Episodic Toxicity in the San Francisco Bay System

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Table of Contents

Background..3

Methods...4

Results: Years One and Two..5

Methods: Year 3...7

Results: Year 3...8

Summary and Conclusions...8

BACKGROUND

Toxicity testing of ambient waters collected as part of the RMP's baseline monitoring has indicated that, in general, there does not appear to be any consistent, wide-spread toxicity problems within the Bay's waters. A major exception to this have been the occurrences of significant toxicity throughout the northern San Francisco Bay system (from the confluence of the Sacramento and San Joaquin Rivers downstream to the Napa River) following major storm events in Jan-Feb of 1996 and 1997 (Table 1a,b). Based upon earlier studies, it was hypothesized that this toxicity was the result of pesticides in stormwater runoff from within the Sacramento and San Joaquin River watersheds. The fact that the toxic water samples were collected immediately following major rainstorms suggests that ambient water toxicity in San Francisco Bay can occur over short time scales, e.g., the result of stormwater runoff and/or other surface water runoff events.

Table 1a. Toxicity of ambient water samples collected from northern San Francisco Bay sites during the February 1996 RMP cruise.

	% Survival
Control #1	**87.5**
Napa River	**2.5***
Grizzly Bay	**60***
Sacramento River	**7.5***
San Joaquin River	**0***

* Significantly different from the Control treatment at $p<0.05$.

Table 1b. Toxicity of ambient water samples collected from northern San Francisco Bay sites during the January 1997 RMP cruise.

	% Survival
Control #1	**95**
Napa River	**72.5***
Grizzly Bay	**77.5***
Control #2	**80**
Sacramento River	**22.5***
San Joaquin River	**0***

* Significantly different from the Control treatment at $p<0.05$.

In response to these observations, an RMP Special Study was initiated to investigate possible episodic toxicity in San Francisco Bay. Beginning in the winter of 1996-1997, we have been collecting and performing toxicity tests on ambient water samples from stormwater runoff entering the Bay at selected sites. The performance and results of the first two years (1996-98) and of this current third year's (1998-99) episodic toxicity monitoring are discussed below.

METHODS

Sampling - Year One. Based upon the hypothesis that stormwater runoff and other surface water runoff events were the primary sources of toxicity in the North Bay ambient waters (and potentially elsewhere in the San Francisco Bay system), the initial episodic toxicity water sampling events took place immediately after significant rainstorm events. During the initial winter of 1996-1997, samples were collected at the mouths of Guadalupe Slough and Alviso Slough in South Bay, and in Napa River and at Mallard Island in North Bay. The goal in the South Bay and Napa River sites was to sample stormwater runoff that had begun to mix with the Bay's water. Mallard Island, located just downstream of the confluence of the Sacramento and San Joaquin Rivers near Chipps Island, is an ideal sampling site as it represents the influence of upstream waters from the Sacramento and San Joaquin watersheds that flow into the Northern Bay system.

Sampling - Year Two. There were some significant changes incorporated into the second year of monitoring (during the winter of 1997-1998). Ambient water sampling was still performed at Guadalupe Slough; however, Pacheco Slough, which receives runoff from the Concord/Walnut Creek area, was substituted for Napa River. Unlike other major urban creek drainages (e.g., Alameda Creek, Guadalupe Slough, etc.), the Pacheco Slough drainage has not yet been subjected to stormwater runoff toxicity characterization, particularly downstream in the mixing zone with Bay water.

In addition, sampling at Mallard Island was increased from strictly episodic stormwater sampling to include a more continuous regime of biweekly sampling from January through May. The objectives of the "continuous" sampling were threefold:

1. to begin to try and characterize the frequency of short-term toxic events;
2. to determine the duration of toxicity of the episodic events (i.e., the observation of toxicity in consecutive samples could be used to infer that the ambient waters in North Bay were continuously toxic over this same period in time);
3. to incorporate monitoring of ambient water toxicity from non-stormwater surface water runoff (e.g., release of agricultural waters).

Ambient Water Toxicity Testing. Upon receipt of each water sample at the testing lab, initial water quality characteristics were determined, after which an aliquot of the sample was used for setting up the toxicity test, with the remainder of the sample being stored inside a sample refrigerator at 4°C until used for test solution renewals. Each of the water samples was evaluated using the short-term chronic *Mysidopsis bahia* testing approach, with survival as the test endpoint. *Mysidopsis bahia* is a small mysid shrimp native to the Gulf of Mexico; it is not resident to San Francisco Bay, but is a readily-obtained EPA standard test species, which is known to be very sensitive to at least one of the regularly-used OP pesticides (chlorpyrifos).

The control water for these tests consisted of reverse-osmosis de-ionized water to which a commercial artificial sea salt (Forty Fathom®, bioassay grade) was added to bring the salinity up to 20 ppt. Each day's aliquot of the ambient water sample was also salted up to 20 ppt using the same sea salt; the ambient water samples were tested at the 100% concentration only. Prior to use in the tests, the treatment waters were allowed to come to test temperature, after which routine water quality characteristics (pH, dissolved oxygen (D.O.), and salinity) were measured for each treatment test solution.

There were eight replicates for each test treatment, each replicate consisting of 200 mL of water in a 400 mL glass beaker. The tests were initiated by the random allocation of five 7-day old mysids into each replicate. The test replicates were then placed within a temperature-controlled water bath at 26°C.

The mysids were fed brine shrimp nauplii twice each day (once in the a.m. and once in the p.m.). Each day, fresh test solutions were prepared and water quality characteristics determined as before. The test replicates were then removed from the water bath and each was examined to determine the number of surviving organisms. Then, any dead animals, uneaten food, and waste material were removed while replacing approximately 80-90% of the test media within each replicate with fresh test solution, after which the test replicates were replaced into the water bath.

After seven days exposure, each replicate was examined and the final number of surviving organisms determined. The resulting percentage survival data for each ambient water sample was compared to the corresponding control treatment to determine whether any differences were statistically significant at the $p<0.05$ level.

RESULTS: YEARS 1 AND 2

*Year One (1996-97)***.** The unusual rainfall pattern during the winter of 1996-97 impacted the progress of this initial year of the study. The results of the Year One study are summarized in Table 2 below.

Table 2. Summary of RMP Episodic Toxicity Testing Pilot Project, 1996-1998

		Guadalupe Slough Area	Napa River	Mallard Island
Year One (1996-1997)	# Tests	16	2	4*
	# Tests w/ Significant Toxicity	3	0	0*

* Sampling conducted in response to rainstorm events; additional non-storm related sampling is not reported here.

South Bay (Guadalupe Slough/Alviso Slough) - Toxicity was observed in three out of the 16 South Bay (Guadalupe Slough area) samples. ELISA analyses of the OP pesticides diazinon and chlorpyrifos revealed that the concentrations of chlorpyrifos exceeded the reported acute LC_{50} of 35 ng/L for *Mysidopsis bahia* for all three of the toxic samples; however, three other samples which also had measured chlorpyrifos levels exceeding the reported LC_{50} did not exhibit significant mysid mortalities, indicating that chlorpyrifos is not the only cause of observed toxicity.

North Bay (Napa River & Mallard Island) - The heavy rains and major flooding in the Sacramento and San Joaquin River watersheds disrupted the planned sampling at the Mallard

Island station, precluding the collection of samples that might have been impacted by upstream agricultural and urban activities (e.g., pesticide spraying) that might have otherwise taken place in a normal rainfall year. None of the Napa River or Mallard Island samples were toxic. All ELISA measurements of diazinon and chlorpyrifos were below the detection limits.

However, ambient water collected from the northern San Francisco Bay sites during the January 1997 RMP cruise all exhibited significant toxicity (Table 1b), indicating that surface water runoff from the Sacramento and San Joaquin River watersheds was contributing significant toxicity to the ambient waters in the Bay on an episodic basis. Unfortunately, due to the unusual rainfall, there were no Mallard Island samples collected at the same time as the cruise samples for comparative purposes.

*Year Two (1997-98) Results***.** The results of the Year Two study, performed during the winter of 1997-98, are summarized in Table 3 below.

Table 3. Summary of RMP Episodic Toxicity Testing Pilot Project, 1997-1998

	Parameter	Guadalupe Slough Area	Pacheco Slough	Mallard Island
Year Two (1997-1998)	# Tests	14	13	70
	# Tests w/ Significant Toxicity	2	5	10

Guadalupe Slough - Two out of 14 samples collected at Guadalupe Slough resulted in significant toxicity (both with $\geq$50% mortality relative to the control). Of the 14 water samples collected, eight had elevated concentrations of diazinon and/or chlorpyrifos. However, only one of the toxic Guadalupe Slough water samples had measured diazinon or chlorpyrifos concentrations exceeding the reported acute LC_{50} for *Mysidopsis bahia*, suggesting that other contaminants were responsible for the observed toxicity.

Pacheco Slough - Five out of 13 samples collected at Pacheco Slough resulted in statistically significant mortality, although only one toxic sample exhibited greater than 50% mortality. Of the 13 water samples collected, 10 had measurable concentrations of diazinon and/or chlorpyrifos. However, only one of the water samples had measured diazinon or chlorpyrifos concentrations exceeding the reported acute LC_{50} for *Mysidopsis bahia*, again suggesting that other contaminants were responsible for some of the observed toxicity.

Mallard Island - As described above, ambient water samples were collected at Mallard Island:

1. on an episodic basis, following significant storm events; and
2. on a continuous basis, biweekly from January through May.

Of the 70 water samples collected, 10 resulted in significant mysid mortality (eight of which exhibited >50% mortality). More importantly, the were two periods of time, February 12-17 and May 5-9, during which three consecutive water samples were toxic (see 1998 Annual Report), suggesting that the ambient waters in North Bay were similarly toxic for at least two extended periods of time during this monitoring.

In order to save costs, ELISA analysis were not performed on the ‘routine’ biweekly water samples collected from Mallard Island (the greatest likelihood of elevated pesticide concentrations in these ambient waters will be during stormwater runoff events, and therefore, diazinon and chlorpyrifos were measured in the Mallard Island water samples only following significant rainstorms and at the same time that Guadalupe Slough and Pacheco Slough water samples were being analyzed). Surprisingly, only two of the toxic water samples from Mallard Island had diazinon or chlorpyrifos concentrations that exceeded the reported LC_{50}; in six of the toxic water samples, including two of the three consecutively toxic samples in February, both diazinon and chlorpyrifos were below the ELISA detection limit (and well below LC_{50}), indicating that other contaminants were responsible for the observed toxicity.

METHODS: YEAR 3

*Sampling***.** It was decided that the previous two years of monitoring had provided adequate evidence of episodic toxicity in surface water entering San Francisco Bay from Guadalupe Slough, which was therefore dropped as a sampling station for Year Three. Episodic sampling (i.e. following storm events) was maintained at Pacheco Slough, and was re-initiated at Napa River. As before, Mallard Island was sampled following storm events, with more continuous sampling (three times per week) taking place from January through June. In addition, and based upon observation of ambient water toxicity for the northern San Francisco Bay samples collected during the July 1996 cruise (Table 4), sampling at Mallard Island was extended on a once-per-week basis from July through September.

Table 4. Toxicity of ambient water samples collected from northern San Francisco Bay sites during the July 1996 RMP cruise.

	% Survival
Control #1	**95**
Grizzly Bay	**72.5***
Sacramento River	**77.5***
San Joaquin River	**72.5***

* Significantly different from the Control treatment at $p<0.05$.

*Testing***.** All ambient water samples were tested using the same 7-day survival test with *Mysidopsis bahia* as was used in the first two years of the study. ELISA analyses for diazinon and chlorpyrifos were also performed, as before.

RESULTS: YEAR 3

The results of the Year Three study, performed during the winter of 1998-99, are summarized in Table 5 below.

Table 5. Summary of RMP Episodic Toxicity Testing Pilot Project - Year Three (1998-1999).

Sample	% Mysid Survival		ELISA Analyses (ng/L)	
	Control	Site Water	Diazinon	Chlorpyrifos
Napa River (3/9/99)	92.5	62.5*	52	< 60
Napa River (5/3/99)	95	0*	< 30	< 60
Pacheco Slough (2/7/99)	90	0*	295	< 60
Pacheco Slough (4/5/99)	97.5	85*	< 30	< 60
Pacheco Slough (5/3/99)	95	67.5*	< 30	< 60
Mallard Island (2/16/99)	95	2.5*	< 30	< 60
Mallard Island (3/26/99)	100	85*	< 30	< 60
Mallard Island (6/1/99)	97.5	85*	< 30	< 60

Napa River - Two of the 10 Napa River samples were toxic to *Mysidopsis bahia.* The toxic sample collected on 5/7/99 resulted in 100% mysid mortality within 48 hrs; a re-test of that same sample with new test organisms resulted in complete mortality within 24 hrs. Both of these samples had measured concentrations of diazinon and chlorpyrifos which were below the reported acute LC_{50}, suggesting that other contaminants were responsible for the observed toxicity.

Pacheco Slough - Three of the 11 samples collected from Pacheco Slough resulted in significant mysid mortalities. All three of these samples had measured concentrations of diazinon and chlorpyrifos which were below the reported acute LC_{50}.

Mallard Island - Only three of the 61 water samples collected through the end of June at Mallard Island resulted in significant reductions in *Mysidopsis bahia* survival. For all three samples, the measured concentrations of diazinon and chlorpyrifos were below the detection limits. Unlike the previous year, there were no sets of consecutively toxic samples indicative of an extended duration of ambient water toxicity.

SUMMARY AND CONCLUSIONS

The Regional Monitoring Program has been assessing aquatic toxicity of ambient waters in the San Francisco Bay system two or three times annually since 1993. It is now known that toxicologically relevant variations in contaminant concentrations occur on smaller time scales due to events such as urban runoff following rainstorms or from similar surface runoff following application of pesticides in agricultural areas, and our monitoring has revealed significant ambient water toxicity following such events.

However, relative to the Year Two results, the current Year Three monitoring results suggest a reduction in the level of toxicity. Only 27% of the Pacheco Slough samples were significantly toxic in 1998-99, relative to 38% toxicity frequency observed in 1997-98. This difference was even more pronounced for Mallard Island, with only 5% of the samples being toxic in 1998-99, relative to 14% toxicity frequency observed in 1997-98. Moreover, the Year Three monitoring of Mallard Island samples did not observe any sets of consecutively toxic samples indicative of an extended period of ambient water toxicity, such as were observed in February and May of 1998.

However, reduced toxicity to *Mysidopsis bahia* may or may not reflect reduced toxicity to the invertebrates actually inhabiting San Francisco Bay. At least one important resident invertebrate, the crustacean *Palaemon macrodactylus,* is reported to be much more sensitive to these pesticides than is *Mysidopsis*; such comparative toxicity information is lacking for most of the resident invertebrates, and it is quite possible that one or more of the resident invertebrate populations are experiencing severe toxic impacts as a result of episodic input of one or more contaminants in surface water runoff from urban and/or agricultural areas. Long-term studies have reported significant declines in zooplankton abundance in the estuary, with recent zooplankton densities being 1-2 orders of magnitude lower than in the early 1970s. Use of pesticides has increased substantially over the last several decades, suggesting a possible link between pesticide toxicity and zooplankton declines.

It can also be argued that if contaminant impacts on resident invertebrate populations are occurring, then impacts on fish may be taking place as a result of reduced food resources. Virtually all of the important fish populations in the San Francisco Estuary rely upon the estuary's invertebrate populations for food, particularly during their vulnerable early life stages. If pulses of toxicity through this ecosystem diminish the available invertebrate resources at critical periods, such as when larval fish are using the invertebrates for food, then adverse effects on fish populations can be expected.

Furthermore, episodic ambient water toxicity may directly impact fish. Ambient water samples collected from the Napa River as part of another study were shown to cause complete mortality to larval fish (*Menidia beryllina*), whereas *Mysidopsis bahia* were unaffected. These test results again indicate that there can be significant differences in toxicant sensitivity of different organisms. Again, this indicates the possibility that samples which were not toxic to *Mysidopsis bahia* in the current study may well have been toxic to other organisms, including fish.

The pesticides diazinon and chlorpyrifos have been most commonly linked with ambient water toxicity in upstream waters of the Sacramento and San Joaquin River watersheds. It has been demonstrated that 'pulses' of pesticides from the Sacramento and San Joaquin River watersheds can and do flow into and through northern San Francisco Bay. Furthermore, we have measured chlorpyrifos in some toxic ambient water samples at concentrations which exceed the reported acute LC50, and we have previously hypothesized that these pesticides, particularly chlorpyrifos, may have been playing a significant role in the ambient water toxicity that we have observed. However, we have also observed that some ambient water samples with similar chlorpyrifos concentrations do not result in significant *Mysidopsis* mortalities. This may be a function of bioavailability, such that under certain conditions, the ELISA method measures some chlorpyrifos that otherwise would not be bioavailable (e.g., the ELISA antibodies may ‘scavenge’ chlorpyrifos molecules which are sorbed to particulates or dissolved ligands).

More importantly, there have been several toxic ambient water samples, including all of the toxic samples collected in the Year Three monitoring, which have had diazinon and chlorpyrifos concentrations well below toxic thresholds,. This clearly indicates that other contaminants are responsible, at least in part (OP pesticide toxicity can be additive), for the observed toxicity. Funding has been made available such that one of the current objectives of the episodic toxicity investigation is the identification of the source of observed toxicity using toxicity identification evaluation (TIE) methods. Identifying the causes (and sources) of episodic ambient water toxicity that occurs in San Francisco Bay will be an essential step forward in the management and control of toxic contaminants entering the Bay ecosystem.