	7.4	8.6	7.65	7.89	500	64	0.003
Napa River at Vallejo Seawall (340)	21675	23.2	23.8	6.8	8.3	7.67	7.81	2640	100	0.001
Montezuma Slough at Nurse Slough (609)	3253	23.2	23.8	7.4	8.4	7.80	7.94	420	92	0.005
Carquinez Strait, West of Benicia army dock (405)	13450	23.2	23.7	7.2	8.1	7.61	7.81	1600	94	0.005
Bottle Blank 052609	343	23.2	23.8	7.5	8.6	7.77	8.18	104	60	-
Field Dup.: Suisun Slough at Rush Ranch	4542	23.2	23.8	7.3	8.7	7.90	8.13	52	154	0.002
Field Dup.: Montezuma Slough at Nurse Slough (609)	3292	23.2	23.8	7.5	8.9	7.78	7.96	420	84	0.006
DIEPAMHR + 25 ppb PBO	339	23.1	23.5	7.5	8.7	7.73	8.24	-	-	-
Low EC Control @ 157.5 µS/cm + 25 ppb PBO	160	23.1	23.5	7.7	8.9	7.46	8.01	-	-	-
High EC Control @ 14.50 mS/cm + 25 ppb PBO	13795	23.0	23.6	7.0	8.4	7.59	7.90	-	-	-
High EC Control @ 23.36 mS/cm + 25 ppb PBO	22405	23.1	23.7	6.9	8.2	7.65	7.88	-	-	-
Suisun Slough at Rush Ranch + 25 ppb PBO	4537.5	23.0	23.8	7.5	8.8	7.81	8.14	-	-	-
Napa River at River Park Blvd. + 25 ppb PBO	10045	23.0	23.9	7.4	8.7	7.90	8.00	-	-	-
Sacramento River at Hood DWR Station + 25 ppb PBO	159.2	23.0	23.8	7.3	8.8	7.63	8.00	-	-	-
Grizzly Bay at Dolphin (602) + 25 ppb PBO	7665	23.1	24.0	7.4	8.9	7.65	7.80	-	-	-
Suisun Bay off Chipps Island (508) + 25 ppb PBO	4036	23.0	24.1	7.4	8.8	7.60	7.94	-	-	-
Napa River at Vallejo Seawall (340) + 25 ppb PBO	21460	23.1	24.0	6.8	8.4	7.68	7.79	-	-	-
Montezuma Slough at Nurse Slough (609) + 25 ppb PBO	3294.5	23.0	23.8	7.6	8.8	7.81	8.07	-	-	-
Carquinez Strait, West of Benicia army dock (405) + 25 ppb PBO	13630	23.0	23.9	7.12	8.6	7.61	7.85	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B25-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 5/29/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/27/09 and 5/28/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	98	2.5	100	0.0	NS
Low EC Control @ 139.6 µS/cm	95	3.1	98	2.5	NS
Rough and Ready DWR station, Stockton	100	0.0	98	2.5	NS
Sacramento R. Deep Water Channel, Light 55[3]	98	2.5	100	0.0	NS
Confluence of Lindsey Sl. and Cache Sl.[3]	98	2.5	100	0.0	NS
Upper Cache Slough at mouth of Ulatis Creek[3]	100	0.0	98	2.5	NS
Sacramento River at tip of Grand Island (711)[3]	93	2.5	98	2.5	NS
Old River at mouth of Holland Cut (915)	95	2.9	100	0.0	NS
San Joaquin River at Potato Slough (815)[3]	98	2.5	100	0.0	NS
Old River, western arm at railroad bridge (902)[3]	100	0.0	98	2.5	NS

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.030	0.005	0.034	0.004	NS
Low EC Control @ 139.6 µS/cm	0.036	0.002	0.036	0.006	NS
Rough and Ready DWR station, Stockton	0.093	0.009	0.087	0.012	NS
Sacramento R. Deep Water Channel, Light 55[3]	0.082	0.008	0.075	0.011	NS
Confluence of Lindsey Sl. and Cache Sl.[3]	0.068	0.010	0.081	0.008	NS
Upper Cache Slough at mouth of Ulatis Creek[3]	0.082	0.003	0.083	0.006	NS
Sacramento River at tip of Grand Island (711)[3]	0.045	0.009	0.074	0.014	NS
Old River at mouth of Holland Cut (915)	0.094	0.009	0.094	0.006	NS
San Joaquin River at Potato Slough (815)[3]	0.079	0.030	0.085	0.009	NS
Old River, western arm at railroad bridge (902)[3]	0.091	0.011	0.100	0.013	NS

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard statistical protocols.

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

3. These low conductivity samples were compared to the Low EC Control.

Table B25-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/27/09 - 5/28/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^{o}C)	pH	DO (mg/L)			
Rough and Ready DWR station, Stockton	435	25.8	7.08	6.0	13.3	0.09	0.001
Sacramento R. Deep Water Channel, Light 55	215	20.9	7.39	8.4	32.2	0.14	0.001
Confluence of Lindsey Sl. and Cache Sl.	188	21.1	7.52	8.4	37.8	0.11	0.001
Upper Cache Slough at mouth of Ulatis Creek	243	21.2	7.5	8.4	63.5	0.04	0.001
Sacramento River at tip of Grand Island (711)	151	21.8	7.54	8.4	11.6	0.22	0.003
Old River at mouth of Holland Cut (915)	286	24.1	7.18	7.7	6.4	0.00	0.000
San Joaquin River at Potato Slough (815)	176	22.7	7.27	8.2	6.3	0.00	0.000
Old River, western arm at railroad bridge (902)	231	23.6	7.29	8.0	7.2	0.00	0.000

Table B25-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 5/29/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 5/27/09 - 5/28/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	339	23.1	23.5	7.3	8.4	7.66	8.08	108	62	-
Low EC Control @ 139.6 µS/cm	145	23.3	23.6	7.2	8.8	7.37	7.83	40	25	-
Rough and Ready DWR station, Stockton	422	23.3	23.5	7.0	8.7	7.70	7.82	96	68	0.002
Sacramento R. Deep Water Channel, Light 55	194	23.3	23.6	6.9	8.7	7.70	7.94	64	66	0.006
Confluence of Lindsey Sl. and Cache Sl.	177	23.3	23.5	6.6	8.7	7.70	7.96	64	62	0.005
Upper Cache Slough at mouth of Ulatis Creek	229	23.3	23.5	6.4	8.9	7.75	8.00	76	80	0.002
Sacramento River at tip of Grand Island (711)	145	23.3	23.5	6.9	8.5	7.62	7.75	52	54	0.006
Old River at mouth of Holland Cut (915)	271	23.3	23.5	6.3	8.6	7.80	7.87	84	70	0.000
San Joaquin River at Potato Slough (815)	172	23.3	23.5	6.4	8.8	7.62	7.92	56	52	0.000
Old River, western arm at railroad bridge (902)	214	23.3	23.5	6.3	8.8	7.69	7.94	68	60	0.000
DIEPAMHR + 25 ppb PBO	338	23.4	23.5	7.0	8.8	7.65	8.09	-	-	-
Low EC Control @ 139.6 µS/cm + 25 ppb PBO	146	23.4	23.5	6.7	8.6	7.31	7.84	-	-	-
Rough and Ready DWR station, Stockton + 25 ppb PBO	423	23.3	23.5	6.7	8.4	7.71	7.80	-	-	-
Sacramento R. Deep Water Channel, Light 55 + 25 ppb PBO	197	23.5	23.5	6.4	8.8	7.69	7.93	-	-	-
Confluence of Lindsey Sl. and Cache Sl. + 25 ppb PBO	181	23.4	23.5	6.6	8.6	7.70	7.96	-	-	-
Upper Cache Slough at mouth of Ulatis Creek + 25 ppb PBO	232	23.4	23.5	6.4	8.8	7.77	8.10	-	-	-
Sacramento River at tip of Grand Island (711) + 25 ppb PBO	144	23.6	23.9	6.3	8.4	7.61	8.00	-	-	-
Old River at mouth of Holland Cut (915) + 25 ppb PBO	266	23.6	23.7	6.2	8.9	7.77	8.40	-	-	-
San Joaquin River at Potato Slough (815) + 25 ppb PBO	164	23.7	23.7	6.2	8.5	7.61	7.90	-	-	-
Old River, western arm at railroad bridge (902) + 25 ppb PBO	209	23.6	23.8	6.1	8.7	7.74	7.99	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B26-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 6/11/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 6/09/09 - 6/10/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	95	2.9	98	2.5	NS
Low EC Control @ 161.5 µS/cm	90	7.1	95	3.1	NS
High EC Control @ 14.06 mS/cm	95	3.1	95	2.9	NS
High EC Control @ 23.81 mS/cm	73	4.8	78	3.9	NS
Suisun Slough at Rush Ranch	100	0.0	100	0.0	NS
Sacramento River at Hood DWR Station[3]	87	4.7	74	6.6	NS
Napa River at River Park Blvd.[4]	98	2.5	95	2.8	NS
Rough and Ready DWR station, Stockton	100	0.0	95	5.0	NS
Napa River at Vallejo Seawall (340)[5]	74	1.6	91	5.4	NS
Montezuma Slough at Nurse Slough (609)	100	0.0	98	2.5	NS
Suisun Bay off Chipps Island (508)	100	0.0	100	0.0	NS
Grizzly Bay at Dolphin (602)	97	2.8	100	0.0	NS
Carquinez Strait, West of Benicia army dock (405)[4]	100	0.0	98	2.5	NS
Trip Blank: DIEPAMHR	97	3.1	-	-	NA
Bottle Blank: DIEPAMHR	92	2.6	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.053	0.009	0.042	0.004	NS
Low EC Control @ 161.5 µS/cm	0.044	0.003	0.036	0.007	NS
High EC Control @ 14.06 mS/cm	0.048	0.006	0.045	0.008	NS
High EC Control @ 23.81 mS/cm	0.033	0.002	0.028	0.004	NS
Suisun Slough at Rush Ranch	0.070	0.005	0.074	0.007	NS
Sacramento River at Hood DWR Station[3]	0.062	0.006	0.051	0.012	NS
Napa River at River Park Blvd.[4]	0.053	0.001	0.040	0.003	S** (75%)
Rough and Ready DWR station, Stockton	0.058	0.009	0.062	0.004	NS
Napa River at Vallejo Seawall (340)[5]	0.038	0.006	0.028	0.003	NS
Montezuma Slough at Nurse Slough (609)	0.068	0.006	0.064	0.005	NS
Suisun Bay off Chipps Island (508)	0.066	0.003	0.063	0.004	NS
Grizzly Bay at Dolphin (602)	0.066	0.011	0.065	0.006	NS
Carquinez Strait, West of Benicia army dock (405)[4]	0.048	0.005	0.057	0.003	NS
Trip Blank: DIEPAMHR	0.040	0.004	-	-	NA
Bottle Blank: DIEPAMHR	0.038	0.007	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control.

*: $P < 0.05$

**: $P < 0.01$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.
3. This low conductivity sample was compared to the Low EC Control.
4. These high conductivity samples were compared to the High EC Control @ 14.06 mS/cm.
5. This high conductivity sample was compared to the High EC Control @ 23.81 mS/cm.

Table B26-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 6/9/09 - 6/10/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Suisun Slough at Rush Ranch	5680	18.0	7.37	7.2	51.3	0.09	0.001
Sacramento River at Hood DWR Station	171	21.3	7.48	8.3	16.4	0.33	0.004
Napa River at River Park Blvd.	13480	21.0	7.85	8.8	18.7	0.03	0.001
Rough and Ready DWR station, Stockton	552	23.3	7.51	6.2	12.7	0.07	0.001
Napa River at Vallejo Seawall (340)	23140	18.1	7.78	8.8	21.3	0.10	0.001
Montezuma Slough at Nurse Slough (609)	4481	19.6	7.7	8.5	63.5	0.12	0.002
Suisun Bay off Chipps Island (508)	2506	19.0	7.85	9.2	30.4	0.12	0.003
Grizzly Bay at Dolphin (602)	7520	18.7	8	9.3	129.3	0.13	0.003
Carquinez Strait, West of Benicia army dock (405)	12010	18.1	7.84	9.2	105.7	0.17	0.003
Trip Blank: DIEPAMHR	-	-	-	-	0.5	0.03	-
Bottle Blank: DIEPAMHR	-	-	-	-	0.3	0.04	-

Table B26-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 6/11/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 6/9/09 - 6/10/09.

Treatment	Laboratory Chemistry							Hardness	Alkalinity	Unionized
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH	(mg/L as $CaCO_3$)	(mg/L as $CaCO_3$)	Ammonia (mg/L)[1]
DIEPAMHR	335	23.0	23.7	7.5	8.4	7.81	8.24	88	60	-
Low EC Control @ 161.5 µS/cm	157	23.0	23.4	7.6	8.6	7.43	8.27	48	22	-
High EC Control @ 14.06 mS/cm	13325	23.0	23.9	7.4	8.2	7.67	8.00	1640	96	-
High EC Control @ 23.81 mS/cm	22670	23.0	23.5	7.0	7.9	7.63	8.00	2800	88	-
Suisun Slough at Rush Ranch	5300	23.0	23.8	7.4	8.7	8.04	8.25	620	160	0.004
Sacramento River at Hood DWR Station	163	23.1	23.5	7.1	8.7	7.67	8.16	60	68	0.021
Napa River at River Park Blvd.	12740	23.1	24.0	7.0	8.3	7.94	8.07	1560	136	0.001
Rough and Ready DWR station, Stockton	545	23.0	23.7	7.2	8.7	7.82	8.20	148	76	0.005
Napa River at Vallejo Seawall (340)	22180	23.0	24.2	6.5	8.2	7.67	7.90	2760	108	0.002
Montezuma Slough at Nurse Slough (609)	4412	23.0	23.9	7.4	8.5	7.62	8.10	480	84	0.006
Suisun Bay off Chipps Island (508)	2269	23.1	24.2	7.6	8.4	7.80	8.03	272	74	0.005
Grizzly Bay at Dolphin (602)	7445	23.1	23.8	7.4	8.9	7.70	7.98	920	76	0.005
Carquinez Strait, West of Benicia army dock (405)	12135	23.1	24.1	7.0	8.2	7.69	7.90	1360	80	0.005
Trip Blank: DIEPAMHR	351	23.1	23.5	7.4	8.7	7.85	8.24	104	62	0.002
Bottle Blank: DIEPAMHR	338	23.1	24.2	7.5	8.6	7.80	8.17	104	64	0.003
DIEPAMHR + 25 ppb PBO	338	23.2	23.9	7.4	8.3	7.82	8.22	-	-	-
Low EC Control @ 161.5 µS/cm + 25 ppb PBO	160	23.2	24.0	7.5	8.4	7.46	8.16	-	-	-
High EC Control @ 14.06 mS/cm + 25 ppb PBO	13375	23.2	23.9	7.3	8.1	7.70	7.98	-	-	-
High EC Control @ 23.81 mS/cm + 25 ppb PBO	22580	23.2	24.1	7.1	8.3	7.74	8.02	-	-	-
Suisun Slough at Rush Ranch + 25 ppb PBO	5380	23.2	24.0	7.4	8.2	8.09	8.29	-	-	-
Sacramento River at Hood DWR Station + 25 ppb PBO	163.85	23.3	24.2	7.1	8.9	7.65	8.09	-	-	-
Napa River at River Park Blvd. + 25 ppb PBO	12835	23.3	24.0	7.0	8.2	7.93	8.03	-	-	-
Rough and Ready DWR station, Stockton + 25 ppb PBO	531.5	23.4	24.2	7.3	8.5	7.83	8.04	-	-	-
Napa River at Vallejo Seawall (340) + 25 ppb PBO	22175	23.3	24.1	6.7	8.3	7.70	7.83	-	-	-
Montezuma Slough at Nurse Slough (609) + 25 ppb PBO	4334.5	23.4	24.1	7.4	8.5	7.86	8.02	-	-	-
Suisun Bay off Chipps Island (508) + 25 ppb PBO	2249.5	23.3	24.2	7.4	8.2	7.77	8.09	-	-	-
Grizzly Bay at Dolphin (602) + 25 ppb PBO	7420	23.4	23.9	7.5	8.2	7.60	7.97	-	-	-
Carquinez Strait, West of Benicia army dock (405) + 25 ppb PBO	12080	23.4	24.1	7.2	8.4	7.72	7.96	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B27-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 6/12/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 6/11/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	87	3.0	95	2.9	S* (109%)
Low EC Control @ 168.2 µS/cm	90	4.1	84*	3.2	NS
Sacramento R. Deep Water Channel, Light 55	95	5.0	89	4.1	NS
Upper Cache Slough at mouth of Ulatis Creek[3]	92	5.3	82	2.6	NS
Confluence of Linsey Sl. And Cache Sl.[3]	81	11.2	95	3.1	NS
Sacramento River at tip of Grand Island (711)[3]	78	5.7	72	8.4	NS
San Joaquin River at Potato Slough (815)[3]	93	7.5	93	2.5	NS
Old River, western arm at railroad bridge (902)[3]	98	2.5	84	5.2	NS
Old River at mouth of Holland Cut (915)	92	5.3	90	7.1	NS
Field Dup.: San Joaquin River at Potato Slough (815)[3]	92	5.3	-	-	NA
Bottle Blank: DIEPAMHR	84	2.6	-	-	NA
Bottle Blank: Clear Plastic	86	5.9	-	-	NA
Bottle Blank: Amber Plastic	95	5.0	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.027	0.009	0.037	0.003	NS
Low EC Control @ 168.2 µS/cm	0.029	0.008	0.042	0.007	NS
Sacramento R. Deep Water Channel, Light 55	0.086	0.006	0.064	0.002	S* (74%)
Upper Cache Slough at mouth of Ulatis Creek[3]	0.075	0.014	0.084	0.012	NS
Confluence of Linsey Sl. And Cache Sl.[3]	0.067	0.003	0.068	0.006	NS
Sacramento River at tip of Grand Island (711)[3]	0.056	0.006	0.073	0.009	NS
San Joaquin River at Potato Slough (815)[3]	0.079	0.005	0.091	0.009	NS
Old River, western arm at railroad bridge (902)[3]	0.081	0.004	0.060	0.009	S* (74%)
Old River at mouth of Holland Cut (915)	0.070	0.003	0.078	0.004	NS
Field Dup.: San Joaquin River at Potato Slough (815)[3]	0.045	0.009	-	-	NA
Bottle Blank: DIEPAMHR	0.048	0.006	-	-	NA
Bottle Blank: Clear Plastic	0.043	0.007	-	-	NA
Bottle Blank: Amber Plastic	0.060	0.003	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard statistical protocols.

*: $P < 0.05$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

3. These low conductivity samples were compared to the Low EC Control.

Table B27-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 6/11/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Sacramento R. Deep Water Channel, Light 55	255	19.6	7.96	8.9	44.7	0.10	0.003
Upper Cache Slough at mouth of Ulatis Creek	214	18.9	7.96	9.0	101.9	0.08	0.002
Confluence of Linsey Sl. And Cache Sl.	183	19.2	7.86	8.8	51.3	0.07	0.002
Sacramento River at tip of Grand Island (711)	170	20.2	7.66	8.6	11.4	0.10	0.002
San Joaquin River at Potato Slough (815)	182	20.7	7.87	8.9	6.7	0.00	0.000
Old River, western arm at railroad bridge (902)	213	21.7	7.80	8.5	6.3	0.00	0.000
Old River at mouth of Holland Cut (915)	271	22.1	7.80	8.1	5.2	0.00	0.000
Field Dup.: San Joaquin River at Potato Slough (815)	182	20.7	7.87	8.9	6.3	0.00	0.000
Bottle Blank: DIEPAMHR	-	-	-	-	0.5	0.00	-
Bottle Blank: Clear Plastic	-	-	-	-	-	-	-
Bottle Blank: Amber Plastic	-	-	-	-	-	-	-

Table B27-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 6/12/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 6/11/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (^oC)	Max Temp (^oC)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	337	22.8	24.3	7.3	8.1	7.72	8.11	88	60	-
Low EC Control @ 168.2 µS/cm	172	22.8	24.2	7.2	8.4	7.54	7.97	52	30	-
Sacramento R. Deep Water Channel, Light 55	248	22.8	24.4	7.2	8.3	7.64	8.10	76	72	0.006
Upper Cache Slough at mouth of Ulatis Creek	199	22.8	24.3	7.4	8.4	7.78	8.22	68	70	0.006
Confluence of Linsey Sl. And Cache Sl.	180	22.9	24.3	7.2	8.4	7.71	8.08	64	64	0.004
Sacramento River at tip of Grand Island (711)	163	23.0	24.4	7.1	8.3	7.68	8.03	56	64	0.005
San Joaquin River at Potato Slough (815)	178	22.9	24.4	7.1	8.4	7.63	8.09	64	56	0.000
Old River, western arm at railroad bridge (902)	210	22.9	24.4	7.3	8.2	7.71	8.12	60	60	0.000
Old River at mouth of Holland Cut (915)	261	23.0	24.3	7.4	8.4	7.74	8.06	72	62	0.000
Field Dup.: San Joaquin River at Potato Slough (815)	181	23.1	24.4	7.1	8.7	7.69	7.96	60	58	0.000
Bottle Blank: DIEPAMHR	340	23.1	24.3	7.3	8.3	7.76	8.11	104	58	0.000
Bottle Blank: Clear Plastic	339	23.2	24.3	7.5	8.4	7.76	8.10	88	60	-
Bottle Blank: Amber Plastic	342	23.2	24.4	7.4	8.8	7.76	8.11	88	60	-
DIEPAMHR + 25 ppb PBO	339	23.2	23.7	7.2	8.2	7.78	8.19	-	-	-
Low EC Control @ 168.2 µS/cm + 25 ppb PBO	172	23.3	23.6	7.3	8.5	7.48	7.94	-	-	-
Sacramento R. Deep Water Channel, Light 55 + 25 ppb PBO	246	23.3	23.9	7.4	8.2	7.78	8.13	-	-	-
Upper Cache Slough at mouth of Ulatis Creek + 25 ppb PBO	201	23.3	23.9	7.6	8.7	7.79	8.20	-	-	-
Confluence of Linsey Sl. And Cache Sl. + 25 ppb PBO	180	23.5	23.7	7.3	8.3	7.73	8.11	-	-	-
Sacramento River at tip of Grand Island (711) + 25 ppb PBO	163	23.4	23.9	7.3	8.6	7.66	8.06	-	-	-
San Joaquin River at Potato Slough (815) + 25 ppb PBO	179	23.5	23.9	7.3	8.4	7.71	8.08	-	-	-
Old River, western arm at railroad bridge (902) + 25 ppb PBO	208	23.6	23.9	7.3	8.4	7.74	8.02	-	-	-
Old River at mouth of Holland Cut (915) + 25 ppb PBO	264	23.6	23.8	7.3	8.4	7.78	8.06	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B28-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 6/25/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 6/23/09 - 6/24/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	92	2.7	95	2.9	NS
Low EC Control @ 140.9 µS/cm	95	2.6	91	5.1	NS
High EC Control @ 12.53 mS/cm	100	0.0	95	5.0	NS
High EC Control @ 17.69 mS/cm	98	2.5	93	4.4	NS
High EC Control @ 20.23 mS/cm	80	12.2	79	4.1	NS
Napa River, near River Park Blvd. [5]	92	5.3	93	7.5	NS
Suisun Slough @ Rush Ranch	98	2.5	97	2.8	NS
Rough and Ready DWR Station, Stockton	97	2.8	100	0.0	NS
Sacramento River at Hood DWR Station [3]	87*	3.0	66**	6.1	S* (76%)
Carquinez Strait, West of Benicia army dock (405) [6]	87	10.2	90	6.7	NS
Montezuma Slough at Nurse Slough (609)	100	0.0	100	0.0	NS
Suisun Bay off Chipps Island (508)	100	0.0	100	0.0	NS
Grizzly Bay at Dolphin (602) [4]	97	2.8	100	0.0	NS
Trip Blank (DIEPAMHR)	97	2.8	-	-	NA
Trip Blank (DIEPAMHR)	98	2.5	-	-	NA
Field Dup: Carquinez Strait, West of Benicia army dock (405) [6]	91	5.4	-	-	NA
Field Dup: Suisun Bay off Chipps Island (508)	100	0.0	-	-	NA

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.041	0.012	0.049	0.004	NS
Low EC Control @ 140.9 µS/cm	0.041	0.004	0.042	0.005	NS
High EC Control @ 12.53 mS/cm	0.044	0.006	0.048	0.006	NS
High EC Control @ 17.69 mS/cm	0.037	0.004	0.042	0.005	NS
High EC Control @ 20.23 mS/cm	0.037	0.001	0.028	0.006	NS
Napa River, near River Park Blvd. [5]	0.043	0.005	0.042	0.005	NS
Suisun Slough @ Rush Ranch	0.089	0.011	0.093	0.017	NS
Rough and Ready DWR Station, Stockton	0.075	0.005	0.133	0.029	NS
Sacramento River at Hood DWR Station [3]	0.035	0.006	0.068	0.015	NS
Carquinez Strait, West of Benicia army dock (405) [6]	0.043	0.012	0.026	0.007	NS
Montezuma Slough at Nurse Slough (609)	0.063	0.005	0.060	0.007	NS
Suisun Bay off Chipps Island (508)	0.052	0.009	0.047	0.010	NS
Grizzly Bay at Dolphin (602) [4]	0.068	0.004	0.060	0.004	NS
Trip Blank (DIEPAMHR)	0.061	0.005	-	-	NA
Trip Blank (DIEPAMHR)	0.046	0.005	-	-	NA
Field Dup: Carquinez Strait, West of Benicia army dock (405) [6]	0.057	0.006	-	-	NA
Field Dup: Suisun Bay off Chipps Island (508)	0.061	0.012	-	-	NA

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control.

*: $P < 0.05$

**: $P < 0.01$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.
3. This low conductivity sample was compared to the Low EC Control.
4. This high conductivity sample was compared to the High EC Control @ 12.53 mS/cm.
5. This high conductivity sample was compared to the High EC Control @ 17.69 mS/cm.
6. These high conductivity samples were compared to the High EC Control @ 20.23 mS/cm.

Table B28-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 6/23/09 - 6/24/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^{o}C)	pH	DO (mg/L)			
Napa River, Near River Park Blvd.	16260	24.2	7.34	6.0	14.0	0.04	0.000
Suisun Slough at Rush Ranch	6460	20.4	7.13	5.5	51.1	0.15	0.001
Rough and Ready DWR Station, Stockton	552	23.3	7.51	6.2	11.5	0.09	0.001
Sacramento River at Hood DWR Station	149	23.2	7.11	7.5	20.4	0.30	0.002
Carquinez Strait, west of Benicia army dock (405)	19430	19.7	7.45	8.9	240.7	0.20	0.001
Montezuma Slough at Nurse Slough (609)	5750	22.0	7.46	7.5	73.6	0.11	0.001
Suisun Bay, off Chipps Island (508)	8510	20.5	7.6	8.8	24.6	0.08	0.001
Grizzly Bay at Dolphin (602)	1190	21.1	7.75	8.9	177.3	0.17	0.004
Trip Blank (DIEPAMHR) 6/23/09	-	-	-	-	0.4	0.02	-
Trip Blank (DIEPAMHR) 6/24/09	-	-	-	-	0.3	0.00	-
Field Dup: Carquinez Strait, west of Benicia army dock (405)	19430	19.7	7.45	8.9	276.3	0.27	0.002
Field Dup: Suisun Bay, off Chipps Island (508)	8510	20.5	7.60	8.8	24.4	0.10	0.001

Table B28-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 6/25/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 6/23/09 - 6/24/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	280	22.7	23.2	7.1	8.4	6.90	8.13	100	58	-
Low EC Control @ 140.9 µS	149	22.8	23.1	7.4	8.7	6.98	7.84	40	26	-
High EC Control @ 12.53 mS	11240	22.9	23.3	7.3	8.6	6.90	7.91	1440	74	-
High EC Control @ 17.69 mS	16970	22.9	23.6	7.1	8.6	6.99	8.00	1960	84	-
High EC Control @ 20.23 mS	19720	22.8	23.4	7.2	8.3	7.20	7.99	2360	84	-
Napa River, near River Park Blvd.	16855	22.9	23.8	7.0	8.5	7.42	7.75	2280	134	0.001
Suisun Slough @ Rush Ranch	6265	22.9	23.9	7.0	8.5	7.56	7.97	710	138	0.004
Rough and Ready DWR Station, Stockton	626	22.9	23.7	7.3	8.9	7.50	8.00	130	82	0.004
Sacramento River at Hood DWR Station	148	22.9	23.7	6.9	8.9	7.07	7.67	48	54	0.007
Carquinez Strait, West of Benicia army dock (405)	18475	22.9	23.6	6.6	8.0	7.04	7.78	2160	86	0.004
Montezuma Slough at Nurse Slough (609)	5585	22.6	23.6	7.3	8.8	7.39	7.78	620	92	0.003
Suisun Bay off Chipps Island (508)	7740	22.9	23.5	5.9	8.5	7.15	7.81	840	70	0.002
Grizzly Bay at Dolphin (602)	11470	22.8	23.7	7.0	8.9	7.26	7.85	1320	76	0.004
Trip Blank (DIEPAMHR)	417	22.9	24.1	7.5	8.7	7.28	8.25	104	58	0.002
Trip Blank (DIEPAMHR)	390	22.9	24.3	7.1	8.6	7.37	8.25	100	58	0.000
Field Dup: Carquinez Strait, West of Benicia army dock (405)	18880	22.9	23.4	7.0	8.4	7.25	7.75	2160	84	0.005
Field Dup: Suisun Bay off Chipps Island (508)	8180	22.8	23.0	7.2	8.7	7.25	7.77	840	70	0.002
DIEPAMHR + 25 ppb PBO	410	22.5	23.0	7.3	8.3	7.13	8.13	-	-	-
Low EC Control @ 140.9 µS + 25 ppb PBO	161	23.1	23.2	7.1	8.6	7.13	7.92	-	-	-
High EC Control @ 12.53 mS + 25 ppb PBO	12060	23.0	23.1	6.9	8.5	6.94	7.97	-	-	-
High EC Control @ 17.69 mS + 25 ppb PBO	16970	23.1	23.3	6.9	8.7	7.23	7.97	-	-	-
High EC Control @ 20.23 mS + 25 ppb PBO	19670	23.2	23.2	7.0	8.4	7.29	8.00	-	-	-
Napa River, near River Park Blvd. + 25 ppb PBO	16855	23.2	23.3	6.8	8.3	7.37	7.83	-	-	-
Suisun Slough @ Rush Ranch + 25 ppb PBO	6395	23.0	23.2	7.0	8.8	7.46	7.96	-	-	-
Rough and Ready DWR Station, Stockton + 25 ppb PBO	630	23.2	23.6	7.2	8.5	7.26	8.14	-	-	-
Sacramento River at Hood DWR Station + 25 ppb PBO	151	23.2	23.8	7.0	8.5	7.15	7.89	-	-	-
Carquinez Strait, West of Benicia army dock (405) + 25 ppb PBO	18965	23.2	23.2	6.6	8.0	7.11	7.77	-	-	-
Montezuma Slough at Nurse Slough (609) + 25 ppb PBO	5640	23.3	23.8	7.4	8.5	7.32	7.81	-	-	-
Suisun Bay off Chipps Island (508) + 25 ppb PBO	8000	23.2	23.4	7.1	8.8	7.18	8.83	-	-	-
Grizzly Bay at Dolphin (602) + 25 ppb PBO	11490	23.3	23.3	7.0	8.5	7.30	7.90	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Table B29-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 6/26/09 examining the toxicity of samples collected by the UC Davis Aquatic Toxicology Laboratory and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 6/25/09.

Treatment	Survival (%)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	97	3.1	95	2.8	NS
Low EC Control @ 132.6 µS/cm	89	6.4	77	6.1	NS
Sacramento River Deep Water Channel, Light 55	87	3.0	88	4.8	NS
Upper Cache Slough at mouth of Ulatis Creek	84	7.1	74	11.6	NS
Confluence of Lindsey Sl. and Cache Sl.	74	15.4	89	0.6	NS
Sacramento River at tip of Grand Island (711) [3]	45**	7.6	61*	4.2	NS
San Joaquin River at Potato Slough (815)	85	11.9	84	9.7	NS
Old River, western arm at railroad bridge (902)	90	7.1	85*	4.2	NS
Old River at mouth of Holland Cut (915)	85	6.4	88	7.5	NS
Bottle Blank Clear (cubitainer)	78	7.9	-	-	NS
Bottle Blank Amber (cubitainer)	93	2.5	-	-	NS

Treatment	Weight (mg/surviving individual)[1]				
	Unmanipulated		25 ppb PBO added		
	mean	se	mean	se	vs Non-PBO[2]
DIEPAMHR	0.046	0.010	0.044	0.005	NS
Low EC Control @ 132.6 µS/cm	0.042	0.007	0.035	0.003	NS
Sacramento River Deep Water Channel, Light 55	0.072	0.011	0.057	0.002	NS
Upper Cache Slough at mouth of Ulatis Creek	0.085	0.005	0.072	0.004	NS
Confluence of Lindsey Sl. and Cache Sl.	0.083	0.007	0.043	0.007	S** (52%)
Sacramento River at tip of Grand Island (711) [3]	0.075	0.013	0.054	0.011	NS
San Joaquin River at Potato Slough (815)	0.067	0.009	0.058	0.006	NS
Old River, western arm at railroad bridge (902)	0.061	0.009	0.077	0.009	NS
Old River at mouth of Holland Cut (915)	0.055	0.010	0.078	0.007	S* (142%)
Bottle Blank Clear (cubitainer)	0.038	0.010	-	-	NS
Bottle Blank Amber (cubitainer)	0.026	0.003	-	-	NS

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard statistical protocols.

*: $P < 0.05$

**: $P < 0.01$

2. NS: Nonsignificant, S: Significant (% non-PBO mean), NA: Not applicable.

3. This low conductivity sample was compared to the Low EC Control @ 312.6 uS/cm.

Table B29-2. Summary of water chemistry at field conditions of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 6/25/09.

Treatment	Field Chemistry				Turbidity (NTU)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)
	SC (uS/cm)	Temp (^oC)	pH	DO (mg/L)			
Sacramento River, Deep Water Channel, Light 55	246	22.0	7.69	8.5	29.7	0.05	0.001
Upper Cache Slough at Mouth of Ulatis Creek	207	20.9	7.62	8.8	60.8	0.04	0.001
Confluence of Lindsey Sl. and Cache Sl.	188	22.1	7.5	8.6	27.9	0.10	0.001
Sacramento River at tip of Grand Island (711)	134	23.2	7.37	8.1	10.6	0.19	0.002
San Joaquin River at Potato Slough (815)	182	21.9	7.47	8.5	6.3	0.03	0.000
Old River, western arm at railroad bridge (902)	204	22.4	7.90	8.5	5.3	0.03	0.001
Old River at mouth of Holland Cut (915)	217	23.3	7.63	7.9	4.3	0.01	0.000

Table B29-3. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 6/26/09 of samples collected by the the UC Davis Aquatic Toxicology Laboratory (UCDATL) and the California Department of Fish and Game (CDFG) for the Department of Water Resources (DWR) on 6/25/09.

Treatment	Laboratory Chemistry							Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unionized Ammonia (mg/L)[1]
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH			
DIEPAMHR	334	22.5	23.1	6.8	8.3	7.52	8.02	-	-	-
Low EC Control @ 132.6 µS/cm	128	22.5	23.4	7.3	8.4	7.26	7.79	40	26	-
Sacramento River Deep Water Channel, Light 55	232	22.6	23.2	6.5	8.5	7.57	8.04	76	74	0.002
Upper Cache Slough at mouth of Ulatis Creek	196	22.3	23.3	7.0	8.6	7.43	8.08	68	74	0.002
Confluence of Lindsey Sl. and Cache Sl.	174	22.5	23.2	6.7	8.6	7.42	7.97	72	66	0.004
Sacramento River at tip of Grand Island (711)	127	22.3	23.3	6.9	8.4	7.39	7.80	60	52	0.006
San Joaquin River at Potato Slough (815)	171	22.3	22.9	6.9	8.7	7.41	8.02	64	60	0.001
Old River, western arm at railroad bridge (902)	194	22.5	23.4	6.8	8.5	7.42	8.00	60	58	0.001
Old River at mouth of Holland Cut (915)	217	22.8	23.3	7.0	8.4	7.53	7.93	72	58	0.000
Bottle Blank Clear (cubitainer)	324	22.4	23.1	6.9	8.5	7.44	8.06	-	-	-
Bottle Blank Amber (cubitainer)	325	22.8	23.2	7.1	8.6	7.39	8.08	-	-	-
DIEPAMHR + 25 ppb PBO	325	22.5	23.3	7.1	8.3	7.59	8.06	-	-	-
Low EC Control @ 132.6 µS + 25 ppb PBO	134	22.2	23.6	7.1	8.5	7.23	7.75	-	-	-
Sacramento River Deep Water Channel, Light 55 + 25 ppb PBO	234	22.7	23.4	6.4	8.6	7.55	8.00	-	-	-
Upper Cache Slough at mouth of Ulatis Creek + 25 ppb PBO	191	22.3	22.4	6.9	8.5	7.40	8.02	-	-	-
Confluence of Lindsey Sl. and Cache Sl. + 25 ppb PBO	172	22.4	23.5	6.9	8.5	7.46	7.99	-	-	-
Sacramento River at tip of Grand Island (711) + 25 ppb PBO	131	22.4	23.5	7.2	8.7	7.41	7.89	-	-	-
San Joaquin River at Potato Slough (815) + 25 ppb PBO	180	22.5	23.3	6.6	8.7	7.33	8.07	-	-	-
Old River, western arm at railroad bridge (902) + 25 ppb PBO	212	22.8	23.6	7.0	8.5	7.38	7.95	-	-	-
Old River at mouth of Holland Cut (915) + 25 ppb PBO	194	22.3	23.3	6.8	8.8	7.53	8.00	-	-	-

1: This unionized ammonia reading is based on the ammonia nitrogen measured upon sample receipt and upon the water chemistry measured at test initiation.

Appendix C

Hypomesus transpacificus
Ambient Sample Toxicity
96-hour and 7-day Survival

Table C1-1. Results of a *H. transpacificus* (Delta Smelt) 7-day test initiated 3/19/09 evaluating the toxicity of ambient water samples collected on 3/17/09, 3/18/09 and 3/19/09. Test animals were 30 days old at test initiation.

Treatment	Survival (%)		EC-specific Statistical Results[1]
	mean	se	
Low EC Control	8.3	5.3	A
Low EC Low Turbidity Control	2.8	2.8	A
Hood[2]	8.7	2.9	A
Light 55[2]	23.6	9.2	A
Cache Lindsey[2]	2.8	2.8	A
Mid EC Control	15.3	6.4	A
Rough and Ready Island[3]	2.8	2.8	A
High EC Control	18.6	7.9	B
High EC Low Turbidity Control	18.1	6.4	B
Suisun[4]	95.0	5.0	A
340[4]	88.8	4.1	A

1. Data were analyzed using separate statistical tests for each EC bracket (low, mid, high). The low and high EC brackets were examined using Tukey's tests, while the intermediate EC bracket was examined using a T-test (all tests were two-tailed, $\alpha = 0.05$). Statistically different groups of treatments are identified by different letters. Due to the poor performance of the controls, USEPA standard statistics were not performed.

2. These low conductivity samples were compared to the Low EC controls.

3. This intermediate conductivity sample was compared to the Mid EC Control.

4. These high conductivity samples were compared to the High EC controls.

Table C1-2. Chemistry of sample waters examined in a *H. transpacificus* (Delta Smelt) 7-day test initiated 3/19/09 evaluating the toxicity of Sacramento River and Delta water samples collected on 3/17/09, 3/18/09 and 3/19/09.

Treatment	Temp (^oC)			EC (uS/cm)			SC (uS/cm)			DO (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Low EC Control	16.3	0.9	8	172	9	8	206	12	8	9.1	0.3	8
Suisun	16.1	1.1	8	3234	148	8	3922	156	8	8.9	0.2	8
Hood	16.0	1.0	8	167	10	8	201	13	8	9.4	0.3	8
Light 55	16.0	1.0	8	296	6	8	357	3	8	9.3	0.2	8
Cache Lindsey	16.0	1.0	8	235	6	8	284	3	8	9.3	0.1	8
Rough and Ready Island	16.1	1.1	8	602	19	8	724	6	8	9.3	0.4	8
Mid EC Control	16.3	0.9	8	661	15	8	792	7	8	8.9	0.4	8
High EC Control	16.4	1.0	8	3192	147	8	3824	139	8	9.0	0.4	8
340	16.2	1.1	8	8531	321	8	10224	262	8	9.1	0.4	8
Low EC Low Turbidity Control	16.3	1.0	8	180	28	8	215	31	8	9.1	0.3	8
Low Turbidity Control	16.3	1.0	8	3247	139	8	3886	146	8	9.1	0.4	8

Treatment	pH			Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)			Turbidity (NTU)			Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*		
Low EC Control	7.81	0.13	8	0.52	0.38	4	0.010	0.008	4	5.57	1.49	7	-	-
Suisun	7.93	0.26	8	0.15	0.02	5	0.004	0.002	5	32.66	2.90	7	620	222
Hood	7.99	0.22	8	0.41	0.07	5	0.011	0.005	5	3.36	0.73	7	72	80
Light 55	8.16	0.16	8	0.19	0.02	5	0.008	0.003	5	7.14	0.86	7	124	124
Cache Lindsey	8.10	0.19	8	0.26	0.04	5	0.009	0.004	5	5.10	0.90	7	100	100
Rough and Ready Island	7.98	0.21	8	0.12	0.03	5	0.003	0.002	5	2.60	0.81	7	176	104
Mid EC Control	7.95	0.06	8	0.35	0.19	4	0.008	0.004	4	5.10	1.40	7	-	-
High EC Control	7.89	0.05	8	0.20	0.06	4	0.004	0.001	4	3.58	1.14	7	-	-
340	7.84	0.09	8	0.12	0.03	5	0.002	0.000	5	10.30	3.73	7	1260	88
Low EC Low Turbidity Control	7.92	0.12	8	0.23	0.10	4	0.006	0.005	4	3.24	1.05	7	-	-
Low Turbidity Control	7.87	0.03	8	0.13	0.03	4	0.002	0.000	4	2.13	1.68	7	-	-

Table C2-1. Results of a *H. transpacificus* 7-day test initiated 4/02/09 evaluating the toxicity of ambient water samples collected on 3/31/09, 4/01/09 and 4/02/09. Test animals were 44 days old at test initiation.

Treatment	Mean Turbidity (NTU)	96-hr Survival (%)[1]				
		Mean	SE	USEPA Statistics: v. SC-specific control	USEPA Statistics: v. SC-specific low turbidity control	SC-specific Tukeys Result
Low SC Control	4.47	85.0	6.5	-	-	A
Low SC Low Turbidity Control	3.52	66.8	5.8	S*	-	A B
Low SC Control + Tannins	1.94	31.8	2.8	S***	S**	C
Sacramento River at Hood DWR Station[2]	2.72	51.0	12.0	S*	NS	B C
Sacramento R. Deep Water Channel, Light 55[2]	5.19	69.3	5.4	NS	NS	A B
Confluence of Lindsey Sl. And Cache Sl.[2]	4.57	53.6	8.7	S*	NS	A B C
Mid EC Control	8.22	81.4	3.7	-	-	A
Rough and Ready DWR station, Stockton[3]	3.21	43.0	6.5	S**	-	B
High SC Control	6.66	86.1	5.8	-	-	A
High SC Low Turbidity Control	2.21	81.6	13.1	NS	-	A
Suisun Slough at Rush Ranch[4]	78.16	97.7	2.3	NS	NS	A
Napa River at Vallejo Seawall (340)[4]	10.68	88.6	8.6	NS	NS	A

Treatment	Mean Turbidity (NTU)	7-day Survival (%)[1]				
		Mean	SE	USEPA Statistics: v. SC-specific control	USEPA Statistics: v. SC-specific low turbidity control	SC-specific Tukeys Result
Low SC Control	4.47	70.0	8.2	-	-	A
Low SC Low Turbidity Control	3.52	43.0	6.0	S*	-	A B
Low SC Control + Tannins	1.94	2.5	2.5	S*	S*	C
Sacramento River at Hood DWR Station[2]	2.72	19.5	6.1	S**	S*	B C
Sacramento R. Deep Water Channel, Light 55[2]	5.19	40.7	3.2	S*	NS	A B
Confluence of Lindsey Sl. And Cache Sl.[2]	4.57	25.0	11.4	S*	NS	B C
Mid EC Control	8.22	69.5	4.9	-	-	A
Rough and Ready DWR station, Stockton[3]	3.21	9.3	3.7	S***	-	B
High SC Control	6.66	64.5	12.8	-	-	A
High SC Low Turbidity Control	2.21	61.6	11.2	NS	-	A
Suisun Slough at Rush Ranch[4]	78.16	95.5	2.6	NS	NS	A
Napa River at Vallejo Seawall (340)[4]	10.68	74.8	9.2	NS	NS	A

1. Data were analyzed using a separate statistical tests for each EC bracket (low, mid, high), and both standard USEPA statistics (one-tailed $\alpha = 0.05$) and ANOVA with Tukeys multiple comparison (two-tailed $\alpha = 0.05$) were performed. The intermediate EC bracket was examined using a T-test instead of Tukey's test. Statistically different groups of treatments are identified by highlighting (USEPA) and by different letters (Tukey).

*: $P < 0.05$

**: $P < 0.01$

***: $P < 0.001$

2. These low conductivity samples were compared to the Low EC controls.

3. This intermediate conductivity sample was compared to the Mid EC Control.

4. These high conductivity samples were compared to the High EC controls.

Table C2-2. Chemistry of sample waters examined in a *H. transpacificus* (Delta Smelt) 7-day test initiated 4/02/09 evaluating the toxicity of Sacramento River and Delta water samples collected on 3/31/09, 4/01/09 and 4/02/09.

Treatment	Temp (^oC)			EC (uS/cm)			SC (uS/cm)			DO (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Low SC Control	16.2	0.6	8	165	16	8	197	18	8	9.6	0.3	8
Low SC Low Turbidity Control	16.2	0.6	8	198	46	8	238	54	8	9.7	0.2	8
Low SC Control + Tannins	16.3	0.6	8	174	25	8	208	29	8	9.8	0.2	8
Sacramento River at Hood DWR Station	16.1	0.7	8	165	26	8	199	31	8	9.8	0.3	8
Sacramento R. Deep Water Channel, Light 55	16.2	0.7	8	238	22	8	286	26	8	9.8	0.3	8
Confluence of Lindsey Sl. And Cache Sl.	16.1	0.6	8	227	24	8	272	29	8	9.8	0.3	8
Mid SC Control	16.5	0.3	8	789	21	8	941	22	8	9.6	0.3	8
Rough and Ready DWR station, Stockton	16.1	0.7	8	748	20	8	901	20	8	9.9	0.2	8
High SC Control	16.5	0.3	8	3158	73	8	3776	80	8	9.6	0.3	8
High SC Low Turbidity Control	16.6	0.4	8	3229	77	8	3848	72	8	9.6	0.3	8
Suisun Slough at Rush Ranch	16.2	0.5	8	3063	43	8	3683	53	8	9.5	0.5	8
Napa River at Vallejo Seawall (340)	16.1	0.6	8	15134	543	8	18245	520	8	9.4	0.5	8

Treatment	pH			Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)			Turbidity (NTU)			Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*		
Low SC Control	7.82	0.10	8	0.38	0.29	4	0.006	0.005	4	4.47	1.79	8	-	-
Low SC Low Turbidity Control	7.92	0.10	8	0.17	0.13	4	0.003	0.002	4	3.52	1.24	8	-	-
Low SC Control + Tannins	7.88	0.10	8	0.06	0.05	4	0.001	0.001	4	1.94	0.56	8	-	-
Sacramento River at Hood DWR Station	8.01	0.22	8	0.32	0.09	4	0.011	0.004	4	2.72	1.92	8	64	72
Sacramento R. Deep Water Channel, Light 55	8.11	0.15	8	0.27	0.03	4	0.011	0.001	4	5.19	0.99	7	104	92
Confluence of Linsey Sl. And Cache Sl.	8.10	0.15	8	0.28	0.04	4	0.010	0.002	4	4.57	0.86	7	92	88
Mid EC Control	8.02	0.12	8	0.14	0.07	4	0.004	0.001	4	8.22	4.76	8	-	-
Rough and Ready DWR station, Stockton	8.12	0.11	8	0.09	0.04	4	0.003	0.002	4	3.21	1.91	8	164	104
High SC Control	7.96	0.07	8	0.18	0.11	4	0.004	0.002	4	6.66	4.89	8	-	-
High SC Low Turbidity Control	8.03	0.07	8	0.13	0.05	4	0.003	0.002	4	2.21	1.92	8	-	-
Suisun Slough at Rush Ranch	8.06	0.24	8	0.11	0.05	4	0.004	0.002	4	78.16	108.08	8	496	176
Napa River at Vallejo Seawall (340)	7.83	0.12	8	0.11	0.02	4	0.002	0.000	4	10.68	11.12	8	1996	94

Table C3-1. Results of a *H. transpacificus* 7-day test initiated 4/16/09 evaluating the toxicity of ambient water samples collected on 4/14/09, 4/15/09 and 4/16/09. Test animals were 54 days old at test initiation.

Treatment	Turbidity (NTU)	96-hr Survival (%)[1] Mean	SE	USEPA Statistics v. SC-specific control	USEPA Statistics v. SC-specific low turbidity control	SC-specific Tukeys Result
Low EC Control	4.51	84.7	2.7	-	-	A
Low EC / Low Turbidity Control	3.47	46.7	5.4	S***	-	B
Low EC Control + Antibiotics	4.94	65.0	12.6	NS	NS	A B
Sacramento River at Hood DWR Station	2.19	67.0	8.1	S*	NS	A B
Sacramento R. Deep Water Channel, Light 55	5.58	71.4	10.0	NS	NS	A B
Confluence of Lindsey Sl. And Cache Sl.	6.84	55.3	2.0	S***	NS	A B
Mid EC Control	4.61	75.6	3.0	-	-	A
Rough and Ready DWR station, Stockton	3.20	59.8	7.1	S*	-	A
High EC Control	5.47	82.5	4.8	-	-	A B
High EC / Low Turbidity Control	1.78	83.3	5.6	NS	-	A B
Suisun Slough at Rush Ranch	31.39	94.7	3.1	NS	NS	A
Napa River at Vallejo Seawall (340)	7.14	62.2	10.9	S*	NS	B

Treatment	Turbidity (NTU)	7-day Survival (%)[1] Mean	SE	USEPA Statistics v. SC-specific control	USEPA Statistics v. SC-specific low turbidity control	SC-specific Tukeys Result
Low EC Control	4.51	58.9	7.2	-	-	A B
Low EC / Low Turbidity Control	3.47	27.4	4.0	S**	-	B
Low EC Control + Antibiotics	4.94	65.0	12.6	NS	NS	A
Sacramento River at Hood DWR Station	2.19	30.1	6.6	S*	NS	A B
Sacramento R. Deep Water Channel, Light 55	5.58	55.8	7.9	NS	NS	A B
Confluence of Lindsey Sl. And Cache Sl.	6.84	46.9	8.5	NS	NS	A B
Mid EC Control	4.61	67.5	4.6	-	-	A
Rough and Ready DWR station, Stockton	3.20	42.2	3.6	S**	-	B
High EC Control	5.47	70.0	5.8	-	-	A B
High EC / Low Turbidity Control	1.78	61.9	3.8	NS	-	B
Suisun Slough at Rush Ranch	31.39	92.2	2.6	NS	NS	A
Napa River at Vallejo Seawall (340)	7.14	62.2	10.9	NS	NS	B

1. Data were analyzed using a separate statistical tests for each EC bracket (low, mid, high). Significant reductions in survival compared to EC-specific controls according to USEPA statistics are indicated by shaded cells, groups of treatments found to be significantly different by Tukey's tests are identified by different letters.
2. These low conductivity samples were compared to the Low EC controls.
3. This intermediate conductivity sample was compared to the Mid EC Control.
4. These high conductivity samples were compared to the High EC controls.

Table C3-2. Chemistry of sample waters examined in a *H. transpacificus* (Delta Smelt) 7-day test initiated 4/16/09 evaluating the toxicity of Sacramento River and Delta water samples collected on 4/14/09, 4/15/09 and 4/16/09.

Treatment	Temp (°C)			EC (uS/cm)			SC (uS/cm)			DO (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Low EC Control	17.0	0.2	8	160	31	8	189	36	8	10.1	0.6	8
Low EC / Low Turbidity Control	17.0	0.3	8	165	31	8	193	34	8	9.8	0.6	8
Low EC Control + Antibiotics	17.0	0.3	8	201	50	8	236	58	8	9.7	0.6	8
Sacramento River at Hood DWR Station	16.7	0.5	8	143	36	8	169	42	8	10.2	0.5	8
Sacramento R. Deep Water Channel, Light 55	16.7	0.4	8	332	6	8	387	18	8	10.0	0.6	8
Confluence of Linsey Sl. And Cache Sl.	16.6	0.4	8	301	6	8	358	7	8	10.2	0.6	8
Mid EC Control	16.8	0.4	8	760	64	8	897	70	8	9.3	0.4	8
Rough and Ready DWR station, Stockton	16.6	0.4	8	766	10	8	909	8	8	10.8	2.3	8
High EC Control	16.7	0.6	8	4101	139	8	4857	159	8	9.8	0.5	8
High EC / Low Turbidity Control	16.8	0.5	8	4212	114	8	4943	149	8	9.7	0.5	8
Suisun Slough at Rush Ranch	16.7	0.4	8	4036	106	8	4785	97	8	9.9	0.9	8
Napa River at Vallejo Seawall (340)	16.8	0.4	8	15918	209	8	18785	222	8	9.6	0.4	8

Treatment	pH			Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)			Turbidity (NTU)			Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*		
Low EC Control	7.89	0.11	8	0.28	0.16	4	0.006	0.003	4	4.51	0.97	7	-	-
Low EC / Low Turbidity Control	7.89	0.17	8	0.22	0.08	4	0.005	0.002	4	3.47	1.42	7	-	-
Low EC Control + Antibiotics	7.87	0.21	8	0.19	0.02	4	0.005	0.003	4	4.94	0.89	7	-	-
Sacramento River at Hood DWR Station	7.98	0.26	8	0.45	0.09	4	0.017	0.006	4	2.19	0.69	7	52	52
Sacramento R. Deep Water Channel, Light 55	8.20	0.12	8	0.14	0.03	4	0.007	0.002	4	5.58	2.23	7	124	108
Confluence of Linsey Sl. And Cache Sl.	8.23	0.12	8	0.15	0.04	4	0.008	0.003	4	6.84	1.58	6	114	118
Mid EC Control	8.01	0.12	8	0.34	0.17	4	0.009	0.004	4	4.61	1.43	7	-	-
Rough and Ready DWR station, Stockton	8.13	0.17	8	0.16	0.03	4	0.007	0.002	4	3.20	1.43	8	190	112
High EC Control	7.97	0.08	8	0.29	0.13	4	0.007	0.003	4	5.47	1.26	7	-	-
High EC / Low Turbidity Control	7.95	0.10	8	0.12	0.05	4	0.003	0.001	4	1.78	0.96	7	-	-
Suisun Slough at Rush Ranch	8.15	0.33	8	0.16	0.03	4	0.008	0.002	4	31.39	4.74	7	650	248
Napa River at Vallejo Seawall (340)	7.89	0.14	8	0.11	0.02	4	0.002	0.001	4	7.14	2.65	7	2340	100

Table C4-1. Results of a *H. transpacificus* (Delta Smelt) 7-day test initiated 4/30/09 evaluating the toxicity of ambient delta water samples collected on 4/28/09, 4/29/09 and 4/30/09. Smelt were XX days post hatch at test initiation.

Treatment	Turbidity (NTU)	96-hr Survival (%)[1]		Comparison to EC-Specific Control	Comparison to EC-specific Low Turbidity Control
		Mean	SE		
Low EC Control: No Antibiotics	6	79.2	4.8	NS	NS
Low EC Control	7	88.2	7.0	-	-
Low EC / Low Turbidity Control	7	92.5	4.8	NS	-
Sacramento River at Hood DWR Station[2]	5	79.5	7.8	NS	NS
Sacramento R. Deep Water Channel, Light 55[2]	16	85.0	5.0	NS	NS
Confluence of Linsey Sl. And Cache Sl.[2]	16	82.5	6.3	NS	NS
Mid-EC Control	7	88.0	4.6	-	-
Rough and Ready DWR station, Stockton[3]	7	90.7	6.4	NS	-
High EC Control	6	100.0	0.0	-	-
Low Turbidity Control	5	88.6	4.4	NS	-
Suisun Slough at Rush Ranch[4]	29	97.5	2.5	NS	NS
Napa River at Vallejo Seawall (340)[4]	16	97.7	2.3	NS	NS

Treatment	Turbidity (NTU)	7-day Survival (%)[1]		Comparison to EC-Specific Control	Comparison to EC-specific Low Turbidity Control
		Mean	SE		
Low EC Control: No Antibiotics	6	69.4	5.5	NS	NS
Low EC Control	7	85.9	8.8	-	-
Low EC / Low Turbidity Control	7	85.2	3.0	-	-
Sacramento River at Hood DWR Station[2]	5	55.3	4.4	S*	S**
Sacramento R. Deep Water Channel, Light 55[2]	16	80.2	10.1	NS	NS
Confluence of Linsey Sl. And Cache Sl.[2]	16	67.5	7.5	NS	S*
Mid-EC Control	7	76.4	4.6	-	-
Rough and Ready DWR station, Stockton[3]	7	88.2	7.0	NS	NS
High EC Control	6	100.0	0.0	-	-
Low Turbidity Control	5	86.1	2.5	-	-
Suisun Slough at Rush Ranch[4]	29	93.1	2.3	NS	NS
Napa River at Vallejo Seawall (340)[4]	16	88.2	7.0	NS	NS

1. Highlighted areas indicate significant reductions in survival, weight or biomass compared to the appropriate EC-specific control. Data were analyzed using both USEPA standard single concentration statistical protocols and ANOVA with Tukey's multiple comparison procedure. Tukey's procedure did not detect any significant differences.
*: $P < 0.05$
**: $P < 0.01$
2. These low conductivity samples were compared to the Low EC control.
3. This intermediate conductivity sample was compared to the Mid-EC control.
4. This high conductivity sample was compared to the High EC control.

Table C4-2. Chemistry of sample waters examined in a *H. transpacificus* 7-day test initiated 4/30/09 evaluating the toxicity of Sacramento River and Delta water samples collected on 4/28/09, 4/29/09 and 4/30/09.

Treatment	Temp (°C)			EC (uS/cm)			SC (uS/cm)			DO (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Low EC Control: No Antibiotics	17.1	1.3	8	158	43	8	182	52	8	9.4	0.2	8
Low EC Control	17.0	1.2	8	171	30	8	199	37	8	9.6	0.2	8
Low EC / Low Turbidity Control	17.0	1.2	8	179	36	8	207	44	8	9.6	0.2	8
Sacramento River at Hood DWR Station	16.9	1.2	8	150	30	8	175	36	8	9.8	0.2	8
Sacramento R. Deep Water Channel, Light 55	16.8	1.5	7	244	24	8	291	30	8	9.8	0.3	8
Confluence of Linsey Sl. And Cache Sl.	16.7	1.4	8	243	22	8	287	30	8	9.9	0.3	8
Mid-EC Control	17.0	1.2	8	716	21	8	845	35	8	9.6	0.3	8
Rough and Ready DWR station, Stockton	16.8	1.2	8	602	19	8	713	31	8	9.5	0.6	8
High EC Control	17.0	1.2	8	3975	74	8	4698	80	8	9.6	0.4	8
Low Turbidity Control	16.9	1.2	8	3774	362	8	4626	54	8	9.7	0.3	8
Suisun Slough at Rush Ranch	16.9	1.2	8	3863	56	8	4598	112	8	9.5	0.4	8
Napa River at Vallejo Seawall (340)	16.8	1.4	8	19420	637	8	23134	572	8	9.1	0.4	8

Treatment	pH			Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)			Turbidity (NTU)			Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*		
Low EC Control: No Antibiotics	7.77	0.12	8	0.1	0.1	4	0.002	0.002	4	6	2	8	-	-
Low EC Control	7.97	0.20	8	0.1	0.1	4	0.002	0.002	4	7	1	8	-	-
Low EC / Low Turbidity Control	7.95	0.14	8	0.1	0.0	4	0.002	0.002	4	7	1	8	-	-
Sacramento River at Hood DWR Station	7.97	0.06	8	0.0	0.0	4	0.001	0.001	4	5	4	8	48	51
Sacramento R. Deep Water Channel, Light 55	8.02	0.04	8	0.1	0.1	4	0.003	0.003	4	16	7	8	84	78
Confluence of Lindsey Sl. And Cache Sl.	8.06	0.07	8	0.1	0.1	4	0.004	0.003	4	16	5	8	64	74
Mid-EC Control	7.93	0.08	8	0.1	0.1	4	0.003	0.002	4	7	2	8	-	-
Rough and Ready DWR station, Stockton	7.95	0.10	8	0.1	0.1	4	0.003	0.002	4	7	3	8	144	93
High EC Control	7.87	0.05	8	0.1	0.1	4	0.002	0.001	4	6	2	8	-	-
Low Turbidity Control	7.83	0.05	8	0.1	0.1	4	0.001	0.001	4	5	3	8	-	-
Suisun Slough at Rush Ranch	7.90	0.17	8	0.1	0.1	4	0.002	0.001	4	29	17	8	640	158
Napa River at Vallejo Seawall (340)	7.71	0.05	8	0.1	0.1	4	0.001	0.001	4	16	17	8	2880	102

Table C5-1. Results of a *H. transpacificus* 7-day test initiated 5/14/09 evaluating the toxicity of ambient delta water samples collected on 5/12/09, 5/13/09 and 5/14/09. Smelt were 41 days post hatch at test initiation.

Treatment	Turbidity (NTU)	96-hour Survival (%)[1] Mean	SE	USEPA Statistics v. SC-specific control	USEPA Statistics v. SC-specific low turbidity control	SC-specific Tukey's Test
Low EC Control	6	76.4	9.3	-	-	A B
Low EC / Low Turbidity Control	5	68.8	5.0	NS	-	A B
Sacramento River at Hood DWR Station[2]	12	62.9	6.0	NS	NS	B
Sacramento R. Deep Water Channel, Light 55[2]	41	84.7	6.4	NS	NS	A B
Confluence of Lindsey Sl. And Cache Sl.[2]	35	94.7	3.1	NS	NS	A
Mid-EC Control	6	80.3	4.5	-	-	A
Rough and Ready DWR station, Stockton[3]	5	56.7*	9.1	S*	-	B
High EC Control @ 4000 uS/cm	5	86.4	4.7	-	-	A
High EC / Low Turbidity Control	3	85.4	2.6	NS	-	A
Suisun Slough at Rush Ranch[4]	21	80.4	12.8	NS	NS	A
High EC Control @ 17000 uS/cm	7	72.1	10.0	-	-	A
Napa River at Vallejo Seawall (340)[5]	19	68.9	5.0	NS	-	A

Treatment	Turbidity (NTU)	7-day Survival (%)[1] Mean	SE	USEPA Statistics v. SC-specific control	USEPA Statistics v. SC-specific low turbidity control	SC-specific Tukey's Test
Low EC Control	6	71.4	11.6	-	-	A
Low EC / Low Turbidity Control	5	59.7	7.6	NS	-	A
Sacramento River at Hood DWR Station[2]	12	52.3	7.8	NS	NS	A
Sacramento R. Deep Water Channel, Light 55[2]	41	85.5	9.8	NS	NS	A
Confluence of Lindsey Sl. And Cache Sl.[2]	35	80.1	5.6	NS	NS	A
Mid-EC Control	6	71.9	3.4	-	-	A
Rough and Ready DWR station, Stockton[3]	5	28.1***	7.3	S***	-	B
High EC Control @ 4000 uS/cm	5	80.8	3.9	-	-	A
High EC / Low Turbidity Control	3	55.2*	10.1	S*	-	A
Suisun Slough at Rush Ranch[4]	21	85.7	14.3	NS	NS	A
High EC Control @ 17000 uS/cm	7	62.5	13.0	-	-	A
Napa River at Vallejo Seawall (340)[5]	19	63.9	3.6	NS	-	A

1. Highlighted areas indicate significant reductions in survival, weight or biomass compared to the appropriate EC-specific control. Data were analyzed using both USEPA standard single concentration statistical protocols and ANOVA with Tukey's multiple comparison procedure.
2. These low conductivity samples were compared to the Low EC control.
3. This intermediate conductivity sample was compared to the Mid-EC control.
4. This high conductivity sample was compared to the High EC control @ 4000 uS/cm.
5. This high conductivity sample was compared to the High EC Control @ 17,000 uS/cm.

Table C5-2. Chemistry of sample waters examined in a *H. transpacificus* (Delta Smelt) 7-day test initiated 5/14/09 evaluating the toxicity of Sacramento River and Delta water samples collected on 5/12/09, 5/13/09 and 5/14/09.

Treatment	Temp (°C)			EC (uS/cm)			SC (uS/cm)			DO (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Low EC Control	17.0	0.3	8	161	16	8	189	18	8	9.5	0.2	8
Low EC / Low Turbidity Control	16.9	0.6	8	198	115	8	224	125	8	9.5	0.2	8
Sacramento River at Hood DWR Station	16.7	0.3	8	164	55	8	390	562	8	9.8	0.2	8
Sacramento R. Deep Water Channel, Light 55	16.9	0.5	8	265	14	8	316	18	8	9.7	0.3	8
Confluence of Lindsey Sl. and Cache Sl.	16.6	0.4	8	226	23	8	268	27	8	9.8	0.2	8
Mid-EC Control	17.0	0.3	8	506	28	8	596	32	8	9.4	0.2	8
Rough and Ready DWR station, Stockton	16.7	0.4	8	454	28	8	541	35	8	9.4	0.6	8
High EC Control @ 4000 uS/cm	17.0	0.3	8	4019	68	8	4773	92	8	9.6	0.2	8
High EC / Low Turbidity Control	17.0	0.5	8	4060	65	8	4810	71	8	9.4	0.3	8
Suisun Slough at Rush Ranch	16.8	0.3	8	3995	65	8	4767	80	8	9.4	0.7	8
High EC Control @ 17000 uS/cm	17.1	0.5	8	14473	204	8	17058	243	8	9.1	0.4	8
Napa River at Vallejo Seawall (340)	16.8	0.3	8	13404	146	8	15951	155	8	9.4	0.4	8

Treatment	pH			Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)			Turbidity (NTU)			Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*		
Low EC Control	7.60	0.25	8	0.2	0.1	4	0.002	0.001	4	6	1	8	-	-
Low EC / Low Turbidity Control	7.80	0.17	8	0.2	0.1	4	0.004	0.005	4	5	2	8	-	-
Sacramento River at Hood DWR Station	7.75	0.23	8	0.2	0.0	4	0.004	0.001	4	12	14	8	44	50
Sacramento R. Deep Water Channel, Light 55	7.98	0.07	8	0.1	0.0	4	0.004	0.002	4	41	25	8	76	72
Confluence of Lindsey Sl. and Cache Sl.	7.98	0.20	8	0.1	0.0	4	0.003	0.002	4	35	39	8	76	74
Mid-EC Control	7.89	0.15	8	0.3	0.1	4	0.006	0.005	4	6	2	8	-	-
Rough and Ready DWR station, Stockton	7.79	0.13	8	0.2	0.0	4	0.004	0.001	4	5	2	8	112	70
High EC Control @ 4000 uS/cm	7.93	0.12	8	0.1	0.1	4	0.003	0.002	4	5	3	8	-	-
High EC / Low Turbidity Control	7.97	0.15	8	0.1	0.0	4	0.003	0.002	4	3	2	8	-	-
Suisun Slough at Rush Ranch	7.80	0.27	8	0.2	0.1	4	0.003	0.002	4	21	17	8	384	198
High EC Control @ 17000 uS/cm	7.90	0.17	8	0.1	0.1	4	0.002	0.002	4	7	2	8	-	-
Napa River at Vallejo Seawall (340)	7.67	0.06	8	0.1	0.0	4	0.001	0.001	4	19	24	8	1920	94

Table C6-1. Results of a *H. transpacificus* (Delta Smelt) 7-day test initiated 5/28/09 evaluating the toxicity of ambient water samples collected on 5/26/09, 5/27/09 and 5/28/09. Test animals were 55 days old at test initiation.

Treatment	96-hr Survival (%)		7-day Survival (%)	
	mean	se	mean	se
Low EC Control	79.2	10.7	76.4	10.2
Low EC / Low Turbidity Control	87.5	4.8	75.0	2.9
Sacramento River at Hood DWR Station[2]	89.7	7.1	71.1	4.7
Sacramento R. Deep Water Channel, Light 55[2]	91.9	5.3	86.9	5.1
Confluence of Lindsey Sl. And Cache Sl.[2]	91.3	3.0	81.3	4.4
Mid-EC Control	70.8	8.3	62.8	10.3
Rough and Ready DWR station, Stockton[3]	86.1	8.3	72.8	5.8
High EC Control @ 4000 uS/cm	92.5	2.5	82.5	4.8
High EC / Low Turbidity Control	92.5	4.8	71.4	10.0
Suisun Slough at Rush Ranch[4]	89.2	4.5	86.4	5.4
High EC Control @ 17000 uS/cm	70.8	17.2	68.1	15.8
Napa River at Vallejo Seawall (340)[5]	67.5	4.8	62.5	2.5

1. Highlighted areas indicate significant reductions in survival, weight or biomass compared to the appropriate EC-specific control. Data were analyzed using both USEPA standard single concentration statistical protocols and ANOVA with Tukey's multiple comparison procedure. Neither statistical procedure detected any significant differences.
2. These low conductivity samples were compared to the Low EC control.
3. This intermediate conductivity sample was compared to the Mid-EC control.
4. This high conductivity sample was compared to the High EC control @ 4000 uS/cm.
5. This high conductivity sample was compared to the High EC Control @ 17,000 uS/cm.

Table C6-2. Chemistry of sample waters examined in a *H. transpacificus* 7-day test initiated 5/28/09 evaluating the toxicity of Sacramento River and Delta water samples collected on 5/26/09, 5/27/09 and 5/28/09.

Treatment	Temp (^oC)			EC (uS/cm)			SC (uS/cm)			DO (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Low EC Control	16.6	0.3	8	144	8	8	171	11	8	9.4	0.4	8
Low EC / Low Turbidity Control	16.4	0.6	8	197	21	8	233	25	8	9.6	0.3	8
Sacramento River at Hood DWR Station	16.4	0.6	8	151	6	8	180	8	8	9.7	0.5	8
Sacramento R. Deep Water Channel, Light 55	16.6	0.5	8	196	11	8	234	15	8	9.7	0.5	8
Confluence of Lindsey Sl. And Cache Sl.	16.5	0.5	8	173	7	8	207	10	8	9.6	0.5	8
Mid-EC Control	16.5	0.3	8	430	24	8	511	27	8	9.7	0.5	8
Rough and Ready DWR station, Stockton	16.4	0.3	8	384	4	8	459	9	8	9.3	1.0	8
High EC Control @ 4000 uS/cm	16.4	0.4	8	4061	115	8	4847	102	8	9.4	0.4	8
High EC / Low Turbidity Control	16.5	0.5	8	3976	122	8	4740	95	8	9.2	0.2	8
Suisun Slough at Rush Ranch	16.5	0.6	8	3843	83	8	4578	75	8	9.3	0.9	8
High EC Control @ 17000 uS/cm	16.4	0.4	8	17080	2945	8	20368	3576	8	9.0	0.4	8
Napa River at Vallejo Seawall (340)	16.2	0.4	8	18059	744	8	21615	723	8	9.1	0.5	8

Treatment	pH			Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)			Turbidity (NTU)			Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*		
Low EC Control	7.85	0.45	8	0.15	0.10	4	0.005	0.005	4	11.95	8.11	8	-	-
Low EC / Low Turbidity Control	7.97	0.60	8	0.13	0.11	4	0.004	0.006	4	7.62	1.71	8	-	-
Sacramento River at Hood DWR Station	7.90	0.33	8	0.38	0.08	4	0.013	0.010	4	4.93	1.54	8	52	54
Sacramento R. Deep Water Channel, Light 55	7.97	0.24	8	0.19	0.05	4	0.007	0.004	4	18.22	5.82	8	64	66
Confluence of Lindsey Sl. And Cache Sl.	7.93	0.22	8	0.21	0.05	4	0.007	0.004	4	20.07	7.32	8	64	62
Mid-EC Control	8.04	0.21	8	0.16	0.10	4	0.005	0.003	4	10.52	8.81	8	-	-
Rough and Ready DWR station, Stockton	7.87	0.28	8	0.13	0.06	4	0.004	0.003	4	8.48	1.65	8	96	68
High EC Control @ 4000 uS/cm	7.90	0.20	8	0.13	0.06	4	0.002	0.001	4	10.43	8.04	8	-	-
High EC / Low Turbidity Control	7.75	0.40	8	0.09	0.06	4	0.001	0.001	4	3.87	2.47	8	-	-
Suisun Slough at Rush Ranch	7.68	0.31	8	0.13	0.05	4	0.002	0.001	4	20.09	2.66	8	520	142
High EC Control @ 17000 uS/cm	7.95	0.11	8	0.05	0.04	4	0.001	0.001	4	10.5	8.1	8	-	-
Napa River at Vallejo Seawall (340)	7.71	0.12	8	0.07	0.04	4	0.001	0.001	4	10.89	9.30	8	2640	100

Appendix D

In Situ Toxicity
Water Chemistry Summary

Table D1. Chemistry of ambient river water at the Rough and Ready DWR Station in Stockton, CA during 7-day in situ exposures.

Test Initiation Date	Ambient SC (uS/cm)					Ambient Turbidity (NTU)				
	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*
3/19/2009	811.5	89.5	725.0	922.0	6	22	15	6	49	9
4/2/2009	870.0	-	-	-	1	33	34	8	111	8
4/16/2009	-	-	-	-	-	13	7	7	29	8
4/30/2009	726.0	-	-	-	1	13	3	10	21	8
5/14/2009	489.0	-	-	-	1	12	3	9	17	8
5/28/2009	425.5	-	-	-	1	23	12	12	42	8

Table D2. Chemistry of ambient water from the Sacramento River at the Hood DWR Station during 7-day in situ exposures.

Test Initiation Date	Ambient SC (uS/cm)					Ambient Turbidity (NTU)				
	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*
3/19/2009	174.6	11.0	164.2	195.0	6	24	24	5	87	9
4/2/2009	179.0	-	-	-	1	12	8	6	29	8
4/16/2009	-	-	-	-	-	14	8	6	31	8
4/30/2009	131.5	-	-	-	1	11	7	5	26	8
5/14/2009	110.8	-	-	-	1	14	4	8	22	8
5/28/2009	136.7	-	-	-	1	12	3	9	18	8

Table D3a. Chemistry of water in exposure chambers during 7-day in situ tests at the Rough and Ready DWR Station in Stockton, CA.

Treatment	Temp (^oC)					EC (uS/cm)					SC (uS/cm)					DO (mg/L)				
	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*
Control Exposure 031909	16.8	0.9	15.1	17.8	8	688	98	584	826	8	803	99	720	970	14	9.6	0.4	8.9	10.3	8
Ambient Exposure 031909	16.2	0.8	15.4	17.8	8	698	72	615	793	8	836	88	730	948	14	9.0	0.8	7.2	9.6	8
Control Exposure 040209	17.6	0.5	16.5	18.1	8	787	14	765	812	8	915	13	895	944	15	9.3	0.4	8.8	9.9	8
Ambient Exposure 040209	17.0	0.5	16.3	17.5	8	783	31	740	851	8	915	29	879	1005	15	9.4	0.4	8.7	9.9	8
Control Exposure 041609	19.7	1.9	16.8	22.3	8	834	37	795	898	7	899	35	849	958	16	8.8	0.5	8.1	9.5	8
Ambient Exposure 041609	19.0	1.8	16.8	21.8	8	767	19	729	791	7	852	47	790	925	14	8.7	0.5	7.7	9.1	7
Control Exposure 043009	19.3	0.5	18.7	20.1	8	591	108	482	832	8	569	199	131	947	16	8.7	0.2	8.4	8.9	8
Ambient Exposure 043009	18.7	0.4	18.2	19.5	8	490	59	429	589	8	546	65	472	668	15	7.2	0.2	7.0	7.5	8
Control Exposure 051409	23.2	1.0	21.5	24.3	8	479	93	388	696	8	495	99	410	744	16	8.1	0.3	7.8	8.6	8
Ambient Exposure 051409	22.7	0.9	21.5	23.9	8	423	29	385	461	8	439	29	405	498	15	7.1	0.3	6.5	7.4	8
Control Exposure 052809	24.4	0.2	24.0	24.7	8	437	25	407	492	8	440	47	290	508	16	8.0	0.2	7.8	8.4	8
Ambient Exposure 052809	24.0	0.4	23.2	24.4	8	420	5	415	430	8	416	55	275	446	15	6.1	0.3	5.6	6.5	8

Treatment	pH					Ammonia Nitrogen (mg/L)					Unionized Ammonia (mg/L)				
	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*
Control Exposure 031909	7.83	0.12	7.66	7.98	11	0.15	0.12	0.03	0.34	8	0.003	0.003	0.000	0.009	8
Ambient Exposure 031909	7.69	0.10	7.56	7.85	11	0.07	0.02	0.06	0.10	8	0.001	0.000	0.001	0.001	8
Control Exposure 040209	7.56	0.19	7.34	7.84	7	0.27	0.18	0.04	0.52	7	0.002	0.001	0.001	0.003	7
Ambient Exposure 040209	7.85	0.08	7.67	7.91	7	0.08	0.04	0.04	0.17	7	0.001	0.000	0.001	0.002	7
Control Exposure 041609	7.71	0.08	7.60	7.85	8	0.30	0.15	0.11	0.50	8	0.005	0.003	0.001	0.010	8
Ambient Exposure 041609	7.94	0.11	7.81	8.14	7	0.09	0.02	0.05	0.11	7	0.002	0.001	0.001	0.003	7
Control Exposure 043009	7.68	0.22	7.50	8.19	8	0.28	0.17	0.09	0.53	8	0.004	0.002	0.001	0.007	8
Ambient Exposure 043009	7.55	0.11	7.41	7.66	7	0.10	0.04	0.07	0.18	7	0.001	0.001	0.001	0.003	7
Control Exposure 051409	7.61	0.07	7.54	7.72	8	0.22	0.16	0.00	0.49	8	0.004	0.003	0.000	0.009	8
Ambient Exposure 051409	7.48	0.04	7.43	7.53	7	0.10	0.11	0.00	0.29	7	0.001	0.001	0.000	0.004	7
Control Exposure 052809	7.83	0.15	7.61	8.05	8	0.12	0.12	0.00	0.30	8	0.003	0.003	0.000	0.008	8
Ambient Exposure 052809	7.37	0.06	7.29	7.46	7	0.10	0.06	0.05	0.22	7	0.001	0.001	0.001	0.002	7

Table D3b. Chemistry of water in exposure chambers during 7-day in situ tests at the Rough and Ready DWR Station in Stockton, CA, cont'd.

Treatment	Turbidity (NTU)					Hardness (mg/L as CaCO3)					Alkalinity (mg/L as CaCO3)				
	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*
Control Exposure 031909	4.42	1.70	2.55	8.26	8	142	16	120	164	7	77	11	56	86	7
Ambient Exposure 031909	8.67	2.04	6.67	12.50	8	178	11	160	190	7	105	3	102	110	7
Control Exposure 040209	2.87	1.04	1.82	4.79	7	134	8	124	148	7	68	3	64	72	7
Ambient Exposure 040209	12.29	2.30	8.49	15.90	7	189	3	184	192	7	112	3	107	116	7
Control Exposure 041609	2.58	0.87	1.60	4.01	8	143	10	128	160	8	75	10	54	84	8
Ambient Exposure 041609	9.72	2.42	7.04	13.80	7	181	10	168	196	7	111	3	106	114	7
Control Exposure 043009	4.71	1.29	3.61	7.72	8	103	16	84	132	7	60	9	48	77	7
Ambient Exposure 043009	13.87	4.44	11.50	23.90	7	125	12	112	144	6	82	4	76	88	6
Control Exposure 051409	2.80	1.08	1.43	4.53	8	83	15	72	116	8	52	5	44	60	8
Ambient Exposure 051409	10.83	2.35	8.57	14.60	7	97	6	88	104	7	67	4	60	72	7
Control Exposure 052809	6.51	4.35	2.46	16.46	8	100	5	92	108	8	71	4	66	76	8
Ambient Exposure 052809	17.29	7.65	9.33	33.23	7	95	14	64	104	7	65	7	52	70	7

Table D4a. Chemistry of water from the Sacramento River in exposure chambers during 7-day in situ tests at the Hood DWR Station.

Treatment	SC (uS/cm)					EC (uS/cm)					SC (uS/cm)					DO (mg/L)				
	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*
Control Exposure 031909	15.4	0.4	15.0	15.9	7	153	8	143	162	7	189	8	172	200	14	9.3	0.5	8.2	9.8	9
Ambient Exposure 031909	14.8	0.6	14.0	15.5	7	140	7	130	153	8	173	7	161	188	13	9.1	0.5	8.0	9.4	8
Control Exposure 040209	15.9	0.4	15.0	16.0	8	152	13	141	179	7	183	19	159	230	15	9.2	0.3	8.8	9.6	7
Ambient Exposure 040209	15.3	0.5	15.0	16.0	8	133	11	115	146	7	160	11	140	177	15	9.0	0.3	8.4	9.5	7
Control Exposure 041609	18.3	1.8	16.0	20.0	8	135	6	126	142	7	153	7	145	167	16	8.9	0.5	8.3	9.6	8
Ambient Exposure 041609	17.4	1.6	15.0	19.0	8	124	6	117	133	8	148	15	133	194	15	8.6	0.5	7.8	9.0	7
Control Exposure 043009	16.3	0.7	15.0	17.0	8	111	7	104	123	8	130	7	122	142	14	9.4	0.3	8.9	9.8	8
Ambient Exposure 043009	15.5	0.5	15.0	16.0	8	104	11	94	119	7	127	9	114	140	15	9.3	0.4	8.5	9.6	7
Control Exposure 051409	20.9	1.0	19.0	22.0	8	120	7	109	131	8	129	6	118	139	16	8.4	0.1	8.3	8.6	8
Ambient Exposure 051409	20.1	0.8	19.0	21.0	8	117	12	102	140	8	129	10	114	152	15	8.2	0.1	8.1	8.5	7
Control Exposure 052809	21.8	0.5	21.0	22.0	8	133	18	111	160	7	148	29	83	211	16	8.2	0.2	8.0	8.7	8
Ambient Exposure 052809	21.0	0.5	20.0	22.0	8	131	14	114	155	7	137	21	89	170	14	7.7	0.2	7.4	8.1	7

Treatment	pH					Ammonia Nitrogen (mg/L)					Unionized Ammonia (mg/L)				
	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*
Control Exposure 031909	7.35	0.36	6.50	7.76	9	0.21	0.17	0.02	0.49	8	0.002	0.001	0.000	0.003	8
Ambient Exposure 031909	7.52	0.17	7.17	7.73	8	0.42	0.12	0.28	0.59	7	0.004	0.001	0.002	0.006	7
Control Exposure 040209	7.35	0.11	7.22	7.48	7	0.15	0.10	0.00	0.26	7	0.001	0.001	0.000	0.002	7
Ambient Exposure 040209	7.40	0.09	7.26	7.49	7	0.42	0.17	0.13	0.64	7	0.003	0.001	0.001	0.005	7
Control Exposure 041609	7.57	0.23	7.23	7.96	8	0.22	0.17	0.03	0.51	8	0.003	0.002	0.000	0.005	8
Ambient Exposure 041609	7.52	0.09	7.34	7.61	7	0.37	0.14	0.24	0.66	7	0.004	0.001	0.003	0.005	7
Control Exposure 043009	7.56	0.11	7.36	7.67	8	0.14	0.11	0.04	0.38	8	0.001	0.001	0.000	0.003	8
Ambient Exposure 043009	7.56	0.16	7.37	7.81	7	0.22	0.15	0.02	0.45	7	0.002	0.001	0.000	0.004	7
Control Exposure 051409	7.36	0.14	7.08	7.49	8	0.24	0.16	0.08	0.46	8	0.002	0.002	0.001	0.005	8
Ambient Exposure 051409	7.32	0.10	7.16	7.46	7	0.29	0.05	0.23	0.35	7	0.002	0.001	0.002	0.004	7
Control Exposure 052809	7.43	0.10	7.30	7.57	8	0.25	0.18	0.00	0.50	8	0.003	0.002	0.000	0.006	8
Ambient Exposure 052809	7.37	0.09	7.25	7.50	7	0.32	0.14	0.18	0.53	7	0.003	0.002	0.001	0.006	7

Table D4b. Chemistry of water from the Sacramento River in exposure chambers during 7-day in situ tests at the Hood DWR Station, cont'd.

Treatment	Hardness (mg/L as CaCO3)					Alkalinity (mg/L as CaCO3)					Turbidity (NTU)				
	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*N*
Control Exposure 031909	41	3	36	44	7	34	9	18	45	7	2.26	0.91	0.59	3.24	7
Ambient Exposure 031909	69	3	64	72	7	71	3	68	76	7	24.99	9.01	15.90	40.20	7
Control Exposure 040209	37	5	32	44	7	32	4	26	36	7	2.05	0.66	1.22	3.28	7
Ambient Exposure 040209	56	6	48	64	7	62	5	56	68	7	17.80	8.86	6.55	30.20	7
Control Exposure 041609	34	5	24	40	8	29	5	22	36	8	3.72	1.86	1.16	6.24	8
Ambient Exposure 041609	52	3	48	56	7	53	8	34	58	7	18.39	8.13	9.25	33.80	7
Control Exposure 043009	25	4	20	32	7	21	5	12	26	7	5.49	1.33	3.54	6.97	7
Ambient Exposure 043009	49	3	44	52	6	53	4	48	58	6	38.53	19.29	10.90	63.20	6
Control Exposure 051409	28	10	12	44	8	23	7	12	32	8	4.44	1.07	2.51	5.71	8
Ambient Exposure 051409	45	4	40	48	7	47	3	44	52	7	23.31	8.50	12.50	34.30	7
Control Exposure 052809	32	3	28	36	8	27	3	24	32	8	2.67	1.08	1.19	4.03	8
Ambient Exposure 052809	50	6	44	60	7	53	4	48	60	7	19.20	5.79	13.70	28.30	7

Appendix E

Eurytemora affinis
7-day Toxicity Test
Water Chemistry Summary

Table E1. Water chemistry during a *E. affinis* 7-day test initiated on 5/01/09 evaluating the toxicity of ambient delta water samples collected on 4/28/09 and 4/30/09.

Treatment	Day 0 - Initial					Day 1 - Final				
	SC (uS/cm)	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH	SC (uS/cm)	EC (uS/cm)	Temp (°C)	DO (mg/L)	pH
L16 @ 1 ppt	1920	1574	16.0	10.0	7.99	1930	1567	15.6	9.5	7.87
L 16 @ 1000 µS/cm	1004	825	16.1	9.7	7.97	1003	828	16.3	9.2	7.88
L 16 @ 500 µS/cm	538	441	16.0	9.7	7.97	517	427	16.3	9.9	7.90
L 16 @ 250 µS/cm	304	248	15.8	9.7	8.00	282	232	16.1	9.8	7.85
L 16 @ 100 µS/cm	160	131	15.8	9.6	7.98	129	106	16.0	9.8	7.79
Light 55	335	276	16.2	9.6	8.01	271	225	16.4	9.5	8.05
711	164	136	16.5	10.0	7.90	136	114	17.0	9.5	7.91
CU	393	325	16.4	10.0	8.24	329	276	16.9	9.6	8.17
Hood	150	124	16.5	10.6	8.02	142	121	17.6	9.4	8.00

Appendix F

Hypomesus Transpacificus
96-hour Survival Sensitivity Tests

Table F1-1. Results of a *H. transpacificus* (Delta Smelt) 7-day test initiated 7/08/09 evaluating the toxicity of ammonia. Test animals were 47 days old at test initiation.

Treatment	Mean Measured Ammonia (mg/L)		96-hr Survival (%)		7-day Survival (%)	
	Total Ammonia Nitrogen	Unionized Ammonia	Mean	SE	Mean	SE
Filtered Hatchery Water @ 900 uS/cm	0.1	0.002	67.5	13.1	15.0	8.7
2.5 ppm Ammonium Chloride	1.9	0.032	75.0	18.9	22.5	7.5
5 ppm Ammonium Chloride	3.7	0.064	80.0	9.1	22.5	4.8
10 ppm Ammonium Chloride	7.1	0.099	61.1	3.2	2.5	2.5
20 ppm Ammonium Chloride	14.4	0.191	27.5	8.5	0.0	0.0
40 ppm Ammonium Chloride	29.0	0.333	0.0	0.0	0.0	0.0
80 ppm Ammonium Chloride	57.8	0.645	0.0	0.0	0.0	0.0

1. The 96-hour endpoint was analyzed using USEPA standard multiple concentration statistical protocols. Highlighted areas indicate significant reductions in survival, weight or biomass compared to the hatchery water control.

Table F1-2. Chemistry of sample waters examined in a *H. transpacificus* (Delta Smelt) 7-day test initiated 7/08/09 evaluating the toxicity of ammonia.

Treatment	Temp (^{o}C)			DO (mg/L)			pH			EC (uS/cm)	SC (uS/cm)
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	Mean
Filtered Hatchery Water @ 900 uS/cm	17.3	0.5	8	8.9	0.7	8	7.84	0.18	8	758	908
2.5 ppm Ammonium Chloride	17.2	0.6	8	8.8	0.9	8	7.76	0.19	8	777	926
5 ppm Ammonium Chloride	17.3	0.8	7	8.8	0.7	8	7.75	0.19	8	792	948
10 ppm Ammonium Chloride	17.2	0.4	8	8.8	0.9	8	7.67	0.16	8	820	979
20 ppm Ammonium Chloride	17.1	0.5	7	9.0	0.7	7	7.66	0.13	7	882	1057
40 ppm Ammonium Chloride	17.0	0.2	4	9.3	0.6	4	7.61	0.11	4	1017	1200
80 ppm Ammonium Chloride	16.9	0.0	2	9.4	0.1	2	7.62	0.04	2	1264	1493

Treatment	Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)			Turbidity (NTU)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
	Mean	SD	*N*	Mean	SD	*N*			
Filtered Hatchery Water @ 900 uS/cm	0.09	0.06	8	0.002	0.001	8	0.84	100	66
2.5 ppm Ammonium Chloride	1.88	0.08	8	0.032	0.013	8	-	-	-
5 ppm Ammonium Chloride	3.74	0.13	8	0.064	0.027	8	-	-	-
10 ppm Ammonium Chloride	7.08	0.72	8	0.099	0.040	8	-	-	-
20 ppm Ammonium Chloride	14.43	0.49	7	0.191	0.056	7	-	-	-
40 ppm Ammonium Chloride	28.95	2.22	4	0.333	0.100	4	-	-	-
80 ppm Ammonium Chloride	57.80	4.81	2	0.645	0.105	2	-	-	-

Table F2-1. Results of a *H. transpacificus* 96-hr test initiated 7/08/09 evaluating the toxicity of chlorpyrifos. Test animals were 47 days old at test initiation.

Treatment	96-hr Survival (%)[1]	
	Mean	SE
Filtered Hatchery Water @ 900 uS/cm	42.5	12.5
Filtered Hatchery Water + Methanol	35.2	11.8
12.5 ppb Chlorpyrifos	33.9	11.5
25 ppb Chlorpyrifos	4.8	2.8
50 ppb Chlorpyrifos	7.0	4.4
100 ppb Chlorpyrifos	5.0	2.9
200 ppb Chlorpyrifos	2.5	2.5

1. Data were analyzed using EPA standard statistical protocols, and no significant reductions in survival were observed. All calculations were based on the solvent control.

Table F2-2. Chemistry of sample waters examined in a *H. transpacificus* (Delta Smelt) 96-hr test initiated 7/08/09 evaluating the toxicity of chlorpyrifos.

Treatment	Temp (°C)			DO (mg/L)			pH			EC (uS/cm)	SC (uS/cm)
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*		
Filtered Hatchery Water @ 900 uS/cm	17.2	0.3	4	9.4	0.3	4	7.92	0.12	4	768	904
Filtered Hatchery Water + Methanol	17.2	0.3	4	9.0	0.9	4	7.86	0.21	4	765	901
12.5 ppb Chlorpyrifos	17.2	0.2	4	9.1	0.9	4	7.80	0.22	4	768	900
25 ppb Chlorpyrifos	17.3	0.1	4	9.3	0.3	4	7.85	0.23	4	767	901
50 ppb Chlorpyrifos	17.4	0.2	4	8.7	1.2	4	7.79	0.30	4	768	894
100 ppb Chlorpyrifos	17.4	0.1	4	9.3	0.5	4	7.89	0.14	4	768	901
200 ppb Chlorpyrifos	17.4	0.1	4	9.2	0.7	4	7.84	0.20	4	774	896

Treatment	Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*
Filtered Hatchery Water @ 900 uS/cm	0.09	0.09	4	0.002	0.002	4
Filtered Hatchery Water + Methanol	0.04	0.04	4	0.001	0.001	4
12.5 ppb Chlorpyrifos	0.02	0.01	4	0.000	0.000	4
25 ppb Chlorpyrifos	0.02	0.01	4	0.000	0.000	4
50 ppb Chlorpyrifos	0.02	0.02	4	0.000	0.000	4
100 ppb Chlorpyrifos	0.03	0.02	4	0.001	0.000	4
200 ppb Chlorpyrifos	0.02	0.01	4	0.000	0.000	4

Table F3-1. Results of a *H. transpacificus* 96-hour test initiated 7/22/09 evaluating the toxicity of esfenvalerate. Test animals were 45 days old at test initiation.

Treatment	96-hr Survival (%)	
	Mean	SE
Hatchery Tap Water	30.2	1.2
Hatchery Tap Water + Solvent	28.3	6.5
94 pptr Esfenvalerate	21.4	3.6
188 pptr Esfenvalerate	24.4	9.6
375 pptr Esfenvalerate	0.0	0.0
750 pptr Esfenvalerate	0.0	0.0
1500 pptr Esfenvalerate	0.0	0.0

1. Data were analyzed using USEPA standard statistical protocols. Highlighted cells indicate significant reductions in survival compared to the solvent control.

Table F3-2. Chemistry of sample waters examined in a *H. transpacificus* (Delta Smelt) 96-hr test initiated 7/22/09 evaluating the toxicity of esfenvalerate.

Treatment	Temp (^{o}C)			EC (uS/cm)			SC (uS/cm)			DO (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Hatchery Tap Water	17.5	0.7	4	769	6	2	906	3	2	9.5	0.2	4
Hatchery Tap Water + Solvent	17.4	0.5	4	757	31	2	890	19	2	9.4	0.6	4
94 pptr Esfenvalerate	17.2	0.5	4	757	13	2	899	6	2	9.2	0.6	4
188 pptr Esfenvalerate	17.2	0.4	4	753	28	2	887	21	2	9.4	0.5	4
375 pptr Esfenvalerate	17.1	0.5	4	757	13	2	900	4	2	9.3	0.6	4
750 pptr Esfenvalerate	17.1	0.6	3	750	30	2	885	16	2	9.6	0.1	3
1500 pptr Esfenvalerate	17.4	0.1	2	769	-	1	900	-	1	9.7	0.2	2

Treatment	pH			Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Hatchery Tap Water	8.22	0.11	4	0.18	0.15	2	0.008	0.007	2
Hatchery Tap Water + Solvent	8.09	0.13	4	0.07	0.02	2	0.002	0.000	2
94 pptr Esfenvalerate	8.08	0.17	4	0.07	0.01	2	0.002	0.000	2
188 pptr Esfenvalerate	8.02	0.17	4	0.05	0.03	2	0.001	0.001	2
375 pptr Esfenvalerate	8.01	0.13	4	0.07	0.04	2	0.001	0.000	2
750 pptr Esfenvalerate	8.08	0.14	3	0.03	-	1	0.001	-	1
1500 pptr Esfenvalerate	8.21	0.04	2	0.04	-	1	0.002	-	1

Table F4-1. Results of a *H. transpacificus* 96-hour test initiated 7/22/09 evaluating the toxicity permethrin. Test animals were 45 days old at test initiation.

Treatment	96-hr Survival (%)	
	mean	se
Hatchery Tap Water	26.0	6.4
313 pptr Permethrin	59.5	1.9
625 pptr Permethrin	38.1	10.0
1250 pptr Permethrin	36.6	3.2
2500 pptr Permethrin	41.4	5.5
5000 pptr Permethrin	35.8	6.3

Table F4-2. Chemistry of sample waters examined in a *H. transpacificus* 96-hour test initiated 7/22/09 evaluating the toxicity of permethrin.

Treatment	Temp (^oC)			EC (uS/cm)			SC (uS/cm)			DO (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Hatchery Tap Water	17.2	0.6	4	763	21	2	901	5	2	9.4	0.3	4
313 pptr Permethrin	17.2	0.3	4	756	27	2	890	22	2	9.3	0.5	4
625 pptr Permethrin	17.2	0.4	4	766	19	2	901	8	2	9.4	0.5	4
1250 pptr Permethrin	17.4	0.4	4	763	22	2	892	14	2	9.2	0.5	4
2500 pptr Permethrin	17.2	0.3	4	753	21	2	887	21	2	9.2	0.6	4
5000 pptr Permethrin	17.2	0.3	4	765	15	2	900	6	2	9.4	0.5	4

Treatment	pH			Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Hatchery Tap Water	8.05	0.16	4	0.20	0.14	2	0.005	0.003	2
313 pptr Permethrin	8.08	0.16	4	0.06	0.01	2	0.001	0.000	2
625 pptr Permethrin	8.07	0.16	4	0.05	0.01	2	0.001	0.000	2
1250 pptr Permethrin	7.98	0.15	4	0.07	0.02	2	0.001	0.000	2
2500 pptr Permethrin	8.01	0.13	4	0.07	0.01	2	0.002	0.000	2
5000 pptr Permethrin	8.05	0.16	4	0.07	0.00	2	0.002	0.001	2

Table F5-1. Results of a *H. transpacificus* 96-hr test initiated 7/29/09 evaluating the toxicity of chlorpyrifos. Test animals were 45 days old at test initiation.

Treatment	96-hr Survival (%)	
	Mean	SE
Hatchery Tap Water	31.6	4.4
Hatchery Tap Water + Solvent	42.5	11.1
18.75 ppb Chlorpyrifos	12.5	4.8
37.5 ppb Chlorpyrifos	5.0	2.9
75 ppb Chlorpyrifos	0.0	0.0
150 ppb Chlorpyrifos	0.0	0.0
300 ppb Chlorpyrifos	0.0	0.0

1. Data were analyzed using EPA standard statistical protocols. Comparisons to both the method control and the solvent control showed a significant reduction in survival at 18.75 ppb.

Table F5-2. Chemistry of sample waters examined in a *H. transpacificus* 96-hour test initiated 7/29/09 evaluating the toxicity of chlorpyrifos.

Treatment	Temp (^{o}C)			EC (uS/cm)			SC (uS/cm)			DO (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Hatchery Tap Water	17.0	0.7	4	747	14	2	877	1	2	9.5	0.2	4
Hatchery Tap Water + Methanol	17.1	0.4	4	379	525	2	878	16	2	9.5	0.5	4
18.75 ppb Chlorpyrifos	17.3	0.4	4	749	19	2	878	10	2	9.4	0.5	4
37.5 ppb Chlorpyrifos	17.2	0.3	4	745	8	2	875	0	2	9.6	0.5	4
75 ppb Chlorpyrifos	17.3	0.6	4	755	8	2	884	6	2	9.4	0.7	4
150 ppb Chlorpyrifos	17.1	0.5	4	745	11	2	880	1	2	9.4	0.6	4
300 ppb Chlorpyrifos	17.2	0.6	3	752	16	2	885	2	2	9.6	0.3	3

Treatment	pH			Ammonia Nitrogen (mg/L)			Unionized Ammonia (mg/L)		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Hatchery Tap Water	7.85	0.12	4	0.04	0.05	4	0.001	0.001	4
Hatchery Tap Water + Methanol	7.83	0.15	4	0.02	0.02	4	0.001	0.000	4
18.75 ppb Chlorpyrifos	7.80	0.15	4	0.02	0.02	4	0.001	0.001	4
37.5 ppb Chlorpyrifos	7.87	0.08	4	0.02	0.02	4	0.000	0.000	4
75 ppb Chlorpyrifos	7.81	0.17	4	0.02	0.01	4	0.000	0.000	4
150 ppb Chlorpyrifos	7.81	0.14	4	0.02	0.01	4	0.000	0.000	4
300 ppb Chlorpyrifos	7.85	0.13	3	0.02	0.02	3	0.000	0.001	3

Appendix G

Pimephales promelas
7-day Survival and Biomass Sensitivity Tests

Table G1-1. Results of a *P. promelas* 7-day test initiated 7/07/09 evaluating the toxicity of Cyfluthrin in laboratory control water and in water collected from the UC Davis Delta Smelt Hatchery in Byron, CA.

Treatment	96-hr Survival (%)[1]		7 Day Survival (%)[1]		Biomass (mg)[1]	
	Mean	SE	Mean	SE	Mean	SE
DIEPAMH	100.0	0.0	100.0	0.0	0.280	0.009
DIEPAMH @ 900 uS/cm (D900)	97.5	2.5	97.5	2.5	0.333	0.017
D900 Solvent Control	100.0	0.0	97.5	2.5	0.311	0.018
D900 + 125 pptr Cyfluthrin	100.0	0.0	97.5	2.5	0.369	0.017
D900 + 250 pptr Cyfluthrin	100.0	0.0	100.0	0.0	0.324	0.013
D900 + 500 pptr Cyfluthrin	100.0	0.0	100.0	0.0	0.341	0.005
D900 + 1000 pptr Cyfluthrin	97.5	2.5	87.5	4.8	0.318	0.025
D900 + 2000 pptr Cyfluthrin	2.5	2.5	0.0	0.0	0.000	0.000
Hatchery Water @ 900 uS/cm (HW)	97.5	2.5	97.5	2.5	0.370	0.019
HW Solvent Control	100.0	0.0	95.0	2.9	0.293	0.021
HW + 125 pptr Cyfluthrin	100.0	0.0	100.0	0.0	0.316	0.015
HW + 250 pptr Cyfluthrin	97.5	2.5	97.5	2.5	0.346	0.002
HW + 500 pptr Cyfluthrin	100.0	0.0	100.0	0.0	0.323	0.010
HW + 1000 pptr Cyfluthrin	95.0	2.9	95.0	2.9	0.343	0.033
HW + 2000 pptr Cyfluthrin	0.0	0.0	0.0	0.0	0.000	0.000

1. Highlighted areas indicate significant reduction in survival or biomass compared to the solvent control. Data were analyzed using USEPA standard statistical protocols.

Table G1-2. Water chemistry data taken during a *P. promelas* 7-day test initiated 7/07/09 evaluating the toxicity of Cyfluthrin in laboratory control water and in water collected from the UC Davis Delta Smelt Hatchery in Byron, CA.

Treatment	Laboratory Chemistry						
	EC (uS/cm)	Min Temp (^{o}C)	Max Temp (^{o}C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMH	285	23.6	24.6	7.5	8.6	7.61	8.04
DIEPAMH @ 900 uS/cm (D900)	887	23.5	24.7	7.3	8.5	7.78	8.14
D900 Solvent Control	880	23.6	24.8	5.8	8.5	7.53	8.19
D900 + 125 pptr Cyfluthrin	881	23.6	24.9	6.7	8.6	7.67	8.19
D900 + 250 pptr Cyfluthrin	879	23.5	24.9	6.5	8.5	7.61	8.18
D900 + 500 pptr Cyfluthrin	884	23.5	24.9	6.0	8.6	7.55	8.19
D900 + 1000 pptr Cyfluthrin	884	23.6	25.0	5.7	8.6	7.40	8.20
D900 + 2000 pptr Cyfluthrin	892	23.5	24.5	5.0	8.6	7.44	8.08
Hatchery Water @ 900 uS/cm (HW)	882	23.8	25.1	7.3	8.6	7.78	8.09
HW Solvent Control	883	23.7	25.2	4.3	8.6	7.43	8.11
HW + 125 pptr Cyfluthrin	884	23.5	25.0	7.1	8.6	7.79	8.09
HW + 250 pptr Cyfluthrin	884	23.5	25.0	6.7	8.6	7.64	8.06
HW + 500 pptr Cyfluthrin	880	23.8	25.0	5.0	8.5	7.48	8.07
HW + 1000 pptr Cyfluthrin	883	23.8	25.1	4.3	8.6	7.44	8.09
HW + 2000 pptr Cyfluthrin	887	23.9	24.4	4.4	8.6	7.42	8.09

Table G2-1. Results of a *P. promelas* 7-day test initiated 7/07/09 evaluating the toxicity of permethrin in laboratory control water and in water collected from the UC Davis Delta Smelt Hatchery in Byron, CA.

Treatment	96-hr Survival (%)[1]		7 Day Survival (%)[1]		Biomass (mg)[1]	
	Mean	SE	Mean	SE	Mean	SE
DIEPAMH	100.0	0.0	100.0	0.0	0.369	0.015
DIEPAMH @ 900 uS/cm (D900)	97.5	2.5	97.5	2.5	0.332	0.012
D900 Solvent Control	100.0	0.0	97.5	2.5	0.377	0.020
D900 + 2 ppb Permethrin	100.0	0.0	100.0	0.0	0.379	0.019
D900 + 4 ppb Permethrin	100.0	0.0	97.5	2.5	0.422	0.020
D900 + 8 ppb Permethrin	72.5	8.5	62.5	11.1	0.470	0.086
D900 + 16 ppb Permethrin	0.0	0.0	0.0	0.0	0.000	0.000
D900 + 32 ppb Permethrin	0.0	0.0	0.0	0.0	0.000	0.000
Hatchery Water @ 900 uS/cm (HW)	100.0	0.0	100.0	0.0	0.324	0.059
HW Solvent Control	100.0	0.0	97.5	2.5	0.355	0.021
HW + 2 ppb Permethrin	97.5	2.5	97.5	2.5	0.387	0.010
HW + 4 ppb Permethrin	100.0	0.0	100.0	0.0	0.380	0.035
HW + 8 ppb Permethrin	92.5	4.8	90.0	4.1	0.378	0.023
HW + 16 ppb Permethrin	0.0	0.0	0.0	0.0	0.000	0.000
HW + 32 ppb Permethrin	0.0	0.0	0.0	0.0	0.000	0.000

1. Highlighted areas indicate significant reduction in survival or biomass compared to the solvent control. Data were analyzed using USEPA standard statistical protocols.

Table G2-2. Water chemistry data taken during a *P. promelas* 7-day test initiated 7/07/09 evaluating the toxicity of Permethrin in laboratory control water and in water collected from the UC Davis Delta Smelt Hatchery in Byron, CA.

Treatment	Laboratory Chemistry						
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMH	329	23.5	25.1	6.5	8.4	7.55	8.22
DIEPAMH @ 900 uS/cm (D900)	875	23.6	25.1	6.4	8.3	7.50	8.19
D900 Solvent Control	870	23.6	25.1	3.9	8.5	7.25	8.16
D900 + 2 ppb Permethrin	872	23.8	25.1	4.1	8.4	7.28	8.18
D900 + 4 ppb Permethrin	869	24.0	25.3	4.0	8.3	7.25	8.39
D900 + 8 ppb Permethrin	877	24.1	24.7	3.5	8.5	7.23	7.99
D900 + 16 ppb Permethrin	877	24.2	24.7	3.9	8.2	7.25	8.01
D900 + 32 ppb Permethrin	875	24.2	24.7	3.9	8.3	7.26	8.02
Hatchery Water @ 900 uS/cm (HW)	878	24.0	25.4	6.6	8.6	7.56	8.37
HW Solvent Control	880	24.0	25.3	2.8	8.6	7.25	8.20
HW + 2 ppb Permethrin	881	23.8	25.4	6.1	8.5	7.48	8.43
HW + 4 ppb Permethrin	879	23.8	25.3	4.2	8.4	7.27	8.12
HW + 8 ppb Permethrin	882	24.0	25.5	2.5	8.3	7.23	8.11
HW + 16 ppb Permethrin	882	24.3	24.8	1.3	8.2	7.11	8.06
HW + 32 ppb Permethrin	880	24.2	24.8	1.3	8.2	7.16	8.05

Treatment	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)[1]
DIEPAMH	80	56	0.00	0.000
DIEPAMH @ 900 uS/cm (D900)	120	64	0.02	0.001
Hatchery Water @ 900 uS/cm (HW)	128	66	0.05	0.002

Appendix H

Hyalella azteca
10-day Survival and Weight Sensitivity Tests

Table H1-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 12/12/08 examining the toxicity of cyfluthrin.

Treatment	96-hour Survival (%)[1]		10-day Survival (%)[1]		Weight (mg/surviving individual)[1]	
	mean	se	mean	se	mean	se
DIEPAMHR (Method Control)	100	0.0	97	2.8	0.060	0.006
DIEPAMHR @ 900 uS/cm (D900)	100	0.0	100	0.0	0.057	0.008
D900 Solvent Control	100	0.0	100	0.0	0.042	0.005
D900 w/ 0.977 pptr Cyfluthrin	98	2.5	98	2.5	0.040	0.006
D900 w/ 1.953 pptr Cyfluthrin	100	0.0	100	0.0	0.053	0.005
D900 w/ 3.906 pptr Cyfluthrin[2]	20	8.2	15	9.6	0.067	0.003
D900 w/ 7.813 pptr Cyfluthrin[2]	3	2.5	3	2.5	0.080	-
D900 w/ 15.625 pptr Cyfluthrin	0	0.0	0	0.0	-	-
Hatchery Water	100	0.0	100	0.0	0.064	0.004
Hatchery Water Solvent Control	98	2.5	98	2.5	0.073	0.004
Hatchery Water w/ 0.977 pptr Cyfluthrin	98	2.5	98	2.5	0.050	0.003
Hatchery Water w/ 1.953 pptr Cyfluthrin	72	6.0	65	8.4	0.057	0.004
Hatchery Water w/ 3.906 pptr Cyfluthrin[2]	20	5.8	8	2.5	0.187	0.020
Hatchery Water w/ 7.813 pptr Cyfluthrin	0	0.0	0	0.0	-	-
Hatchery Water w/ 15.625 pptr Cyfluthrin	0	0.0	0	0.0	-	-

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control.
2. These treatments were excluded from analysis of weight effects because of the difficulty of weighing a small number of surviving animals.

Table H1-2. Summary of water chemistry during a *H. azteca* initial screening toxicity test initiated on 12/12/08 examining the toxicity of cyfluthrin.

Treatment	Laboratory Chemistry						
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMHR (Method Control)	346	20.2	24.6	7.2	8.8	7.72	8.03
DIEPAMHR @ 900 uS/cm (D900)	650	20.2	24.8	7.3	8.5	7.70	7.95
D900 Solvent Control	643	20.5	25.0	5.0	8.2	7.41	8.00
D900 w/ 0.977 pptr Cyfluthrin	919	20.1	24.9	6.9	8.6	7.56	7.92
D900 w/ 1.953 pptr Cyfluthrin	916	19.9	24.8	6.9	8.3	7.69	7.95
D900 w/ 3.906 pptr Cyfluthrin	909	20.3	24.9	7.2	8.6	7.74	7.94
D900 w/ 7.813 pptr Cyfluthrin	890	20.3	24.8	6.6	8.4	7.72	7.94
D900 w/ 15.625 pptr Cyfluthrin	833	21.1	23.3	7.9	8.1	7.92	8.07
Hatchery Water	885	20.0	24.4	6.7	8.2	7.96	8.09
Hatchery Water Solvent Control	871	19.9	24.5	3.6	8.2	7.49	8.10
Hatchery Water w/ 0.977 pptr Cyfluthrin	888	20.3	24.5	6.7	8.4	7.89	8.13
Hatchery Water w/ 1.953 pptr Cyfluthrin	894	19.4	24.3	7.0	8.4	7.89	8.14
Hatchery Water w/ 3.906 pptr Cyfluthrin	889	20.3	24.3	7.2	8.5	7.94	8.14
Hatchery Water w/ 7.813 pptr Cyfluthrin	846	21.5	23.6	8.0	8.0	8.06	8.25
Hatchery Water w/ 15.625 pptr Cyfluthrin	845	21.3	23.7	8.0	8.1	8.01	8.24

Table H2-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 12/30/08 examining the toxicity of diazinon.

Treatment	96-hr Survival (%)[1]		10-day Survival (%)[1]		Weight (mg/surviving individual)[1]	
	mean	se	mean	se	mean	se
DIEPAMHR (Method Control)	100	0.0	93	2.5	0.029	0.009
DIEPAMHR @ 900 uS/cm (D900)	100	0.0	100	0.0	0.047	0.002
D900 w/ 0.05% Methanol Control	100	0.0	100	0.0	0.043	0.006
D900 w/ 0.50 ppb Diazinon	100	0.0	100	0.0	0.040	0.003
D900 w/ 1.00 ppb Diazinon	100	0.0	100	0.0	0.038	0.004
D900 w/ 2.00 ppb Diazinon	100	0.0	75	9.5	0.019	0.005
D900 w/ 4.00 ppb Diazinon[2]	58	8.5	13	4.8	0.020	0.005
D900 w/ 8.00 ppb Diazinon	5	3.1	0	0.0	-	-
Hatchery Water	98	2.5	98	2.5	0.037	0.004
Hatchery Water w/ 0.05% Methanol Control	100	0.0	100	0.0	0.045	0.003
Hatchery Water w/ 0.05 ppb Diazinon	100	0.0	100	0.0	0.049	0.003
Hatchery Water w/ 1.00 ppb Diazinon	98	2.5	98	2.5	0.039	0.002
Hatchery Water w/ 2.00 ppb Diazinon	100	0.0	95	2.8	0.033	0.002
Hatchery Water w/ 4.00 ppb Diazinon[2]	68	13.1	23	2.5	0.013	0.004
Hatchery Water w/ 8.00 ppb Diazinon	5	2.9	0	0.0	-	-

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control.

2. These treatments were excluded from analysis of weight effects because of the difficulty of weighing a small number of surviving animals.

Table H2-2. Summary of water chemistry during a *H. azteca* toxicity test initiated on 12/30/08 examining the toxicity of diazinon.

Treatment	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMHR (Method Control)	314	20.4	24.1	7.3	8.8	7.83	8.24
DIEPAMHR @ 900 uS/cm (D900)	854	20.3	24.0	7.2	8.9	7.71	8.22
D900 w/ 0.05% Methanol Control	852	20.6	24.2	4.1	8.6	7.47	8.13
D900 w/ 0.50 ppb Diazinon	853	20.5	24.1	6.9	8.8	7.68	8.22
D900 w/ 1.00 ppb Diazinon	856	20.5	23.9	7.1	8.8	7.70	8.11
D900 w/ 2.00 ppb Diazinon	855	20.6	24.1	7.0	8.7	7.67	8.17
D900 w/ 4.00 ppb Diazinon	866	20.6	25.1	4.6	8.6	7.51	8.13
D900 w/ 8.00 ppb Diazinon	810	20.5	24.4	5.1	8.6	7.61	8.16
Hatchery Water	866	19.3	23.5	7.5	8.7	7.97	8.16
Hatchery Water w/ 0.05% Methanol Control	864	20.2	24.0	3.4	8.7	7.53	8.14
Hatchery Water w/ 0.05 ppb Diazinon	872	20.5	23.7	7.5	8.8	7.89	8.16
Hatchery Water w/ 1.00 ppb Diazinon	863	20.7	23.6	7.1	8.7	7.87	8.13
Hatchery Water w/ 2.00 ppb Diazinon	879	20.7	23.4	4.0	8.8	7.57	8.11
Hatchery Water w/ 4.00 ppb Diazinon	867	20.6	23.5	4.0	8.7	7.56	8.16
Hatchery Water w/ 8.00 ppb Diazinon	822	20.4	23.9	3.9	8.6	7.55	8.13

Table H3-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 1/14/09 examining the toxicity of bifenthrin.

Treatment	Measured Bifenthrin (pptr)	96-hr Survival (%)[1]		10-day Survival (%)[1]		Weight (mg/surviving individual)[1]	
		mean	se	mean	se	mean	se
DIEPAMHR (Method Control)		100	0.0	100	0.0	0.062	0.006
DIEPAMHR @ 900 uS/cm (D900)		100	0.0	100	0.0	0.078	0.008
D900 w/ 0.05% Methanol Control	ND	98	2.5	98	2.5	0.069	0.004
D900 w/ 1.0 pptr Bifenthrin	0.6	100	0.0	100	0.0	0.062	0.010
D900 w/ 2.0 pptr Bifenthrin		98	2.5	98	2.5	0.053	0.004
D900 w/ 4 pptr Bifenthrin	2.0	100	0.0	100	0.0	0.045	0.003
D900 w/ 8 pptr Bifenthrin[2]		75	5.0	35	6.5	0.028	0.007
D900 w/ 16 pptr Bifenthrin	8.0	8	4.8	0	0.0	-	-
Hatchery Water	ND	100	0.0	100	0.0	0.101	0.004
Hatchery Water w/ 0.05% Methanol Control	ND	100	0.0	100	0.0	0.088	0.005
Hatchery Water w/ 1 pptr Bifenthrin	ND	100	0.0	100	0.0	0.089	0.010
Hatchery Water w/ 2 pptr Bifenthrin	ND	100	0.0	100	0.0	0.055	0.010
Hatchery Water w/ 4 pptr Bifenthrin	1.0	100	0.0	98	2.5	0.050	0.002
Hatchery Water w/ 8 pptr Bifenthrin[2]	3.0	90	4.1	33	19.2	0.055	0.016
Hatchery Water w/ 16 pptr Bifenthrin[3]	6.0	13	6.3	3	2.5	0.090	-

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control.

2. These treatments were excluded from analysis of weight effects because of the difficulty of weighing a small number of surviving animals.

3. This treatment was excluded from analysis of weight because surviving animals were found in only one replicate.

Table H3-2. Summary of water chemistry during a 10-day *H. azteca* test initiated on 1/14/09 examining the toxicity of bifenthrin.

Treatment	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMHR (Method Control)	321	21.8	24.4	7.5	8.6	7.82	8.28
DIEPAMHR @ 900 uS/cm (D900)	846	22.0	24.1	7.6	8.4	7.84	8.16
D900 w/ 0.05% Methanol Control	840	22.0	24.4	4.1	8.6	7.49	8.13
D900 w/ 1 pptr Bifenthrin	856	22.3	24.2	7.6	8.5	7.88	8.11
D900 w/ 2 pptr Bifenthrin	839	22.1	24.5	7.4	8.6	7.81	8.14
D900 w/ 4 pptr Bifenthrin	854	22.2	24.4	7.4	8.6	7.87	8.18
D900 w/ 8 pptr Bifenthrin	841	22.2	24.6	7.7	8.6	7.85	8.18
D900 w/ 16 pptr Bifenthrin	829	22.4	24.4	7.2	8.5	7.82	8.09
Hatchery Water	841	22.3	23.8	7.5	8.7	7.93	8.19
Hatchery Water w/ 0.05% Methanol Control	840	22.3	23.9	3.6	8.7	7.45	8.18
Hatchery Water w/ 1 pptr Bifenthrin	855	22.2	25.5	7.6	8.8	7.92	8.14
Hatchery Water w/ 2 pptr Bifenthrin	853	22.1	24.0	7.6	8.9	5.08	8.20
Hatchery Water w/ 4 pptr Bifenthrin	850	21.9	24.1	7.6	8.7	7.98	8.29
Hatchery Water w/ 8 pptr Bifenthrin	842	22.1	24.0	7.7	8.8	7.92	8.13
Hatchery Water w/ 16 pptr Bifenthrin	839	22.2	24.0	7.5	8.6	7.91	8.15

Table H4-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 1/15/08 examining the toxicity of chlorpyrifos.

Treatment	Measured Chlorpyrifos (pptr)	96-hr Survival (%)[1]		10-day Survival (%)[1]		Weight (mg/surviving individual)[1]	
		mean	se	mean	se	mean	se
DIEPAMHR (Method Control)		100	0.0	100	0.0	0.073	0.005
DIEPAMHR @ 900 uS/cm (D900)		100	0.0	100	0.0	0.089	0.005
D900 w/ 0.05% Methanol Control	ND	100	0.0	100	0.0	0.071	0.004
D900 w/ 31.25 pptr Chlorpyrifos	14	100	0.0	98	2.5	0.080	0.005
D900 w/ 62.5 pptr Chlorpyrifos		98	2.5	98	2.5	0.092	0.006
D900 w/ 125 pptr Chlorpyrifos[2]	128	68	13.1	31	13.1	0.096	0.002
D900 w/ 250 pptr Chlorpyrifos		3	2.5	0	0.0	-	-
D900 w/ 500 pptr Chlorpyrifos	540	0	0.0	0	0.0	-	-
Hatchery Water	ND	100	0.0	100	0.0	0.102	0.006
Hatchery Water w/ 0.05% Methanol Control	ND	100	0.0	100	0.0	0.097	0.007
Hatchery Water w/ 31.25 pptr Chlorpyrifos	17	100	0.0	100	0.0	0.101	0.007
Hatchery Water w/ 62.5 pptr Chlorpyrifos	66	100	0.0	100	0.0	0.089	0.008
Hatchery Water w/ 125 pptr Chlorpyrifos[2]	133	59	4.2	21	8.2	0.123	0.011
Hatchery Water w/ 250 pptr Chlorpyrifos	252	0	0.0	0	0.0	-	-
Hatchery Water w/ 500 pptr Chlorpyrifos	420	0	0.0	0	0.0	-	-

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control.
2. These treatments were excluded from analysis of weight effects because of the difficulty of weighing a small number of surviving animals.

Table H4-2. Summary of water chemistry during a *H. azteca* 10-day test initiated on 1/15/09 examining the toxicity of chlorphyrifos.

Treatment	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMHR (Method Control)	323	19.9	23.4	7.9	8.7	7.94	8.28
DIEPAMHR @ 900 uS/cm (D900)	845	20.0	23.5	7.9	8.6	7.86	8.19
D900 w/ 0.05% Methanol Control	848	20.4	23.6	4.2	8.5	7.65	8.17
D900 w/ 31.25 pptr Chlorpyrifos	843	20.4	23.8	7.6	8.7	7.78	8.22
D900 w/ 62.5 pptr Chlorpyrifos	853	22.1	23.8	7.2	8.6	7.82	8.17
D900 w/ 125 pptr Chlorpyrifos	863	20.7	23.8	6.8	8.5	7.74	8.22
D900 w/ 250 pptr Chlorpyrifos	818	22.2	23.7	4.9	8.4	7.63	8.10
D900 w/ 500 pptr Chlorpyrifos	882	22.9	23.5	6.8	8.4	7.72	8.06
Hatchery Water	859	21.9	23.5	7.4	8.8	8.02	8.15
Hatchery Water w/ 0.05% Methanol Control	855	22.4	23.3	3.8	8.8	7.58	8.16
Hatchery Water w/ 31.25 pptr Chlorpyrifos	865	21.7	23.6	7.7	8.7	8.02	8.18
Hatchery Water w/ 62.5 pptr Chlorpyrifos	879	22.1	23.6	7.7	8.9	8.02	8.15
Hatchery Water w/ 125 pptr Chlorpyrifos	863.5	21.9	23.7	7.6	8.9	7.93	8.17
Hatchery Water w/ 250 pptr Chlorpyrifos	822	20.8	23.8	6.9	8.8	7.71	8.10
Hatchery Water w/ 500 pptr Chlorpyrifos	877.5	23.1	23.5	4.5	8.5	7.59	8.15

Table H5-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 1/21/09 examining the toxicity of permethrin in a variety of matrices.

Treatment	Measured Permethrin (pptr)	96-hr Survival (%)[1]		10-day Survival (%)[1]		Weight (mg/surviving individual)[1]	
		mean	se	mean	se	mean	se
DIEPAMHR (Method Control)		100	0.0	100	0.0	0.054	0.004
DIEPAMHR @ 900 uS/cm (D900)	16	100	0.0	100	0.0	0.062	0.004
D900 w/ 0.05% Methanol Control		100	0.0	100	0.0	0.060	0.005
D900 w/ 6.25 pptr Permethrin	6	100	0.0	98	2.5	0.045	0.003
D900 w/ 12.5 pptr Permethrin		100	0.0	100	0.0	0.041	0.007
D900 w/ 25 pptr Permethrin	19	100	0.0	100	0.0	0.044	0.011
D900 w/ 50 pptr Permethrin		100	0.0	98	2.5	0.051	0.005
D900 w/ 100 pptr Permethrin[2]	90	45	17.1	15	5.0	0.090	0.037
Hatchery Water	ND	100	0.0	100	0.0	0.061	0.007
Hatchery Water w/ 0.05% Methanol Control	ND	100	0.0	100	0.0	0.038	0.004
Hatchery Water w/ 6.25 pptr Permethrin	15	100	0.0	100	0.0	0.072	0.007
Hatchery Water w/ 12.5 pptr Permethrin	14	100	0.0	100	0.0	0.062	0.004
Hatchery Water w/ 25 pptr Permethrin	19	100	0.0	94	5.6	0.041	0.008
Hatchery Water w/ 50 pptr Permethrin	40	95	2.9	93	2.5	0.033	0.010
Hatchery Water w/ 100 pptr Permethrin	69	98	2.5	72	6.0	0.038	0.004

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control.

2. These treatments were excluded from analysis of weight effects because of the difficulty of weighing a small number of surviving animals.

Table H5-2. Summary of water chemistry during a *H. azteca* 10-day test initiated on 1/21/09 examining the toxicity of permethrin.

Treatment	Laboratory Chemistry						
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMHR (Method Control)	325	20.2	23.6	8.0	8.6	7.76	8.24
DIEPAMHR @ 900 uS/cm (D900)	821	20.2	23.4	8.2	8.7	7.56	8.40
D900 w/ 0.05% Methanol Control	843	21.0	23.7	4.0	8.8	7.56	8.10
D900 w/ 6.25 pptr Permethrin	831	20.9	23.8	8.0	8.7	7.75	8.13
D900 w/ 12.5 pptr Permethrin	870	21.2	23.7	8.1	8.9	7.78	8.16
D900 w/ 25 pptr Permethrin	836	21.1	23.9	8.0	8.7	7.69	8.20
D900 w/ 50 pptr Permethrin	859	21.2	23.6	7.9	8.8	7.80	8.16
D900 w/ 100 pptr Permethrin	856	21.3	23.8	7.6	8.8	7.71	8.19
Hatchery Water	871	21.8	23.9	7.9	8.9	7.98	8.19
Hatchery Water w/ 0.05% Methanol Control	867	21.3	23.7	3.5	8.7	7.56	8.19
Hatchery Water w/ 6.25 pptr Permethrin	771	21.8	23.9	8.0	8.8	7.98	8.18
Hatchery Water w/ 12.5 pptr Permethrin	881	21.3	23.9	8.0	8.9	7.99	8.15
Hatchery Water w/ 25 pptr Permethrin	857	21.6	23.9	7.7	8.8	7.99	8.18
Hatchery Water w/ 50 pptr Permethrin	864	21.7	23.9	8.0	8.8	7.99	8.14
Hatchery Water w/ 100 pptr Permethrin	861	21.8	24.0	7.9	8.8	7.98	8.18

Table H6-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 4/10/09 examining the toxicity of ammonia.

Treatment	Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)	96-hour Survival (%)[1]		10-day Survival (%)[1]		Weight (mg/surviving individual)[1]	
			Mean	SE	Mean	SE	Mean	SE
DIEPAMHR (Method Control)	0.02	0.001	98	2.5	95	2.9	0.057	0.006
DIEPAMHR @ 900 µS/cm (D900)	0.03	0.002	100	0.0	98	2.5	0.083	0.003
D900 w/ 6.25 mg/L NH_4Cl	4.70	0.236	100	0.0	100	0.0	0.084	0.004
D900 w/ 12.5 mg/L NH_4Cl	9.05	0.368	100	0.0	100	0.0	0.063	0.012
D900 w/ 25 mg/L NH_4Cl	19.0	0.658	97	2.8	92	8.3	0.066	0.004
D900 w/ 50 mg/L NH_4Cl	37.0	1.010	92	5.3	89	4.6	0.046	0.010
D900 w/ 100 mg/L NH_4Cl	78.0	1.512	69	3.6	49	4.3	0.033	0.005
D900 w/ 200 mg/L NH_4Cl	158.4	2.107	23	9.9	0	0.0	-	-
Hatchery Water	0.1	0.007	100	0.0	100	0.0	0.056	0.005
Hatchery Water w/ 6.25 mg/L NH_4Cl	4.85	0.279	100	0.0	100	0.0	0.072	0.010
Hatchery Water w/ 12.5 mg/L NH_4Cl	10.15	0.554	100	0.0	100	0.0	0.055	0.006
Hatchery Water w/ 25 mg/L NH_4Cl	19.4	0.793	100	0.0	98	2.5	0.071	0.004
Hatchery Water w/ 50 mg/L NH_4Cl	39.2	1.378	92	5.3	84	3.1	0.059	0.009
Hatchery Water w/ 100 mg/L NH_4Cl	76.0	1.702	86	5.5	50	9.6	0.034	0.005
Hatchery Water w/ 200 mg/L NH_4Cl[2]	156.8	2.500	44	5.2	11	7.9	0.125	0.045

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard statistical protocols.
2. This treatment was excluded from weight dose-response calculations because of a lack of precision in weighing the few surviving test animals.

Table H6-2. Water chemistry during a 10-day *H. azteca* water column toxicity test initiated on 4/10/09 examining the toxicity of ammonia.

Treatment	Initial EC (uS/cm)	Temp (°C)		DO (mg/L)		pH		Ammonia Nitrogen (mg/L)		Unionized Ammonia (mg/L)	
		Min	Max	Min	Max	Min	Max	Initial	Final	Initial	Final
DIEPAMHR (Method Control)	338	23.1	23.7	5.8	8.7	7.43	8.18	0.02	0.71	0.001	0.019
DIEPAMHR @ 900 µS/cm (D900)	881	23.2	23.8	5.6	8.6	7.40	8.17	0.03	0.65	0.002	0.010
D900 w/ 6.25 mg/L NH_4Cl	917	23.2	23.8	5.8	8.7	7.41	8.06	4.70	1.78	0.236	0.043
D900 w/ 12.5 mg/L NH_4Cl	956	23.2	23.7	6.2	8.8	7.35	7.97	9.05	1.79	0.368	0.035
D900 w/ 25 mg/L NH_4Cl	1039	23.2	24.0	6.4	8.7	7.39	7.89	19.0	1.69	0.658	0.037
D900 w/ 50 mg/L NH_4Cl	1214	23.2	23.6	6.5	8.7	7.36	7.80	37.0	1.69	1.010	0.030
D900 w/ 100 mg/L NH_4Cl	1567	23.1	24.1	6.6	8.9	7.29	7.64	78.0	1.67	1.512	0.022
D900 w/ 200 mg/L NH_4Cl	2157	23.9	23.9	6.7	8.6	7.10	7.49	158.4	-	2.107	-
Hatchery Water	883	23.1	23.5	5.5	8.9	7.57	8.19	0.10	0.63	0.007	0.024
Hatchery Water w/ 6.25 mg/L NH_4Cl	910	23.3	23.9	6.6	8.4	7.54	8.12	4.85	1.69	0.279	0.063
Hatchery Water w/ 12.5 mg/L NH_4Cl	958	23.2	23.8	6.5	8.5	7.55	8.10	10.15	1.66	0.554	0.051
Hatchery Water w/ 25 mg/L NH_4Cl	1046	23.4	23.5	6.3	8.7	7.52	7.98	19.4	1.55	0.793	0.042
Hatchery Water w/ 50 mg/L NH_4Cl	1213	23.4	23.7	6.6	8.3	7.50	7.91	39.2	1.67	1.378	0.035
Hatchery Water w/ 100 mg/L NH_4Cl	1567	23.7	23.9	6.6	8.4	7.39	7.71	76.0	1.74	1.702	0.027
Hatchery Water w/ 200 mg/L NH_4Cl	2204	23.5	23.9	6.5	8.6	7.22	7.57	156.8	1.60	2.500	0.018

Table H7-1. Summary of 10-day *H. azteca* water column toxicity test initiated on 4/10/09 examining the toxicity of copper.

Treatment	96-hour Survival (%)[1]		10-day Survival (%)[1]		Weight (mg/surviving individual)[1]	
	Mean	SE	Mean	SE	Mean	SE
DIEPAMHR (Method Control)	100	0.0	92	2.6	0.068	0.004
DIEPAMHR @ 900 µS/cm (D900)	100	0.0	100	0.0	0.080	0.003
D900 w/ 0.125 ppm Copper	100	0.0	90	4.1	0.027	0.004
D900 w/ 0.250 ppm Copper	98	2.5	7	7.1	-	-
D900 w/ 0.500 ppm Copper	42	7.8	4	3.6	-	-
D900 w/ 1.000 ppm Copper	5	3.1	0	0.0	-	-
D900 w/ 2.000 ppm Copper	0	0.0	0	0.0	-	-
Hatchery Water	100	0.0	100	0.0	0.095	0.012
Hatchery Water w/ 0.125 ppm Copper	100	0.0	100	0.0	0.047	0.009
Hatchery Water w/ 0.250 ppm Copper	98	2.5	85	6.5	0.016	0.003
Hatchery Water w/ 0.500 ppm Copper	69	11.2	0	0.0	-	-
Hatchery Water w/ 1.000 ppm Copper	6	3.6	0	0.0	-	-
Hatchery Water w/ 2.000 ppm Copper	0	0.0	0	0.0	-	-

1. Highlighted areas indicate a significant reduction in survival or weight compared to the appropriate control. Data were analyzed using USEPA standard statistical protocols.

Table H7-2. Water chemistry during a 10-day *H. azteca* water column toxicity test initiated on 4/10/09 examining the toxicity of copper.

Treatment	Laboratory Chemistry						
	EC (uS/cm)	Min Temp (°C)	Max Temp (°C)	Min DO (mg/L)	Max DO (mg/L)	Min pH	Max pH
DIEPAMHR (Method Control)	333	22.7	24.0	7.0	8.8	7.73	8.22
DIEPAMHR @ 900 µS/cm (D900)	873	23.1	23.7	7.8	8.6	7.73	8.21
D900 w/ 0.125 ppm Copper	873	22.8	24.0	7.6	8.7	7.72	8.12
D900 w/ 0.250 ppm Copper	877	22.6	24.0	7.4	8.9	7.88	8.15
D900 w/ 0.500 ppm Copper	872	22.7	24.3	7.7	9.1	7.84	8.12
D900 w/ 1.000 ppm Copper	854	23.9	23.9	7.7	8.5	7.80	7.94
D900 w/ 2.000 ppm Copper	861	23.9	23.9	7.9	8.5	7.76	7.91
Hatchery Water	870	22.7	24.0	7.9	9.0	7.61	8.16
Hatchery Water w/ 0.125 ppm Copper	866	22.8	23.8	7.7	8.9	7.82	8.16
Hatchery Water w/ 0.250 ppm Copper	875	22.9	24.1	7.6	8.8	7.89	8.14
Hatchery Water w/ 0.500 ppm Copper	859	22.7	24.2	7.6	8.9	7.94	8.17
Hatchery Water w/ 1.000 ppm Copper	868	22.6	24.2	7.9	9.0	7.94	8.08
Hatchery Water w/ 2.000 ppm Copper	855	24.1	24.1	7.9	8.1	7.96	7.96

Treatment	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Total Ammonia Nitrogen (mg/L)	Unionized Ammonia (mg/L)[1]
DIEPAMHR (Method Control)	100	56	0.02	0.001
DIEPAMHR @ 900 µS/cm (D900)	168	60	0.03	0.002
Hatchery Water	148	82	0.10	0.006

Appendix I

Analyte Method Detection
&
Reporting Limits

Table I-1. List of organophosphate pesticide analytes with corresponding method detection and reporting limits.

Organophosphate Pesticides	Method Detection Limit (µg/L)	Reporting limit (µg/L)
Azinphos methyl	0.030	0.050
Chlorpyrifos	0.010	0.020
Diazinon	0.005	0.020
Dimethoate	0.030	0.050
Disulfoton	0.010	0.050
Malathion	0.030	0.050
Methidathion	0.030	0.050
Parathion, Methyl	0.010	0.050
Phorate	0.030	0.050
Phosmet	0.030	0.050

Table I-2. List of pyrethroid pesticide analytes with corresponding method detection and reporting limits.

Pyrethroid Pesticides	Method Detection Limit (µg/L)	Reporting limit (µg/L)
Bifenthrin	0.001	0.002
Cyfluthrin	0.002	0.004
Cypermethrin	0.002	0.004
Deltamethrin	0.002	0.004
Esfenvalerate/Fenvalerate	0.001	0.002
Fenpropathrin	0.002	0.004
Lambda Cyhalothrin	0.001	0.002
Permethrin, Cis	0.003	0.005
Permethrin, Trans	0.003	0.005

Table I-3. List of carbamate pesticide analytes with corresponding method detection and reporting limits.

Carbamate Pesticides	Method Detection Limit (µg/L)	Reporting limit (µg/L)
Aldicarb	0.002	0.005
Captan	0.002	0.005
Carbaryl	0.001	0.002
Carbofuran	0.0005	0.001
Diuron	0.002	0.005
Linuron	0.002	0.005
Methiocarb	0.002	0.005
Methomyl	0.0005	0.001

Table I-4. List of Fipronil and Metabolites analytes with corresponding method detection and reporting limits.

Fipronil & Metabolites	Method Detection Limit (µg/L)	Reporting limit (µg/L)
Fipronil	0.100	0.200
Fipronil Desulfinyl	0.100	0.200
Fipronil Sulfide	0.100	0.200
Fipronil Sulfone	0.100	0.200

Table I-5. List of Trace Metal analytes with corresponding method detection and reporting limits.

Trace Metals	Method Detection Limit (µg/L)	Reporting limit (µg/L)
Aluminum	1.70	5.00
Arsenic	0.01	0.03
Cadmium	0.004	0.01
Chromium	0.10	0.30
Copper	0.03	0.10
Lead	0.002	0.006
Manganese	0.01	0.03
Nickel	0.01	0.03
Selenium	0.45	1.00
Silver	0.001	0.003
Zinc	0.05	0.15

Table I-6. List of PAH analytes with corresponding method detection and reporting limits.

PAHs	Method Detection Limit (µg/L)	Reporting limit (µg/L)
Naphthalene	0.00474	0.005
Methylnaphthalene, 2-	0.00457	0.005
Methylnaphthalene, 1-	0.00437	0.005
Dimethylnaphthalene, 2,6-	0.00293	0.005
Trimethylnaphthalene, 2,3,5-	0.00726	0.010
Naphthalenes, C1-	-	0.005
Naphthalenes, C2-	-	0.005
Naphthalenes, C3-	-	0.005
Naphthalenes, C4-	-	0.005
Biphenyl	0.00293	0.005
Acenaphthylene	0.00456	0.005
Acenaphthene	0.00251	0.005
Fluorene	0.00372	0.005
Methylfluorene, 1-	0.00656	0.010
Fluorenes, C1-	-	0.005
Fluorenes, C2-	-	0.005
Fluorenes, C3-	-	0.005
Dibenzothiophene	0.00195	0.005
Methyldibenzothiophene, 4-	0.00371	0.005
Dibenzothiophenes, C1-	-	0.005
Dibenzothiophenes, C2-	-	0.005
Dibenzothiophenes, C3-	-	0.005
Phenanthrene	0.00317	0.005
Methylphenanthrene, 1-	0.00762	0.010
Dimethylphenanthrene, 3,6-	0.00552	0.005
Phenanthrene/Anthracene, C1-	-	0.005
Phenanthrene/Anthracene, C2-	-	0.005
Phenanthrene/Anthracene, C3-	-	0.005
Phenanthrene/Anthracene, C4-	-	0.005
Anthracene	0.00281	0.005
Fluoranthene	0.00340	0.005
Methylfluoranthene, 2-	0.00410	0.005
Fluoranthene/Pyrenes, C1-	-	0.005
Pyrene	0.00379	0.005
Benz(a)anthracene	0.00364	0.005
Chrysene	0.00259	0.005
Chrysenes, C1-	-	0.005
Chrysenes, C2-	-	0.005
Chrysenes, C3-	-	0.005
Benzo(b)fluoranthene	0.00380	0.005
Benzo(k)fluoranthene	0.00377	0.005
Benzo(e)pyrene	0.00285	0.005
Benzo(a)pyrene	0.00345	0.005
Perylene	0.00313	0.005
Indenol(1,2,3-c,d)pyrene	0.00950	0.010
Dibenz(a,h)anthracene	0.00498	0.005
Benzo(g,h,i)perylene	0.00276	0.005