2008/2009 Summary of Don Weston's (UC Berkeley) 2008/2009 Pyrethroid Source Evaluation Study Results from SRWTP Effluent

Date: 10-Jul-09 (Draft)

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INTRODUCTION

The draft information below is a brief summary of effluent (DFE) grab sample chemical analysis and toxicity data intended to identify whether SRWTP is a potential source of pyrethroids to Delta surface waters. This effort was a part of Dr. Don Weston's (UC Berkeley) study for the CVRWQCB and Surface Water Ambient Monitoring Program (SWAMP) to investigate 19 potential sources of pyrethroids at agricultural, urban, and wastewater treatment plant discharges (Figure 1). Samples were collected during three (3) dry weather events and three (3) rain events between January 2008 and February 2009. **The study does not determine the toxicity of discharged pyrethroids in ambient Delta surface waters or sediments**. Once discharged, pyrethroids would likely sorb to suspended particulate matter (if not already part of the suspended particulate phase) and settle out of solution. Pyrethroids also degrade in the environment with half-lives ranging from 30-60 days.

Toxicity was assessed with *Hyalella azteca* – an amphipod known to occur on Delta surface sediments and is very sensitive to pyrethroids. Toxicity was evaluated in split samples by UC Berkeley and Pacific EcoRisk Lab (PER, Vacaville, CA). *H. azteca* survival less than 50% in 96-hour whole effluent toxicity tests triggered toxicity identification evaluation (TIE) re-testing to determine if pyrethroids contributed to the toxicity. TIEs for this study were not designed to determine what other toxicants could be contributing to toxicity. **It is important to note that toxicants other than pyrethroids could be present in 100% effluent samples.**

Chemical analysis of pyrethroids in SRWTP effluent samples was conducted in split samples by the University of Southern Illinois (Dr. Mike Lydy) and CalTest (Napa, CA). Reporting limits differed between the chemical analysis labs, with Dr. Lydy indicating 3 ng/L (parts per trillion) with GC/ECD and CalTest reporting limits ranging from 5 to 10 ng/L using GC/MS/SIM for the nine measured pyrethroids (bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin, and permethrin).

SUMMARY OF RESULTS

- *H. azteca* survived well (36-100%) in 100% effluent (Table 1).
- Split sample toxicity testing varied between labs and did not confirm any incidences of elevated toxicity (i.e., >50% mortality) at either lab.
  - PER found *H.azteca* survival was <50% in only 2/6 sampling events (1 wet and 1 dry); and was significantly reduced in only 4/6 WET tests (2 wet and 2 dry samples)
  - Don Weston found - *H.azteca* survival <50% in only 1/6 samples (dry) and was significantly reduced in in 3/6 tests (1 wet and 2 dry samples).
- Despite low levels of mortality, many effluent samples caused organism paralysis, or nearly dead organisms, by the end of the test. This observation was used as a rationale for TIE testing to determine if pyrethroids may have contributed to the observed toxicity.
- TIEs by both Dr. Weston and PER suggested the presence of pyrethroids in SRWTP effluent.
- Chemical analysis by Dr. Lydy's lab reported pyrethroid concentrations near detection limits in SRWTP effluent (concentrations reported by the Lydy lab were typically less than the CalTest reporting limit).
- CalTest could not validate the detected concentrations of pyrethroids reported by Dr. Lydy'd lab because pyrethroids were below their reporting limits in all SRWTP effluent samples (note that some samples were stored up to the maximum hold-time before analysis).

Table 1. Summary from Pyrethroid Testing with SRWTP Effluent Grab Samples

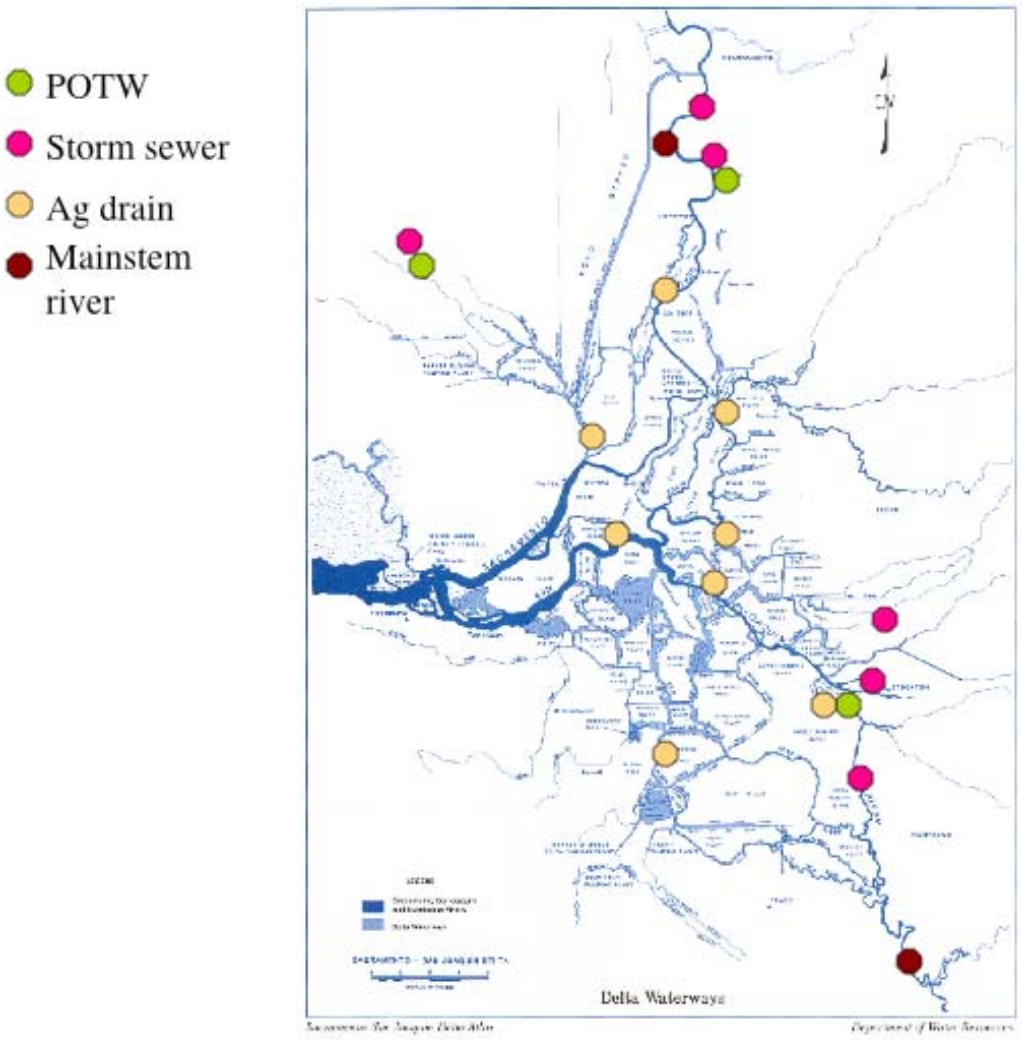
Sample Date	Condition	Survival >50% (Weston/PER)*	TIE Indicates Pyrethroids Present (Weston/PER)	Pyrethroids Detected (Lydy/CalTest)
Jan 27rd	Wet	No / No	No/-	Yes/No
May 27th	Dry	**Yes** / No	Yes/Yes	No*/No
July 15th	Dry	No / No	- /-	Yes/No
Sept 19th	Dry	No / No	Yes/Yes	Yes/No
Nov 2nd	Wet	No / **Yes**	Yes/Yes	No/No
Feb 17th	Wet	No / No	Yes / -	Yes/No

Paralysis toxicity characteristic of all SRWTP effluent samples
*One pyrethroid (bifenthrin) detected below reporting limit.

TIE treatments varied between sample dates but could include any or all of the following treatments which were intended to identify whether pyrethroids contributed to the observed toxicity.

- **Temperature** (lower temp) - increases toxicity if pyrethroids are present. Enzyme detoxification of the pyrethroids slows down at lower temperatures. Tests were conducted at temperatures matching ambient conditions (typically 17 degrees Celsius in the winter and 23 degrees Celsius in the summer).
- **PBO** (piperonyl butoxide) - increases toxicity if pyrethroids are present, but also reduces toxicity from any organophosphate (OP) insecticides present.
- **Enzymes –** specific enzymes can be used to detoxify specific pyrethroids or OP chemicals. PER did not have access to enzyme treatments used by Dr. Weston.

Figure 1. Dr. Weston's Pyrethroid Source Evaluation Sampling Locations



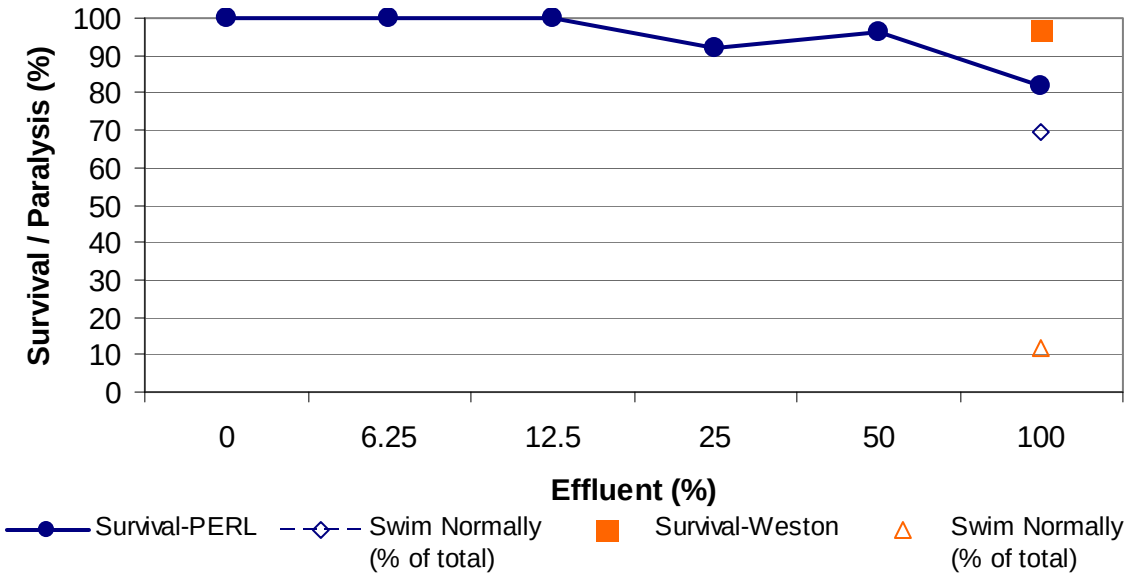
TOXICITY TESTING RESULTS

January 27th, 2008 – WET Event 1

- Toxicity was low (Figure 2)
  - Weston survival was 97% (controls 98%)
  - PER survival was 82% (controls 100%)
- "Paralysis" or immobilized organisms at the end of the test indicated there was an effect from the effluent, but the organisms were not quite dead, yet.
- Don Weston attempted to perform a TIE on this sample based on the paralysis endpoint, but low oxygen resulted from the methanol carrier solution and the test was not valid.
- Don Weston reported that toxicity (paralysis) could not be confirmed as concluded to be from a pyrethroid in this test.
- Chemical analysis (Lydy lab) detected 3 pyrethroids
  - 5.5 ng/L lamda-cyhalothrin

- 7.0 ng/L permethrin
- 1.7 ng/L cyfluthrin (J qualified – estimated concentration below the RL and above the method detection limit)

Figure 2. Whole Effluent Toxicity of WET Weather DFE Grab Sample to *H. azteca*, 1/27/08.



May 27th, 2008 – Dry Event 1

- Toxicity was inconsistent between labs (Figure 3)
  - Weston survival was 36% (controls 80%)
  - PER survival was 100% (controls 100%)
- Low toxicity reported by Don Weston was complicated by poorly performing controls; however, TIEs were performed by Weston and PER.
- TIEs indicated pyrethroids were present (increased toxicity in low temperature and PBO treatments; reduced toxicity in enzyme treatment); however, pyrethroids are likely not the only toxicant present and the proportion of toxicity attributable to pyrethroids is low (Figures 4 and 5).
- Chemical analysis (Lydy lab) did not detect any pyrethroids above reporting limits.
  - 2.7 ng/L bifenthrin J qualified (estimated concentration below the RL and above the method detection limit)

Figure 3. Whole Effluent Toxicity of DRY Weather DFE Grab Sample to *H. azteca*, 5/27/08.

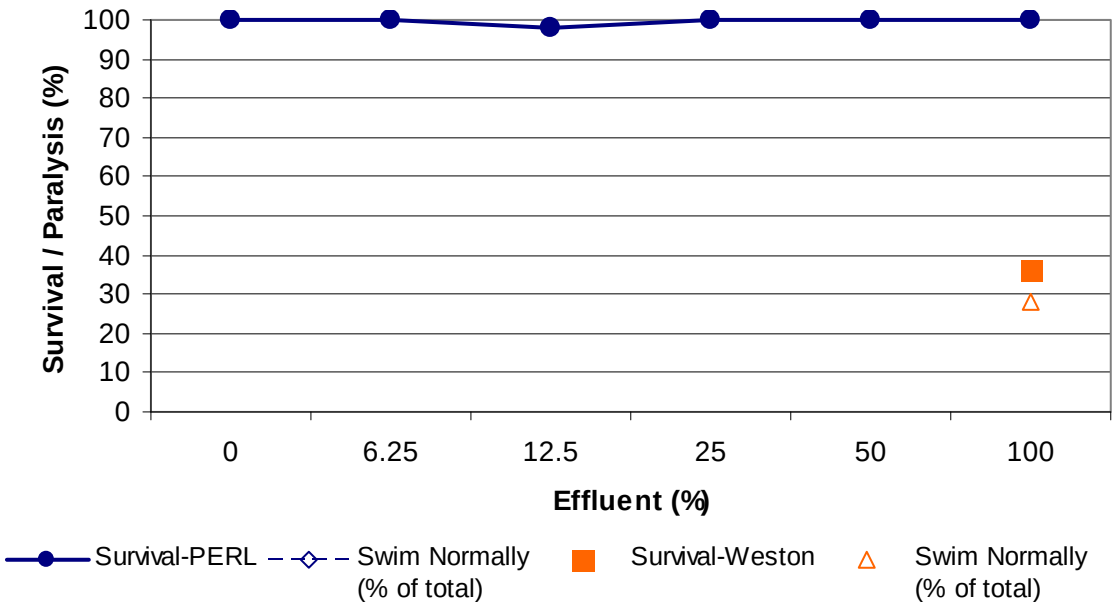


Figure 4. Weston TIE for *H. azteca* Toxicity from DRY Weather DFE Grab Sample, 05-27-08.

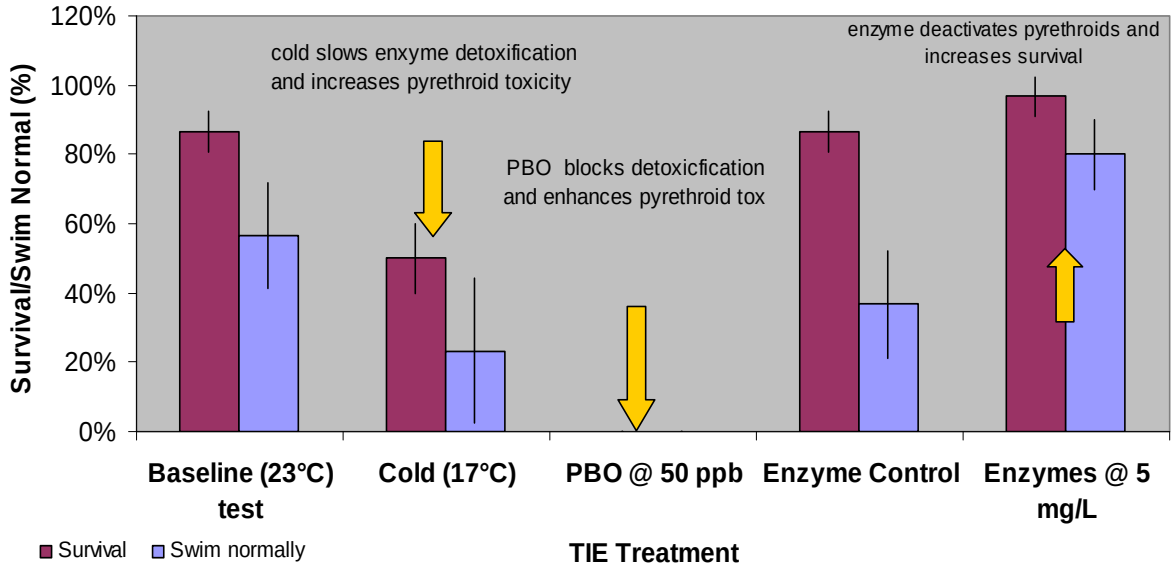
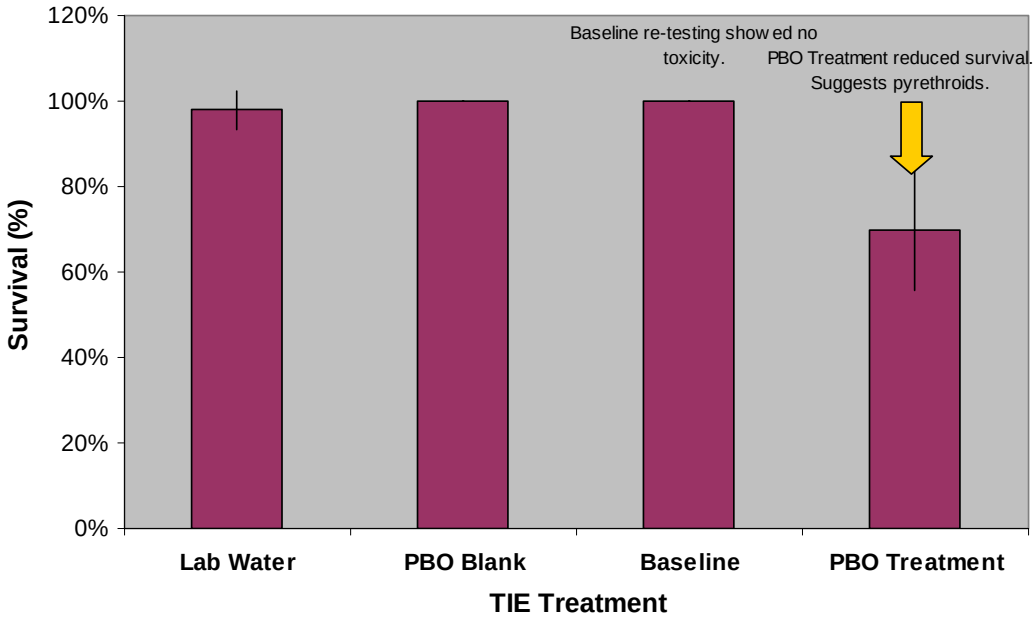


Figure 5. PER's TIE for *H. azteca* Survival in DRY Weather DFE Grab Sample, 05-27-08.



July 15th, 2008 – Dry Event 2

- Toxicity was inconsistent between labs (Figure 6)
  - Weston survival was 88% (controls 96%)
  - PER survival was 40% (controls 100%)
- PER was the only lab to perform a TIE. Results showed persistent toxicity in the re-tested baseline sample (28% survival)
  - Toxicity increased with the addition of PBO (a pyrethroid synergist). These results suggest pyrethroids contributed to effluent toxicity (Figure 7).
- Chemical analysis (Lydy lab) detected 2 pyrethroids
  - 3.5 ng/L lamda-cyhalothrin
  - 12.2 ng/L permethrin
- There are several possible explanations why the Lydy lab reported concentrations (typically permethrin) at concentrations above the CalTest RL while Caltest did not detect them.
  - The lack of validation may suggest the Lydy lab is detecting false positives. This has been reported in the literature for the method used by Lydy's lab (Tony Pirondini pers. Comm.)
  - Maximum holding times by CalTest may have lost some of the sample signal. Pyrethroids sorb to plastics and glass to a lesser degree.

Figure 6. Whole Effluent Toxicity of DRY Weather DFE Grab Sample to *H. azteca*, 7/15/08.

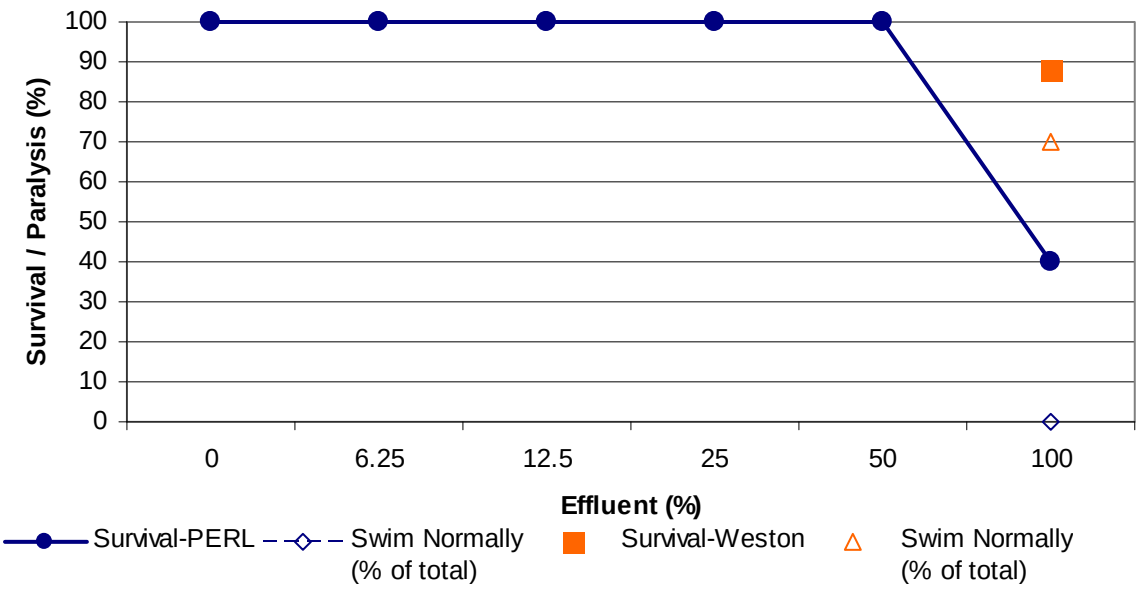
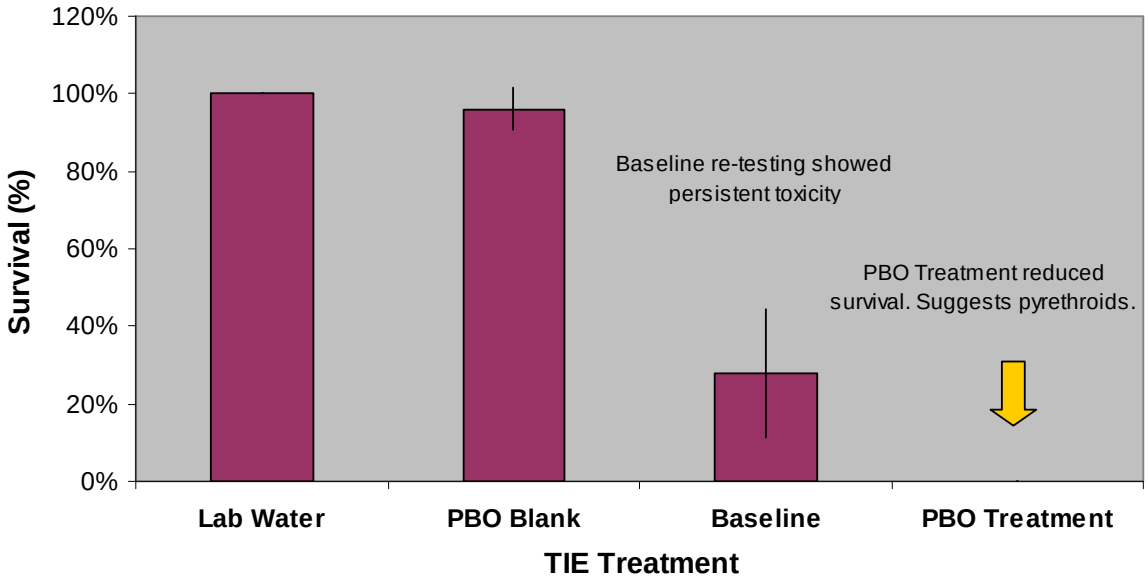


Figure 7. PER's TIE for *H. azteca* Survival in DRY Weather DFE Grab Sample, 07-15-08.



September 19th, 2008 – Dry Event 3

- Toxicity was comparable between labs (Figure 8)
  - Weston survival was 56% (controls 96%)
  - PER survival was 66% (controls 100%)
- TIEs were performed by both labs despite toxicity not meeting the 50% trigger.
  - Don Weston found that found toxicity increased with the addition of PBO (a pyrethroid synergist) and toxicity was significantly reduced with the addition of an enzyme designed to break the ester bond in all pyrethroids. These results suggest pyrethroids contributed to effluent toxicity (Figure 9).

- PER found toxicity increased with the addition of PBO (a pyrethroid synergist). These results suggest pyrethroids contributed to effluent toxicity (Figure 10).

- Chemical analysis (Lydy lab) detected 2 pyrethroids
  - 3.7 ng/L esfenvalerate
  - 17.2 ng/L permethrin
  - Note that baseline organism survival was >50% despite these detected concentrations.

Figure 8. Whole Effluent Toxicity of DRY Weather DFE Grab Sample to *H. azteca*, 9/22/08.

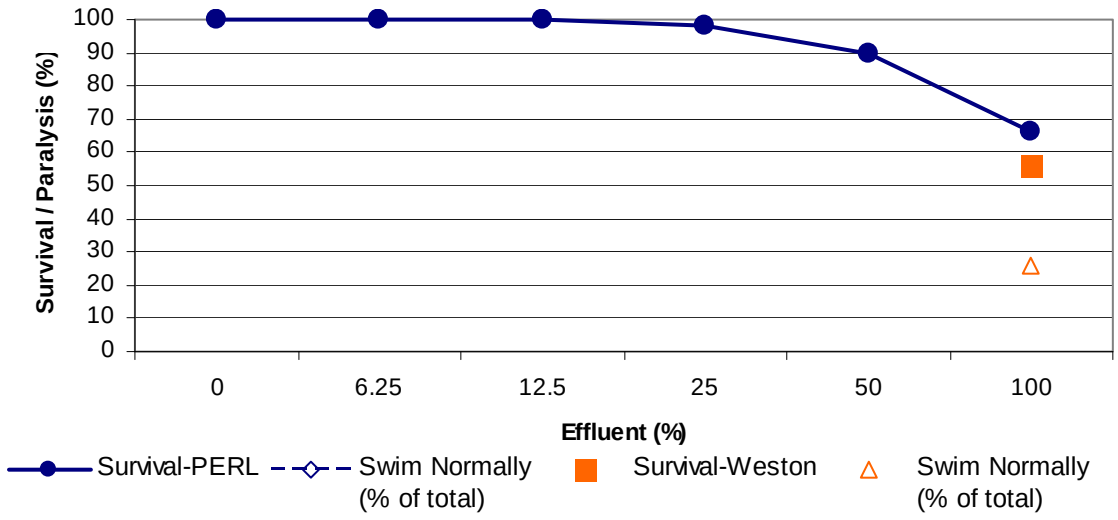


Figure 9. Weston TIE for *H. azteca* Toxicity from DRY Weather DFE Grab Sample, 09-22-08.

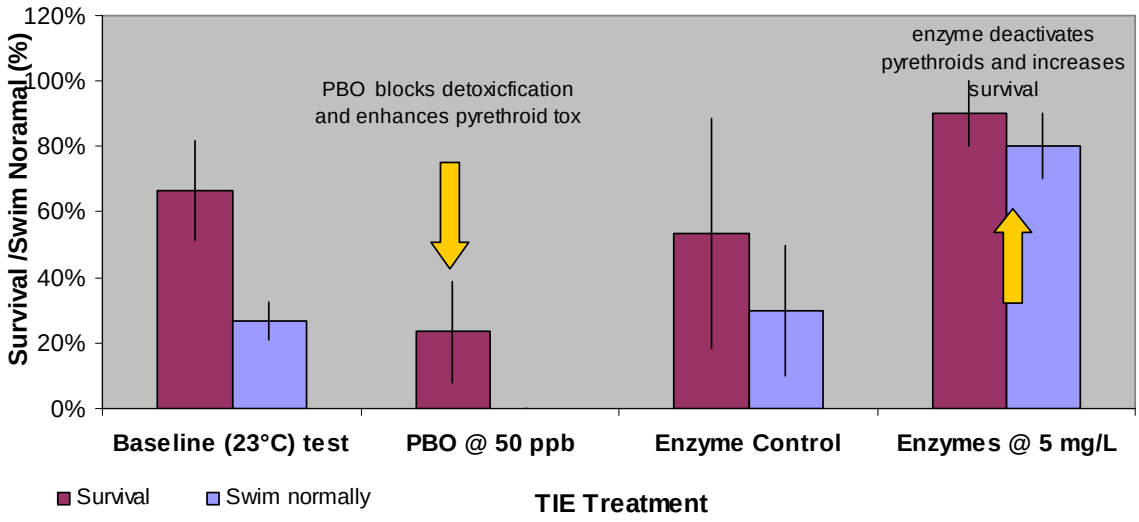
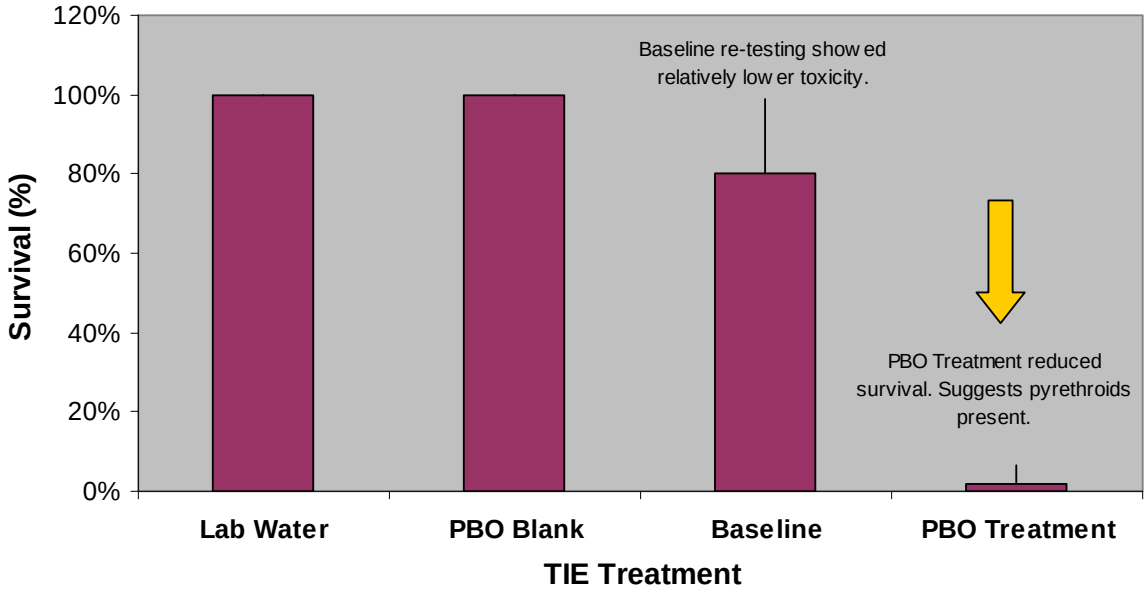


Figure 10. PER's TIE for *H. azteca* Survival in DRY Weather DFE Grab Sample, 09-22-08.



November 2nd, 2008 – Wet Event 2

- Toxicity was comparable between labs (Figure 11)
  - Weston survival was 60% (controls 80%)
  - PER survival was 46% (controls 100%)
- TIEs were performed by both labs.
  - Don Weston found that found toxicity increased with the addition of PBO (a pyrethroid synergist) **but the addition of an enzyme designed to break the ester bond in all pyrethroids did not increase survival**. These **results do not confirm pyrethroid contribution to effluent toxicity** (Figure 12).
  - PER found toxicity increased with the addition of PBO (a pyrethroid synergist), but testing at low temperature was not more toxic than at higher temperatures. These **results do not confirm pyrethroid contribution to effluent toxicity** (Figure 13).
- Chemical analysis (Lydy lab) **did not detect any pyrethroids** in this first flush sampling event.

Figure 11. Whole Effluent Toxicity of WET Weather DFE Grab Sample to *H. azteca*, 11/2/08.

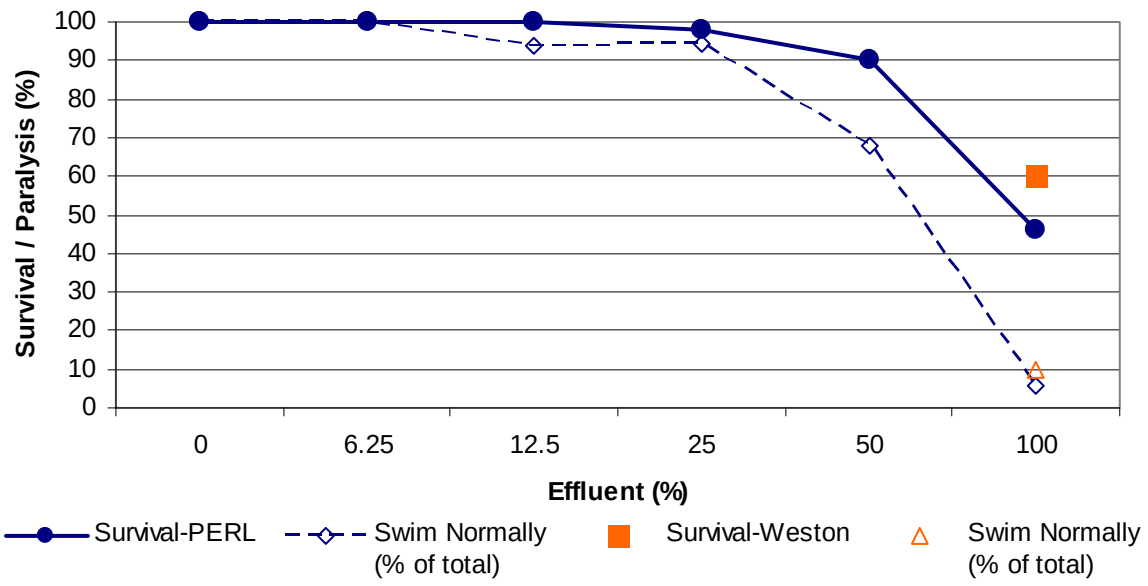


Figure 12. Weston TIE for *H. azteca* Toxicity from DRY Weather DFE Grab Sample, 11-02-08.

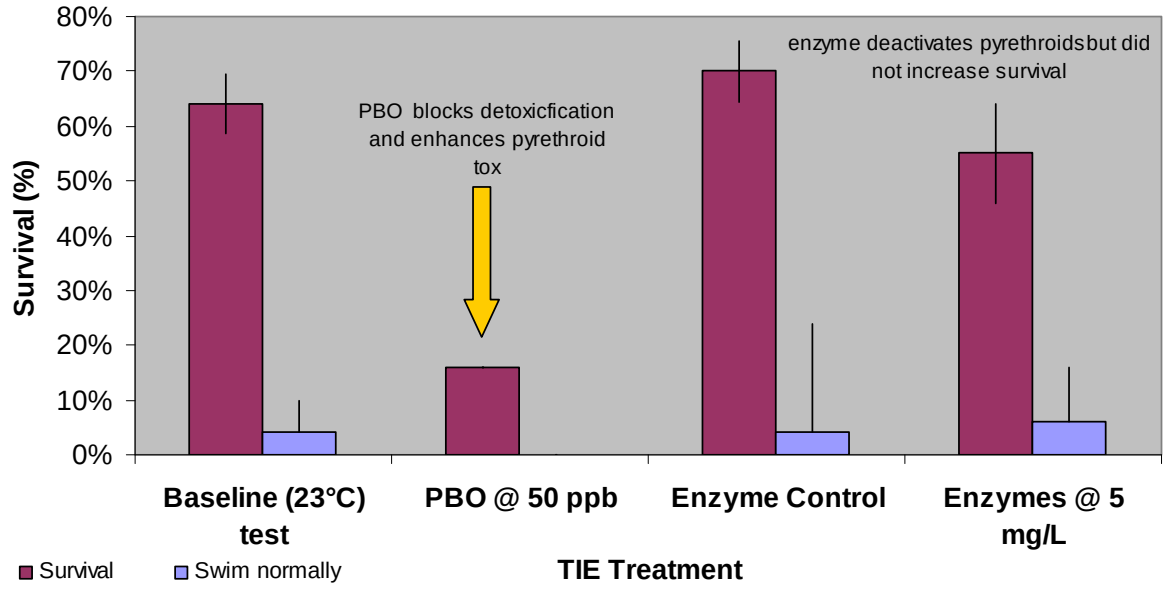
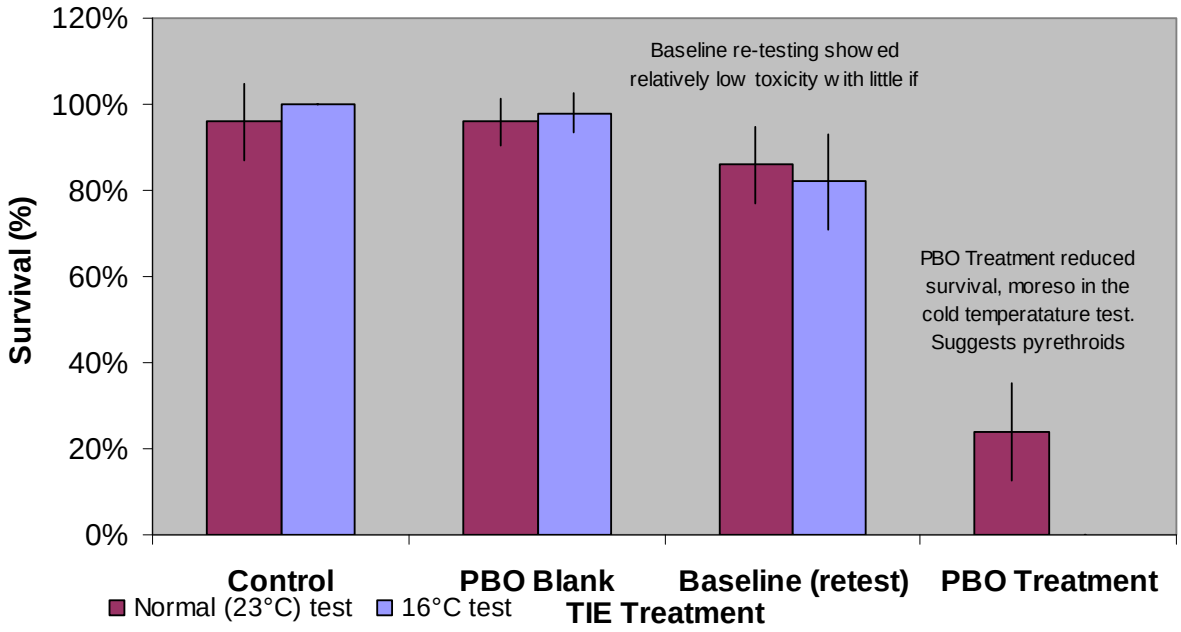


Figure 13. PER's TIE for H. azteca Survival in DRY Weather DFE Grab Sample, 11-02-08.



February 17th, 2009 – Wet Event 3

- Toxicity was low (Figure 14)
  - Weston survival was 96% (controls 96%)
  - PER survival was 62% (controls 96%)
- TIEs were conducted by both labs despite low toxicity not meeting the TIE trigger level.
  - Don Weston found that found toxicity increased with the addition of PBO (a pyrethroid synergist). These **support a conclusion that pyrethroids contribute to effluent toxicity** (Figure 15).
  - PER conducted a TIE concurrent with initial testing. The purpose of this TIE was to evaluate the potential for ammonia toxicity contributing to overall effluent toxicity. Ammonia removal treatment (zeolite) did remove toxicity (Figure 16); however, this treatment could also have removed some degree of organic contaminants. This was not a comprehensive TIE design (due to cost contstraints), but it showed that **other effluent chemicals could account for some of the observed toxicity.**
- Chemical analysis (Lydy lab) detected two pyrethroids
  - 9.4 ng/L permethrin
  - 17 ng/L cypermethrin

Figure 14. Whole Effluent Toxicity of WET Weather DFE Grab Sample to *H. azteca*, 2/18/09.

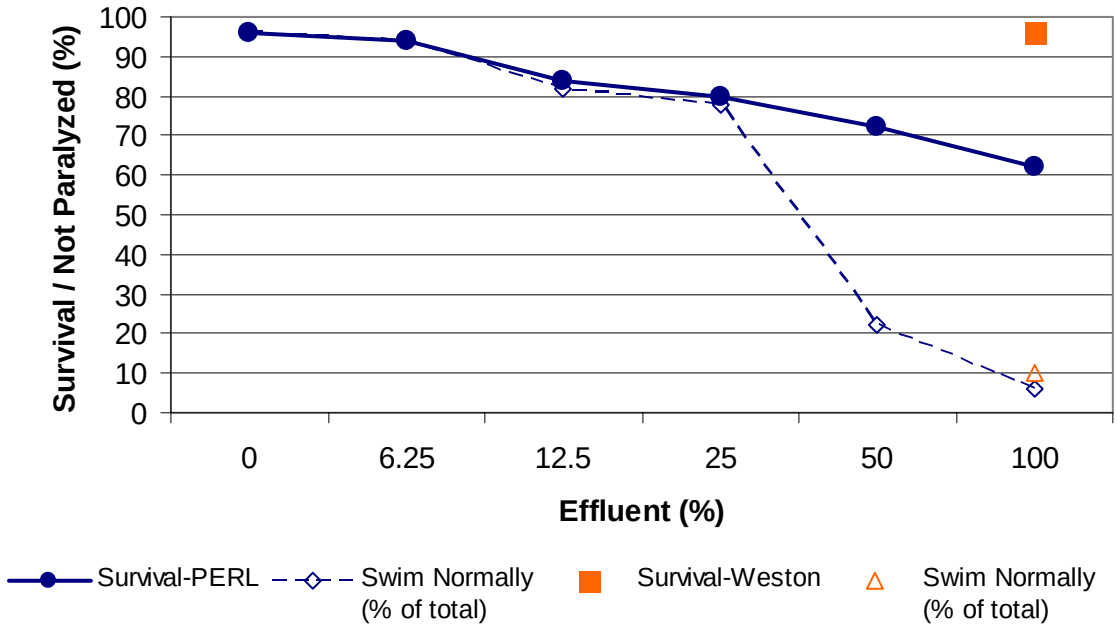


Figure 15. Weston TIE for *H. azteca* Toxicity from WET Weather DFE Grab Sample, 02-17-09

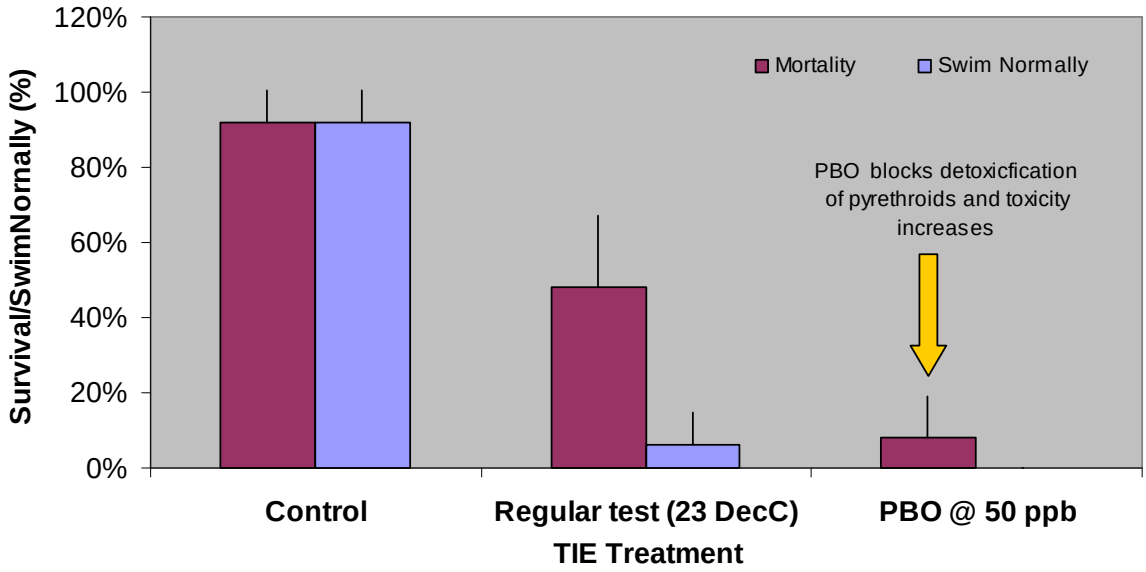
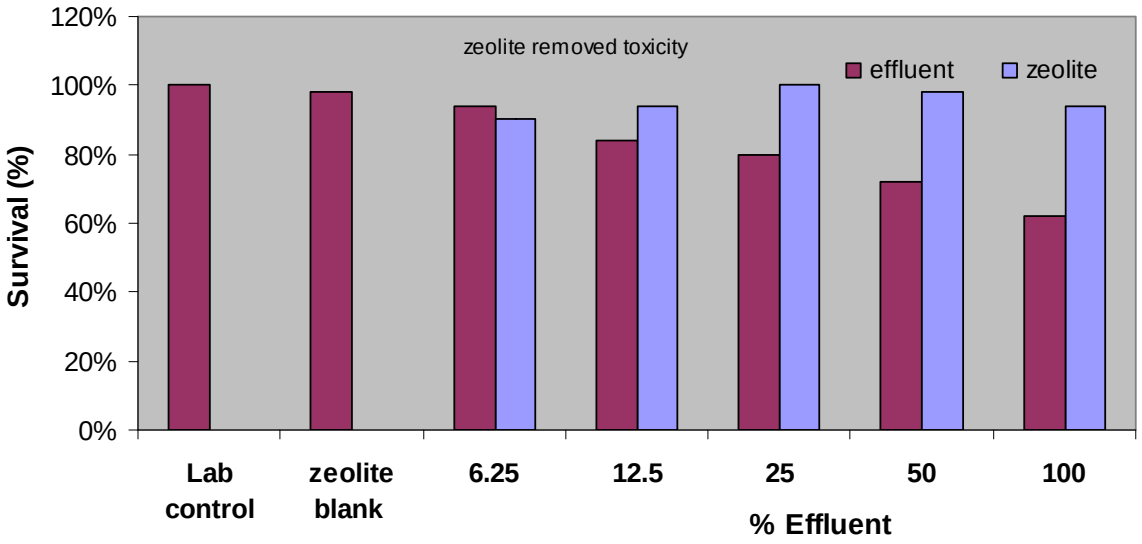


Figure 16. PER's TIE for *H. azteca* Survival in WET Weather DFE Grab Sample, 02-17-09.



CHEMICAL ANALYSIS

Chemical analysis of split effluent samples were conducted by CalTest and the Lydy Lab. CalTest did not report any detected concentrations of pyrethroids in any effluent sample (not shown). Results of the Lydy lab used a different method with intensive clean-up steps. Their reported results for the nine pyrethroids of interest are provided in Table 2.

Wet/Dry sampling conditions did not affect the reported pyrethroid concentrations greatly. The two samples with lowest concentrations (0 detected, although 1 qualified result) occurred during wet weather sampling. Permethrin and other pyrethroids may be diluted in wet conditions. The effects of season may also play a role due to varying application periods of the different insecticides. These **concentrations are well below the 50 – 500 ng/L total pyrethroids reported in urban creek samples**.

Concentrations in 100% effluent are typically below the EC50s for *H. azteca* in water only exposures (Table 3). Cypermethrin exceeded the EC50 by 10 times in the only sample where it was detected (2/17/09).

Table 2. Pyrethroid Concentrations in SRWTP Effluent Grab Samples (Lydy Lab results).

Sample Date	Units	1/27/2008	5/27/2008	7/15/2008	7/15/2008 (duplicate)	9/19/2008	11/2/2008	2/17/2009
Conditions		WET	DRY	DRY	DRY	DRY	WET	WET
chlorpyrifos*	ng/L	no data	0	4.8	5.3	24.1	0	5.8
bifenthrin	ng/L	0	(2.7)	0	0	0	0	0
lamda-cyhalothrin	ng/L	**5.5**	0	3.5	**6.4**	0	0	0
esfenvalerate	ng/L	0	0	0	0	3.7	0	0
delatamethrin	ng/L	0	0	0	0	0	0	0
permethrin	ng/L	**7.0**	0	**12.2**	**14.2**	**17.2**	0	**9.4**
cyfluthrin	ng/L	(1.7)	0	0	0	0	0	0

cypermethrin	ng/L	0	0	0	0	0	0	**17**
fenpropathrin	ng/L	0	0	0	0	0	0	0
Summed Pyrethroids	ng/L	*14.2*	*(2.7)*	*15.7*	*20.6*	*20.9*	*0*	*26.4*

Notes:

Chlorpyrifos is an OP pesticide included in the analyses reported by Dr. Lydy.

Values in bold also exceeded the CalTest RLs but were not detected.

Values in brackets were qualified as estimated concentrations above the MDL but below the RL.

Concentrations could range from 0-3 ng/L for non-detects; therefore summed pyrethroids could vary from 0 – 24 ng/L even when none are detected.

Table 3. H. azteca 96-h EC50s (impaired swimming) and LC50s from Weston lab, UC Berkeley. Values are medians of 3-4 separate tests for each pyrethroid.

Pyrethroid	EC50 (ng/L) based on nominal conc.	EC50 (ng/L) based on actual conc.	LC50 (ng/L) based on nominal conc.	LC50 (ng/L) based on actual conc.
bifenthrin	5.0	3.3	11.5	7.7
lamda-cyhalothrin	Not available	Not available	Not available	Not available
esfenvalerate	Not available	Not available	Not available	Not available
delatamethrin	Not available	Not available	Not available	Not available
permethrin	Not available	Not available	Not available	Not available
cyfluthrin	Not available	Not available	Not available	Not available
cypermethrin	2.8	1.9	3.4	2.3
fenpropathrin	2.5	1.7	3.5	2.3
bifenthrinthrin	Not available	Not available	Not available	Not available

Notes: Because of adsorption to the glass walls of the container, the actual concentration of pyrethroid may be less than the nominal concentration. Over the duration of the exposure (48 hr, after which water is changed and fresh pyrethroids added for another 48 hr), concentrations declined by about two-thirds. Therefore, one-third less than nominal is approximately the average concentration to which the animals were exposed, and that factor has been used in the table to convert nominal to actual values.

Estimated Pyrethroid loading to the Sacramento River

- Estimated river concentrations are very low (< 1 ng/L summed pyrethroids) due to SRWTP effluent dilution (Table 4). These estimated concentrations are below any reported effect concentration.
- There is a high degree of error associated with estimates of river concentrations and loads based on diluted effluent. There are several reasons for this high degree of error:
    - There is a large volume of water discharged from SRWTP and only a single grab was collected during each sampling event. The representativeness of these grabs are not known.
    - Measured concentrations were near reporting limits where there is greater uncertainty than concentrations well above the reporting limit.
    - Measurement errors (±30% recoveries) are amplified when multiplied by the large volumes discharged by SRWTP.
    - For example, the 11/02/09 sample contained no detectable concentrations of pyrethroids. However, the summed concentrations could range from 0 – 24 ng/L if pyrethroids were present below the reporting limit. Likewise, river concentrations could range from 0 – 0.71 ng/L and 0-23 g/day loads. These uncertain concentration ranges span the entire dataset for detected pyrethroids.

Table 4. Estimated Pyrethroid Concentrations in the Sacramento River based on SRWTP Effluent Concentrations (Lydy Lab results).

Sample Date	Units	1/27/2008	5/27/2008	7/15/2008	7/15/2008 (duplicate)	9/19/2008	11/2/2008	2/17/2009
Conditions	-	WET	DRY	DRY	DRY	DRY	WET	WET
Effluent Flow	MGD	193.5	143.8	149.3	149.3	158.8	248.8	215.7
dilution	(:1)	94	47.7	59.6	59.6	39.2	33.6	95.4
bifenthrin	ng/L	0	(0.057)	0	0	0	0	0
lamda-cyhalothrin	ng/L	**0.06**	0	0.06	**0.11**	0	0	0
esfenvalerate	ng/L	0	0	0	0	0.094	0	0
delatamethrin	ng/L	0	0	0	0	0	0	0
permethrin	ng/L	**0.07**	0	**0.20**	**0.24**	**0.44**	0	**0.10**
cyfluthrin	ng/L	(0.018)	0	0	0	0	0	0
cypermethrin	ng/L	0	0	0	0	0	0	**0.18**
fenpropathrin	ng/L	0	0	0	0	0	0	0
Summed Pyrethroids	ng/L	*0.15*	*(0.057)*	*0.26*	*0.35*	*0.53*	*0*	*0.28*

Notes:
Values in bold exceeded the CalTest RLs but were not detected by CalTest.
Values in brackets were based on qualified results.
Concentrations could range from 0-3 ng/L for non-detects in effluent samples; therefore summed pyrethroid concentrations in the river could vary from 0 – 0.71 ng/L even when none are detected.

- Pyrethroid loads were variable and ranged from 0 to 22 g/day
- There were no obvious relationships between loads and sample condition (i.e., wet/dry weather samples).
- Don Weston presented an example (at SETAC, 2008) where 1 million gallons of surface water would dilute 8 mg bifenthrin to concentrations that were non-toxic to H. azteca. Under this example, 150 MGD would dilute 1200 mg or 1.2 g of bifenthrin (load) to below toxic levels.

Table 5. Estimated Pyrethroid Loads into the Sacramento River based on SRWTP Effluent Concentrations and Discharge (Lydy Lab results).

Sample Date	Units	1/27/2008	5/27/2008	7/15/2008	7/15/2008 (duplicate)	9/19/2008	11/2/2008	2/17/2009
Conditions		WET	DRY	DRY	DRY	DRY	WET	WET
Effluent Flow	MGD	193.5	143.8	149.3	149.3	158.8	248.8	215.7
dilution	(:1)	94	47.7	59.6	59.6	39.2	33.6	95.4
bifenthrin	g/day	0	(0.031)	0	0	0	0	0
lamda-cyhalothrin	g/day	**4.0**	0	1.98	**3.6**	0	0	0
esfenvalerate	g/day	0	0	0	0	2.2	0	0
delatamethrin	g/day	0	0	0	0	0	0	0
permethrin	g/day	**5.1**	0	**6.9**	**8.0**	**10**	0	**7.7**
cyfluthrin	g/day	(0.01)	0	0	0	0	0	0
cypermethrin	g/day	0	0	0	0	0	0	**13.9**
fenpropathrin	g/day	0	0	0	0	0	0	0
Summed Pyrethroids	g/day	9.2	<0.1	8.9	11.7	12.6	0	21.6

Notes:
Values in bold exceeded the CalTest RLs but were not detected by CalTest.
Values in brackets were based on qualified results.
Concentrations could range from 0-3 ng/L for non-detects in effluent samples; therefore summed pyrethroid loads could vary from 0 – 23 g/day even when none are detected.

Lab Validation Sample, August 15th (dry weather)

SRWTP effluent samples were collected for chemical validation study at CalTest and the Lydy lab. New analytical equipment at CalTest was hoped to provide lower reporting limits for effluent samples, similar to the Lydy lab. Unfortunately, method development for the new GC/MS/NCI was not validated prior to samples exceeding holding times, and CalTest was unable to complete their method validation.

SUMMARY

Toxicity testing results point to the presence of one or more pyrethroids in SRWTP effluent at least some of the time. Concentrations may be just slightly above toxic levels because toxicity to H. azteca is lost quickly in diluted effluent. However, other chemicals in the effluent could also be contributing to toxicity and the relative proportion of toxicity attributable to pyrethroids was not determined. Diluted concentrations in the Sacramento river would be one or two orders of magnitude lower than those in the effluent. The potential for toxicity in the Sacramento River from discharged pyrethroids was not determined by this study, although could be suggested as an area of future research.